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Plant physiology and biochemistry

EFFECT OF DONOR PLANT GROWTH ENVIRONMENT ON *IN VITRO* ANDROGENESIS IN WHEAT (*TRITICUM AESTIVUM* L.)

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The effect of the donor plant growing conditions on *in vitro* androgenesis was studied using several winter wheat varieties. The results indicate that significant differences can be expected in variety responses in different experiments due to the changes in donor plant growing conditions. The varieties also differ in their sensitivity to changes in the growth environment of the donor plants. Both genotype and donor plant environment play a significant role in determining *in vitro* androgenesis. Callus formation and green plant regeneration are more significantly influenced by genotype than by environmental factors. In contrast, environmental factors and the genotype x environment interaction are chiefly responsible for the extent of total plant regeneration.

Keywords: wheat, anther culture, genotype, donor plant growing condition

Introduction

The frequency of *in vitro* androgenesis is significantly influenced by the genotype and also by the donor plant growth environment. Many authors have reported that there was a significant difference in the androgenetic abilities of the varieties in different years (Dunwell, 1976; Foroughi-Wehr and Mix, 1979). The importance of growth conditions was demonstrated by comparing *in vitro* androgenesis of field-grown plants to that of plants raised in the greenhouse or growth chamber (Lazar et al., 1984; Jones and Petolino, 1987; Barnabás et al., 1989; Björnstad et al., 1989). Several researchers have studied the effect of genotype and donor plant growth environment on microspore embryogenesis, with a simultaneous study of the interactions. The results indicate that the environment of the donor plant also plays a significant role in the determination of callus induction. It has less influence than the genotype and makes its effect on callus formation through interaction with the genotype (Charmet and Bernard, 1984; Lazar et al., 1984; Jones and Petolino, 1987; Björnstad et al., 1989). With respect to plant regeneration ability, most of the results indicate that total plant regeneration is not significantly influenced either by the genotype, or by the donor plant environment, or by interactions between these two factors (Andersen et al., 1987; Knudsen et al., 1989; Tuvesson et al., 1989). By contrast, Lazar et al. (1984) found a genotype effect significant at the P=0.001 level, while environmental factors did not appear to have any influence on this ability. At the same time, the frequency of green plant regeneration was more influenced by genotype than by environmental factors.

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It is thus important to investigate the genotypic reliability of the androgenetic ability and the extent of variation in anther culture due to the different donor plant growth conditions. To this end, five winter wheat varieties were used to examine the effect of donor plant growth environment on *in vitro* androgenesis.

Materials and methods

The results obtained for two varieties Mv 15 and Mv 18 in anther cultures in five different experiments, were compared in order to determine the stability of the variety response. In order to study the variety x donor plant growth condition interaction, anther cultures were initiated simultaneously from three varieties (Mv 16, Mv 18 and Fatima) in three experiments. In the first experiment, in May 1987, the anthers were isolated from field-grown plants, while the donor plants used in December 1987 and January 1988 were raised in the greenhouse.

In the case of field experiments the seeds of donor plants were sown in autumn in the field and anther cultures were initiated in May of the next year.

The growing conditions of the donor plants raised in greenhouse were similar in each case. After a seven-week vernalization, the seedlings were transplanted into pots. At the beginning the temperature was kept at 15/10 °C for helping good tillering of the plants, then was raised to 18/15 °C. An artificial light of six hours at night was added to the natural light conditions.

The anther culture method used was the same in each independent experiments. The ears were isolated in the medium to late uninucleate microspore stage of development. Sterilization was carried out in 0.1% $HgCl_2$ solution, after which the anthers were placed on P2 (Ouyang, 1986) medium. The cultures were kept in the dark at 29 °C for 30 days. The calli obtained were evaluated and transferred to 190-2 regeneration medium (Zhuang and Jia, 1983). Plant regeneration took place at 26 °C with 16-hour illumination. After 30 days we recorded the ratio of green to albino plants.

The experiments were arranged completely at random. For the statistical evaluation, the mean responses given by anthers excised from two–four ears represented one replication; there were a total of eight replications. Single factor variance analysis was applied to analyse the response exhibited by the variety Mv 18 in different years, while the effects of variety and donor plant growth condition on anther cultures were compared with the aid of bifactorial variance analysis (Sváb, 1981).

Results

Anther cultures were initiated in five different experiments for two winter wheat varieties, Mv 15 and Mv 18. The varying androgenetic abilities of the two varieties are illustrated in Tables 1 and 2. On studying the variety reaction shown in anther culture in different experiments, it was found that there were significant differences in the frequency of anther response and of callus induction in Mv 15. For both properties there was a threefold difference between the lowest and highest values. By contrast, the plant regeneration frequency for this variety was similar on all five occasions. In the case of the other variety, Mv 18, the effect of donor plant growing conditions was significant for all four properties studied. Variability was particularly great for the frequency of anther response and for green plant regeneration.

Table 1

In vitro *androgenesis of the variety Mv 15 in different experiments (Martonvásár)*

Date	Environment	Anther response (%)	Callus induction (%)	Plant regeneration total (%)	Plant regeneration green (%)
Dec. 1987	Greenhouse	16.77	41.14	18.57	11.40
Jan. 1988	Greenhouse	7.29	19.10	27.69	11.46
May 1988	Field	9.24	33.20	22.36	9.71
Dec. 1988	Greenhouse	5.84	12.15	21.38	7.48
Feb. 1989	Greenhouse	9.37	22.23	22.49	12.11
	L.S.D. (P=0.05)=5.69		14.45	11.87	9.55

Table 2

In vitro *androgenesis of the variety Mv 18 in different experiments (Martonvásár)*

Date	Environment	Anther response (%)	Callus induction (%)	Plant regeneration total (%)	Plant regeneration green (%)
May 1987	Field	11.88	33.44	36.81	0.00
Dec. 1987	Greenhouse	11.95	24.28	18.84	4.58
Jan. 1988	Greenhouse	9.32	23.00	19.56	0.93
May 1989	Field	9.57	23.61	23.54	6.93
May 1990	Field	18.26	44.29	39.34	13.63
	L.S.D. (P=0.05)=3.65		10.68	10.10	4.90

It is thus important to determine the role of interaction between genotype and the growth environment of the donor plant in *in vitro* androgenesis. To this end, anther cultures were initiated simultaneously from three wheat varieties (Mv 16, Mv 18 and Fatima) in three different experiments. The results of variance analysis (Table 3) indicate that both the growth environment of the donor plant and the genotype have a significant effect (at P=0.001) on the frequencies of anther response and callus induction and on the total plant regeneration. For the first two properties, the interaction between these two factors also played a significant role. In the case of callus induction, genotype was of primary importance. The frequency of total plant regeneration, on the other hand, was influenced to a greater extent by the growing condition of donor plants. The majority of the variance observed for green plant regeneration could be attributed to the genotype, but the growth conditions had no effect in themselves, only in interaction with the genotype.

Table 3

Variance analysis on the androgenetic responses of three winter wheat varieties in three different experiments (Martonvásár)

Factor	Df	Anther response	Callus induction	Plant regeneration total	Plant regeneration green
		MS	MS	MS	MS
Experiment	2	165.52***	1868.36***	3949.90***	47.80
Variety	2	361.31***	9562.91***	1409.71**	4318.10**
Experiment x Variety	4	50.06*	851.64**	487.35	321.22*
Error	56	18.73	156.79	246.26	101.85

Significant at the *P=0.05; **P=0.01; ***P=0.001 levels

The *in vitro* androgenesis of the three winter wheat varieties is presented in Table 4. With respect to anther response and callus induction, the best variety, Mv 16, exhibited great variability, while the other two varieties, Mv 18 and Fatima, gave similar results in all three experiments. In the first experiment the anther response and callus induction frequencies of the three varieties differed significantly from each other, the values decreasing in the order Mv 16, Mv 18, Fatima. On the second occasion, Mv 16 differed significantly from the other two varieties, but in the third experiment this difference disappeared. When averaged over the three varieties, this latter experiment gave significantly the poorest results.

Table 4

In vitro *androgenesis of three winter wheat varieties in three experiments (Martonvásár)*

Variety	Experiment	Anther response (%)	Callus induction (%)	Plant regeneration total (%)	Plant regeneration green (%)
Mv 16	May 1987	16.05	70.78	52.13	7.82
	Dec. 1987	21.03	62.46	21.85	12.49
	Jan. 1988	10.19	32.44	22.47	2.84
Mv 18	May 1987	11.88	33.44	36.81	0.00
	Dec. 1987	11.95	24.28	18.84	4.58
	Jan. 1988	9.32	23.00	19.56	0.93
Fatima	May 1987	7.50	16.63	50.86	24.31
	Dec. 1987	9.45	19.43	23.73	21.96
	Jan. 1988	7.20	14.03	46.56	36.07
	L.S.D. (P=0.05)=4.33		12.54	15.72	10.11

With respect to total plant regeneration, the varieties did not differ significantly from each other in two of the experiments; while in the third, Fatima proved to be significantly better than the other two varieties. The green plant regeneration abilities of Mv 16 and Mv 18 did not differ in the course of the experiments. On all three occasions, Fatima produced a significantly larger number of green plants than the other two varieties. The results are presented in Table 5 over the average of the three varieties. In two of the three experiments, where anther cultures were initiatied in one case from field-grown plants and in the other from plants raised in the greenhouse, the frequencies of anther response and callus induction were similar. In the third anther culture experiment initiated in January, significantly lower values were obtained. As regards total plant regeneration, field-grown plants gave the best results. When averaged over the varieties, there was no significant difference between the years for green plant regeneration ability.

Table 5

Effect of donor plant growing conditions on in vitro androgenesis, averaged over three winter wheat vatieties (Martonvásár)

Experiment	Anther response (%)	Callus induction (%)	Plant regeneration total (%)	Plant regeneration green (%)
May 1987	11.81	40.28	46.60	10.71
Dec. 1987	14.14	35.39	21.47	13.71
Jan. 1988	8.90	23.15	29.53	13.28
L.S.D. (P=0.05)=	2.50	7.24	9.07	5.83

Discussion

The differences observed in the *in vitro* androgenetic abilities of the varieties indicate that the process is genotypically determined. This factor, however, only specifies the possible limits to the extent of *in vitro* androgenesis, while other factors also play a significant role in the creation of the results obtained. In addition to studies on genotype, detailed investigations were also made on the growth environment of the donor plant. The results of anther cultures initiated for the two varieties on five different occasions indicate that the frequency of callus induction might be 2–3 times as great in one experiment as in the other. The varieties also differed from each other in their sensitivity to changes in the donor plant environment. While the plant regeneration ability of the variety Mv 15 was similar on all five occasions, that of Mv 18 exhibited a high degree of variability.

Several authors attempted to determine the optimum raising conditions for the donor plant, and emphasized the advantages of a field environment (Lazar et al.,

1984; Jones and Petolino, 1987; Björnstad et al., 1989). The present results, obtained when initiating anther cultures for several varieties at different times from donor plants raised under different conditions, indicate that no generally valid conclusions can be drawn with respect to the optimum environment. Within a single variety, significant differences were found in some cases for anther cultures arising from the same environment but in different years. This can be attributed partly to the significant interactions between genotype and growth condition experienced, and partly to the effect of differences in the biotic and abiotic environmental factors affecting the donor plants, since neither the greenhouse nor the field environment can be standardized. If high frequency of *in vitro* androgenesis is to be achieved, it is important for the development of the donor plant to be uniform and intense, so any growing conditions which can ensure this development are suitable for the initiation of anther cultures.

In studies on the variety x year interaction, the results obtained for frequencies of anther response and callus induction were similar to those previously published (Andersen et al., 1987; Knudsen et al., 1989; Tuvesson et al., 1989), indicating that both the genotype, the donor plant environment, and the interaction between these two played a role in the development of these properties. The effect of genotype was the most pronounced, especially as regards the frequency of callus induction. In contrast to the reports cited above, however, the role of the interaction between these two factors was found to be small for both properties. This can be explained by the fact that, of the three varieties tested, only one (Mv 16) exhibited substantial variability between the experiments. The significant genotype x environment interaction indicates that the environmental effects which are optimum for anther culture may differ from one genotype to another. It is thus unlikely that a system of environmental conditions could be elaborated for use with all genotypes. The significant interaction also explains why considerable differences are found in the *in vitro* androgenesis of the same genetic material in different years.

The results obtained for plant regeneration also differed in part from those reported in the above-mentioned publications, since significant variety and donor plant growing condition effects were observed for total plant regeneration. Of the two factors, the donor plant growing condition effect played the greater role. However, this effect observed for plant regeneration can probably be attributed not only to the donor plant environment, but also to effects acting during the *in vitro* stage; these have a fundamental influence on the quality of the callus formed and thus on the regeneration ability. At the same time, in determining the frequency of green plant regeneration the most pronounced role was played by genotype, while the donor plant environment was only significant in interaction with the genotype, and even then to a much slighter extent, in agreement with results published by Andersen et al. (1987), Tuvesson et al. (1989) and Knudsen et al. (1989). This means that changes in the donor plant environment may improve the total plant frequency, but have no real influence on the frequency of green plant regeneration.

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HISTOLOGICAL STUDIES ON *IN VITRO* AND *EX IN VITRO* LEAVES DURING THE ADAPTATION PHASE OF *RHODODENDRON* 'PINK PEARL'*

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Histological investigations with *Rhododendron* 'Pink Pearl' plants representing separate stages of adaptation showed marked differences in the anatomy of their leaves. The large leaves of two-year-old field-grown plants were characterized by high density of stomata (313–317 pro mm^2), multilayered epidermis and palisade tissue, and by the presence of cuticle. Leaves of plants from *in vitro* phase were very small, with low density of stomata (13 pro mm^2), small respiration caves and a poorly developed mesophyll.

Leaves of the adaptation phase had intermediate characteristics between the *in vitro* and field-grown leaves. Both *in vitro* and adaptation leaves had a one-layered upper epidermis without cuticle. Acclimatization at lower relative air-humidities resulted in an increase of leaf thickness, stomatal density, and an improved differentiation of the palisade tissue, as compared to the high (90%) humidity conditions.

Keywords: *Rhododendron*, micropropagation, adaptation, leaf histology, embedding technique, Technovit

Introduction

The transfer of micropropagated plantlets from *in vitro* to *ex vitro* (glasshouse) conditions is especially difficult with woody species. Besides the sudden contamination occurring after the excision of micro-plantlets from the glass (test-tube), poor conditions during the period of adaptation may also contribute to their decay (Conner and Thomas, 1981; Read and Fellman, 1985). Another reason for failure is the after-effect of the last *in vitro* medium (Hasegawa, 1979), but the survival-rates depend on the subsequent soil mixture as well. With thornless blackberries for example, it was 60% (Broome and Zimmerman, 1978); with roses 50–81% (Hasegawa, 1979; Skirvin and Chu, 1979); and with *Acacia koa* it varied between 0% and 100% (Skolmen and Mapes, 1978). To facilitate the acclimatization of plantlets from the almost 100% air humidity conditions of *in vitro* culture to those of a glasshouse and (later) of the open field, different methods such as adaptation under polyethylene cover mist or fog, are recommended (Griffis et al., 1983; Read and Fellman, 1985). In the experiments of Sutter and Hutzell (1984), the use of silicon-based antitranspirants resulted into decreased growth, as compared to protection by a plastic tunnel. A phytotoxic effect of the antitranspirant was not excluded either. According to Fuchigami et al. (1981), sudden drying of *in vitro*

*This research was carried out within the framework of OTKA 4231, supported by the Hungarian Academy of Sciences

propagated *Prunus instititia* 'Pixi' was probably due to the absence of cuticle (Sutter, 1988). However, as demonstrated with *Malus*, *Prunus* and *Liquidambar*, significant water losses may occur even through an existing cuticle. Besides cuticular transpiration, poor stomata-reaction of unadapted plants is suggested to be also responsible for their wilting (Brainerd and Fuchigami, 1981; Brainerd et al., 1981; Wetzstein and Sommer, 1983).

Reuther (1986) and Smith et al. (1986) measured low assimilation capacities in *in vitro* grown plants. An increase of light intensity did not result in a respective increase of photosynthetic production.

Histological modifications in the leaf-structure of *in vitro* material, such as smaller cells of mesophyllum, poorly developed palisade-tissue and spongy parenchyma, may be further reasons for problematic acclimatization (Fabbri et al., 1986; Smith et al., 1986).

The adaptation of *Rhododendron* plantlets is usually successful under air-humidities lower than that for *Rosa*, *Syringa*, and *Prunus*. The aim of the present studies was to investigate the mechanism of this phenomenon by following the most important anatomical changes and determining the anatomical features in *in vitro* and *ex vitro Rhododendron* leaves, and also to study the effect of different air-humidities during the adaptation period on their histological structures.

Materials and methods

The investigations were carried out with leaves from micropropagated *Rhododendron* 'Pink Pearl' plants having different ages and phases of adaptation. This cultivar is a cross between cultivars 'George Hardy' (*R. griffithianum* x *R. catawbiense*) and 'Broughtonii' (*R. arboreum* x *R. ?*).

In vitro culture

Shoot tips were cultivated in vitro on the medium of Anderson (1984) modified by additions of 2.5 ppm 2 ip, 5 ppm IAA, 3.0% of saccharose and 0.7% Agar. The pH was adjusted to 4.5. The cultivation took place in a climatized room under 16 h/day illumination with 2500 luxes light intensity and at a constant temperature of 25 °C. After the proliferation phase, root formation was induced on the medium of Ma and Wang (1974) with no growth regulators. The content of saccharose was reduced to 2.0%, and that of Agar to 0.56%. Temperature and daylength were the same as before, but light intensity was reduced to 500 luxes.

Adaptation and further cultivation

After careful washing of roots, *in vitro* plantlets were transplanted in a peatmoss substrate to which 2 grams per litre of $CaCO^3$ was added. During this operation, the plantlets were frequently sprayed with water against dessication.

The subsequent adaptation phase lasted 8 weeks and took place in April in climate chambers at natural daylength and light, at a temperature around 25 °C, and at different air-humidities as follows:

40–50% (under mist)
60–70% (under mist)
75–85% (under mist)
above 90% (under fog)

Finally, the acclimatized (hardened-off) plants were potted in 7 cm diameter pots and grown in a glasshouse during the rest of the vegetation period. After winter dormancy, they were transplanted into containers and grown for one more season in the open field.

Histological studies

Histological studies were carried out on leaves taken from:

- the middle parts of *in vitro* cultivated plantlets
- the middle parts (cca 8th node) of young *ex vitro* plants just after an 8-week adaptation phase
- the basal and apical parts of two-year-old field-grown plants.

The leaves of *in vitro* plantlets were 2–5 mm long and of juvenile appearance, while those of freshly adopted ones 2–3 cm, and of transitional type with broad ovate form. Plants grown in the second year in the field had typical leathery (adult) *Rhododendron*-leaves: 5–10 cm long, 2–4 cm wide and with oblanceolate form. Whole leaves were investigated from tiny-leaved *in vitro* material and representative samples from all the rest: small leaf-parts (cca 5–10 mm) cut out diagonally from the middle of the leaf blade near the main nerve. External and internal microscopical investigations were made of them:

- External investigations: the samples, either whole small leaves or their epidermis (in fresh or fixed condition), were slightly stained with toluidine blue, laid directly upon the glass-slides, covered in glycerine-gelatin and examined under microscope from both the upper and lower side.
- Internal investigations: leaves or leaf-samples were first fixed in an ethanol-formol-ice-acetic-acid mixture (90 : 5 : 5), dehydrated by an ethanol-series (70–80–90–95 and 100% concentrations), and finally preinfiltrated and embedded into (the embedding material) Technovit 7100.

Technovit 7100 is a synthetical resin, consisting of coldpolymerised 2-hydroxyethyl-methacrylate, using X-Cl as co-catalisator; and polyethyleneglycol 400. This mixture is marketed in liquid form. Hardeners No. 1 and No. 2 are added during and after the infiltration, respectively. The new embedding technology, described in detail by Grunewaldt-Stöcker (1985), as well as by Ruetze and Schmitt (1986), has the advantage of being faster and milder to the tissues than the traditional paraffin. Also, the sections stick easier to the slides and can be stained both with hydrophilous or hydrophobic pigment solutions.

Cross-sections (cca 15 mm thickness) were made from the embedded material with a sledge-microtome, stained for about 15 minutes in water-soluted toluidine-blue, and after fixing to the slides, covered in glycerine-gelatin.

Microscopic measurements were made:

1. Externally, on the upper and lower surface; counting
   number of epidermis-cells per mm^2,
   number of stomata per mm^2,
   number of leaf-hairs per mm^2.
2. Internally, on cross-sections; comprising thickness of the whole leaf blade, and that of the epidermis; palisade and spongy parenchyma, respectively; number (per mm) of epidermis- and palisade-cells and number of stomata, all in transversal direction; diameter of lacunae.

Results

1. External investigations of the leaf surface

As shown in Table 1, higher air humidities during the adaptation of young *in vitro* material resulted in a higher number (smaller sizes) of epidermis-cells per mm^2, but decreased the number of stomata. *In vitro* material had more epidermis-cells per mm^2 and much less stomata than any of the adaptation-variants. By far the highest

number of both the epidermis-cells per mm^2 (measured only on the upper side), and that of stomata, was measured in the 2nd-year field-grown adult leaves, taken either from the basal or the apical parts of plants.

Table 1

Leaf surfaces of micropropagated Rhododendron 'Pink Pearl' plants subjected to different acclimatization conditions

Origin of leaves	Epidermis-cells per mm^2 upper surface	lower surface	Stomata per mm^2 upper surface	lower surface	Hairs per mm^2 upper surface	lower surface
in vitro plantlets	270	500	0	13	9	7
after adaptation under						
90% rH*	126	305	0	64	2	1
75–85% rH+	117	192	0	68	3	3
60–70% rH+	103	169	1	76	3	3
40–50% rH+	90	131	0	86	2	2
2nd-year field-grown plants						
base	620	–	0	317	10	17
top	626	–	0	313	12	36

rH relative air humidity; * fog + mist; - not measured

A striking difference was observed in the shape of epidermis-cells: on young *in vitro* and adaptation leaves they had strongly undulated anticlinal walls giving an interlacing pattern, while the epidermis-cells of adult leaves had an almost isodiametric shape (Fig. 1a, b).

From leaf hairs (trichomes), using the terminology of Seithe (1960), two types were identified: glandular and branched ("wooly") hairs. *In vitro* leaves had glandular hairs along the leaf-margin and glandular and semi-branched types on the leaf surface (Fig. 2a). Adaptation leaves had glandular, transitional, and branched hairs as well (Fig. 2b), while the hairs of adult leaves were all branched and very often shed, leaving a round scar on both sides of the leaf. The differences in hair-density between the basal and apical leaves were small.

2. *Internal studies on cross-sections*

There were marked differences between the internal structure of *in vitro* and field-grown leaves (Table 2). Leaves from 2nd-year field-grown plants had a two-layered upper epidermis, and their palisade-tissue consisted of two, three, or

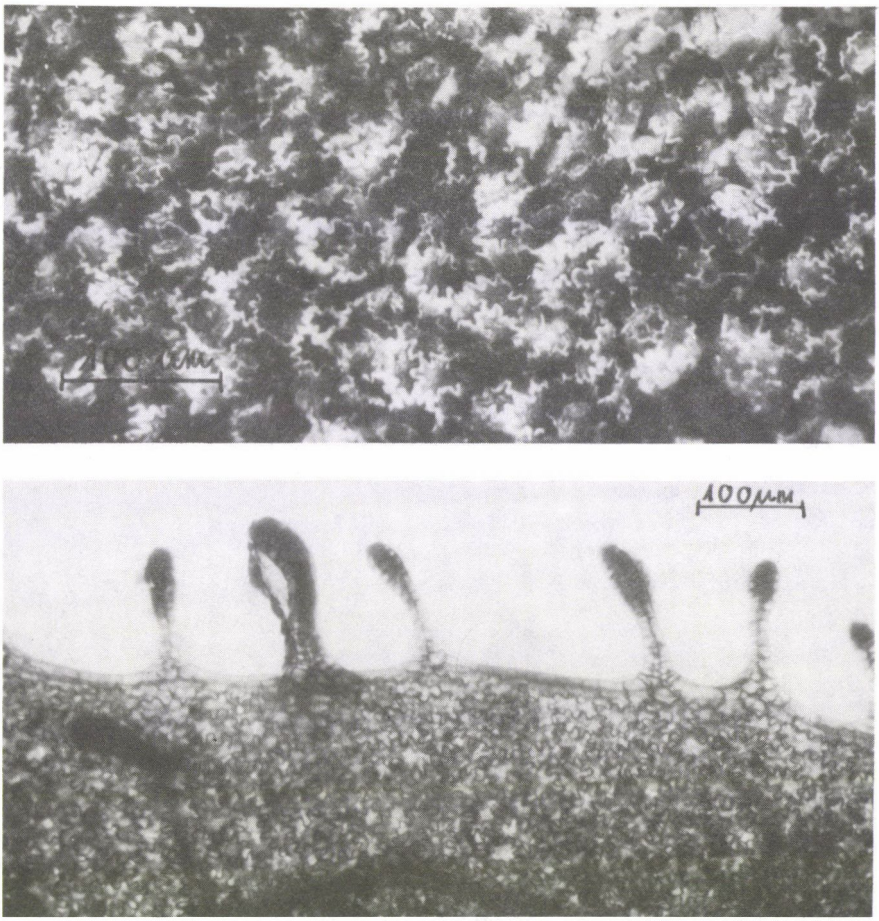

Fig. 1. Epidermal surface of *in vitro* leaves (a) and leaves of two-year-old liners (b)

occasionally four layers of cells (Fig. 3). On the other hand, leaves from the *in vitro* phase had only one layer of epidermis- and palisade-cells, respectively (Fig. 4).

Adaptation leaves had many characteristics similar to those of *in vitro* leaves, but differed from them with their more developed stomata and substomatal chambers, and also with a more advanced stage of differentiation (Table 3). From the different adaptation-variants, plants grown under fog (air-humidity 90%) had a thicker palisade-layer, but an imperfect spongy parenchyma, as compared to those grown under mist (humidities between 40 and 80%). There was, however, an increasing tendency in total leaf-thickness under lower air-humidities, due mainly to the marked increase of spongy parenchyma.

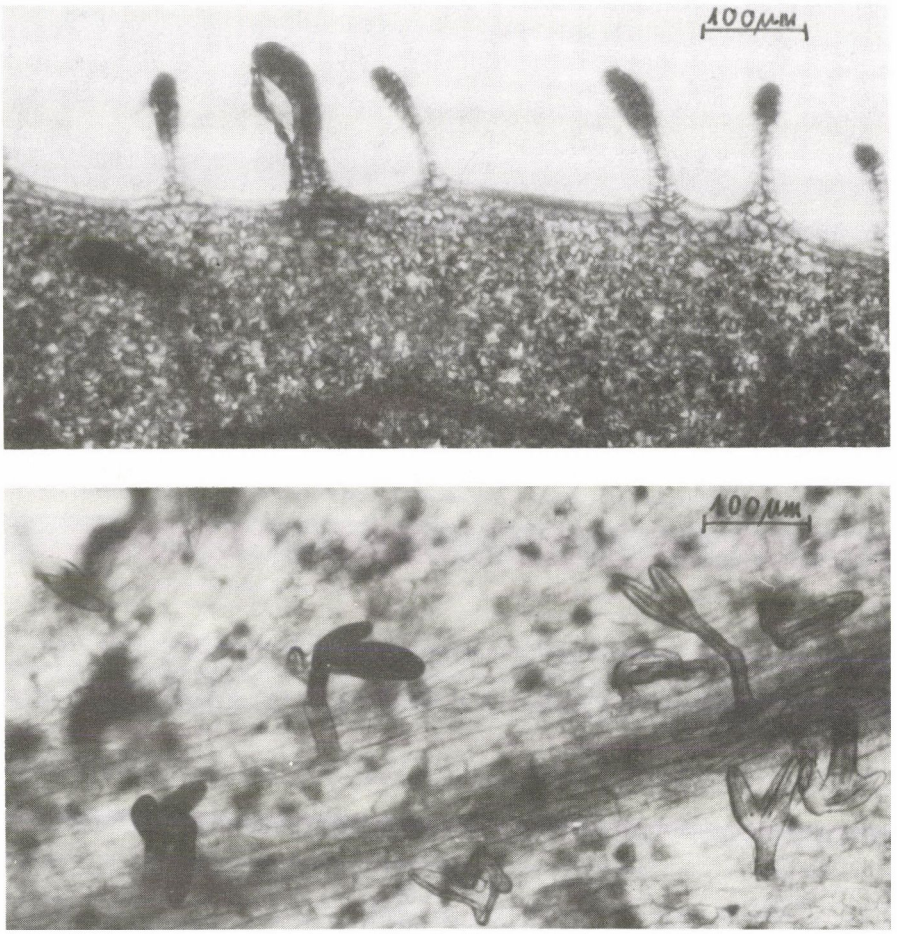

Fig. 2. Glandular (a) and branched (b) hair class types of *Rhododendron* 'Pink Pearl'

The number of epidermis-cells per mm^2 was increasing as follows: adaptation leaves, *in vitro* leaves, field-grown (adult) leaves. It means that their size decreased with aging. Cuticula was found only on the upper epidermis of field-grown (adult) leaves.

Discussion

Strong water losses through evaporation may lead to irreversible leaf damages of microprogagated plants taken out from the laboratory conditions to those of a glasshouse. In adaptation experiments of Sutter (1988) with *Malus, Prunus* and *Liquidambar,* almost 40% of leaves were damaged if the plantlets were kept for hours under 87% relative humidity.

Table 2

Histological differences in the cross-section of in vitro and ex vitro cultivated Rhododendron 'Pink Pearl' leaves

Characteristics measured in transversal direction	*in vitro* leaves	*ex vitro* (2nd year) leaves	
		base	top
Cuticle*	–	+	+
Epidermis layers*	1	2	2
Palisade layers	1	2.5	2.6
Thickness (μm) of			
whole leaf-blade	122	450	526
epidermis	18	40	42
palisade tissue	25	116	102
spongy parenchyma	72	256	326
Number of cells per mm in			
epidermis I	28	29	30
epidermis II	–	26	23
palisade tissue	38	47	44
Respiration cavities			
number per mm	6.5	5.3	5.4
diameter (μm)	60	163	230

* upper leaf-surface

The reason for this contradiction is probably the different anatomical leaf-structure of the above species and the related different suspectibility to the conditions of adaptation phase. A sudden wilting may be caused by the absence of cuticula, or by an insufficient (if any) reaction of stomata. Different adaptative abilities of stomata were reported by Brainerd and Fuchigami (1981) on *Malus* 'Mac 9' and by Fuchigami et al. (1981) on *Prunus* 'Pixi'. Non-adapted non- or malfunctioning stomata fail to close under stress-conditions. A high density of such stomata is a disadvantage during the adaptation phase. In our studies, *in vitro Rhododendron* leaves had a low stomata-density (13/mm^2) and their number increased only with the gradual decrease of air humidity. Lee et al. (1988) measured 305–370 stomata/mm^2 in the *in vitro* leaves of *Liquidambar* grown under different light intensities. These large differences in the number of stomata may give a partial explanation for the relatively easy acclimatization of *Rhododendron* as compared to other genera.

Histological studies on *Rhododendron* leaves are very few. Hayes et al. (1951), and later Breitfeld (1988) attempted to create an infragenetic classification based on the leaf anatomy. Taxonomical works, however, within the genus *Rhododendron* are extremely difficult because of the large number and variability of species and hybrids. From leaf characteristics, the type and density of hairs (trichomae)

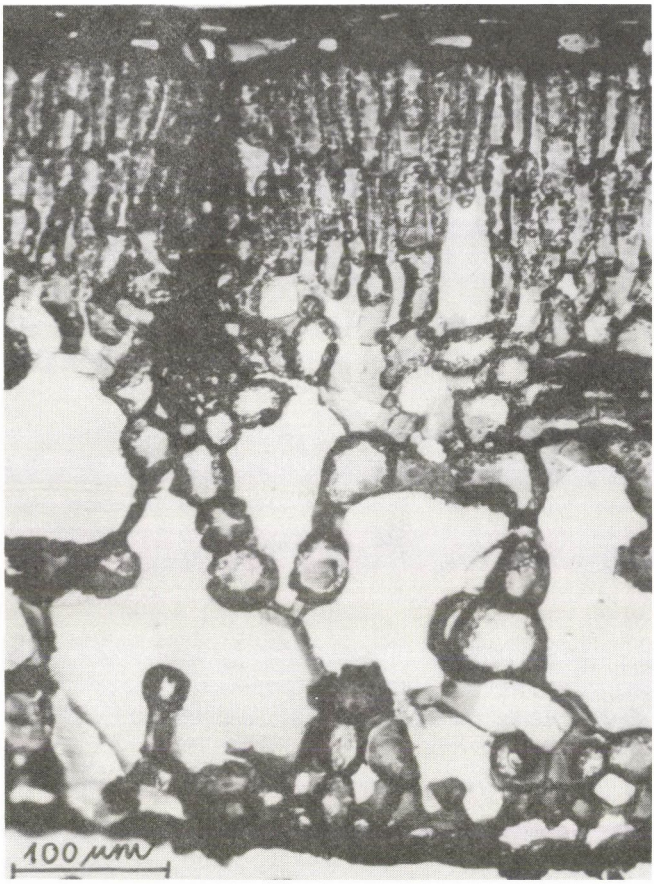

Fig. 3. Leaf cross section of a two-year-old leaf

and the number of epidermis-layers are considered to be of taxonomical importance (Seithe, 1960). According to Spethmann (1987), the transition from one to multilayered epidermis is a sign of phylogenetical development.

All the parents and grandparents of *R.* 'Pink Pearl' are species and/or cultivars with multilayered epidermis (Salley and Greer, 1986; Hayes et al., 1951). *In vitro* plantlets of the same cultivar, however, had an one-layered epidermis, which remained of the same type in the adaptation (transitional) leaves as well. The large and thick palisade layers and the elongated substomatal chambers in the adaptation leaves, however, are first signs of a higher stage of differentiation.

In the experiments of Lee et al. (1988), an increase of light intensity resulted into thicker leaf blades and a more developed palisade-tissue of *in vitro* cultivated *Liquidambar* plantlets, but could not reduce their water-losses nor to increase their assimilation during the beginning of adaptation.

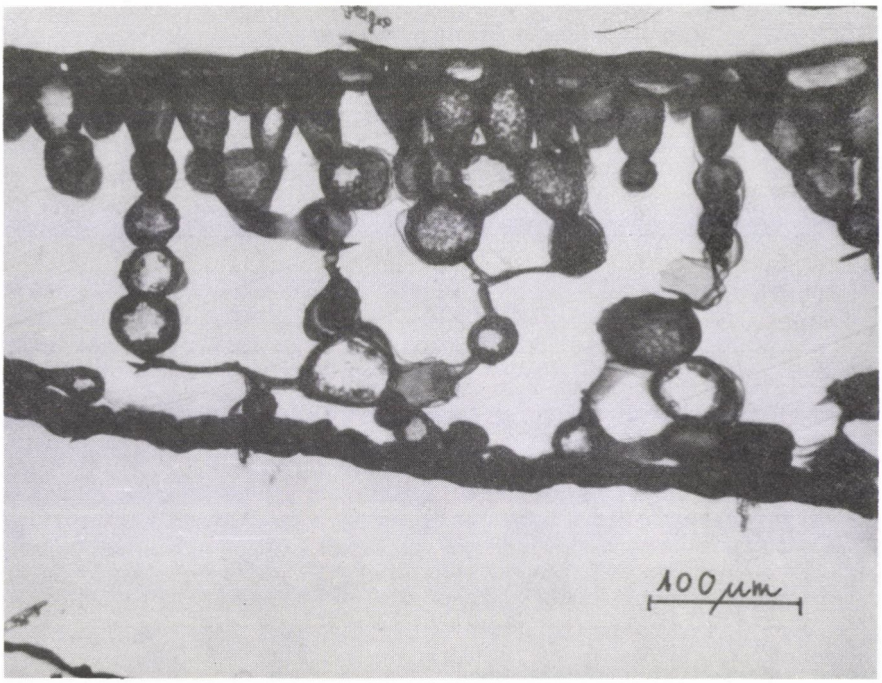

Fig. 4. Leaf cross section of an *in vitro* developed leaf

According to Reuther (1986), Lakso et al. (1986), and Smith et al. (1986), an increase of photosynthesis and that of CO_2-uptake begins only 2–3 weeks after the transfer from *in vitro* to *ex vitro* conditions.

In our studies, stronger structure of *R.* 'Pink Pearl' leaves adopted under lower air-humidities were assumed to be a symptom of more perfect change from heterotrophic to autotrophic way of life.

The relatively high number (small size) of epidermis cells and low number of stomata per mm^2 at the highest (90%) air-humidity was probably due to the fact that this treatment most resembled the humidity of *in vitro* phases. Under such conditions, cell-divisions were carried on further and cell-differentiation started later than in the treatments with lower humidities. The one-layered epidermis of *in vitro* and adaptation leaves probably represents a lower stage of development than the two-layered epidermis of adult ones, but this in not enough proof for the correlation with their phylogenetics.

From their studies comparing microprogated *in vitro* and *ex vitro* (glasshouse)-cultivated, *Betula* with young *Betula* seedlings Smith et al. (1986) suggested that the histological differences between *in vitro* and *in vivo* plants should not be deduced from an increased stage of juvenility. The validity of the above conclusion to *R.* 'Pink Pearl' still needs to be cleared. First, it is necessary to determine the exact cultural phase in which the leaves change from one-layered to two-layered epidermis, and further histological studies are needed to compare the micropropagated *in vitro* plantlets with young seedlings.

Table 3

Effect of different adaptation conditions on the anatomy of micropropagated Rhododendron 'Pink Pearl' leaves

Characteristics, measured in transversal direction	Relative air humidity during the adaptation phase			
	> 90%**	75–85%+	60–70%+	40–50%+
Cuticle*	–	–	–	–
Epidermis-layers*	1	1	1	1
Palisade-layers*	1	1	1	1
Thickness (µm) of				
whole leaf-blade	140	178	188	222
epidermis	18	24	24	24
palisade-tissue	60	38	42	54
spongy parenchyma	58	104	112	133
Number of cells per mm				
epidermis	21	20	20	21
palisade-tissue	32	28	29	30
Respiration cavities				
number per mm	6.4	5.5	5.7	6.0
diameter (µm)	29	118	110	144

* upper leaf surface; **fog; +mist

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COMPARISON OF APPLE VARIETIES BY THE APPLICATION OF THE INDEX OF FLOWERING (*INDEX-V*)

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In the case of apple, the exact assessment of the course of flowering has several practical aspects, foremost among them the simultaneous flowering of pollinating and fertilized flowers. The aim of present experiments was to analyse the flowering characteristics of a fruit crop, the apple (*Malus domestica* L.) by applying the *Index-V*, as the numerical expression of flowering. In apple similar regularities could be described in the changing of the number of buds, open and withered flowers, as were described for *Adonis vernalis* L., *Capsicum annum* L. and *Matricaria chamomilla* L. Consequently, the index can be applied also for this crop.

Mass flowering of apple is characterized by Index values in the range V= –0.51 – +0.51. Simultaneous flowering is most optimal if the 'mass flowering' of varieties occurs on the same day or within a one or two days' interval. The duration of mass flowering, however, seems to be significantly influenced by the climatic factors. The *Index-V*, by its numerical expression of the course of flowering, can facilitate long-term phenological observations.

Keywords: apple, *Malus domestica* L., flowering of cultivars, numerical expression of flowering, *Index-V*

Introduction

In the case of most horticulture crops, the determination of phenophases is a frequent necessity.

In apple, the exact assessment, for knowledge of the flowering time has several practical aspects, among them the simultaneous flowering of pollinating and fertilized flowers being the most important.

Differences in the flowering response of varieties have been studied by Soltész (1982) in detail. It has been established that years with the early onset of flowering are more favourable for the determination of differences between varieties, as under such conditions flowering is generally prolonged, thus providing enough time for the comparison of varieties.

The time and duration of flowering is determined by the combined effect of several factors. According to Nyéki (1980), the most important of these factors can be ranked into the group of both ecological factors (geographic localization, climatic, edaphic, and agrotechnical factors), and biological as well as morphological traits (variety, rootstock, tree age, etc.).

Soltész (1982) also established that the duration of flowering is more decisively influenced by the climatic conditions than by the relative sequence of anthesis of

various varieties. On the basis of observations on 66 varieties spanning over several years he also states that the duration of flowering varies between 13 and 22 days.

Since the success of fertilization, i.e. the best possible simultaneous flowering of pollinating and fertilized flowers, is one of the main preconditions of high yields, the need to study the course of flowering has been recognized. As the chance of mutual fertilization is also only possible among simultaneously flowering varieties, with a possible 1–4 days overlapping in the time of main flowering (Nyéki, 1989), the exact characterization of the flowering process seems to be a priority.

To date, the selection of pollinating apple varieties has been made mainly on the basis of phenograms, a result of several year's observations, with the extent of simultaneous flowering expressed as a rate (%) of overlapping areas under the curve of flowering.

As in previous years, favourable results were obtained with the *Index-V* (index of flowering) elaborated for the characterization of the course of flowering of *Adonis vernalis* L. plant individuals, as well as for the comparison of populations (Máthé, 1977; Máthé and Máthé, 1979; Máthé et al., 1985; Franz et al., 1985; Máthé and Bahadli, 1989) the aim of our present experiments was to analyse the flowering characteristics of a fruit crop, the apple (*Malus domestica* L.). By applying the *Index-V* for describing the flowering dynamics of apple, it is also endeavoured to facilitate the determination of simultaneosuly flowering varieties.

Materials and methods

The experiments were carried out between 1988 and 1990, in the orchards of the Debrecen State Farm, at Pallag, Tamási and Gut.

In the experiments the following apple varieties of different flowering time were studied on MM 106 and M 4 rootstocks: 'Golden Delicious', 'Granny Smith', 'Duncan Red Delicious', 'Mutsu', 'Red Winesap', 'Redspur Delicious', 'Watson Jonathan'.

The date of flowering was characterized by determining the onset and the culmination, as well as end, of flowering; the onset being that 1–5% of the flowers had opened up; the culmination, that most of the flowers were open; and the end of flowering that 95–100% of flowers were beyond flowering, in which the fall of petals had begun.

In order to characterize thy dynamics of flowering, the number of flowers in the various stages of anthesis was determined daily at the same time; between 10:00 and 12:00 hours, 200–500 flowers on the northern, western, southern and eastern side of the tree were examined.

The rate of simultaneous flowering was expressed as the percentage of time of the total period in which their flowering overlapped.

The flowering index (*Index-V*) was calculated as follows:

$$Index\text{-}V = \frac{t - b}{b + v + t}$$

where b = number of flowers at the beginning of flowering,
v = number of flowers in full flowering (anthesis),
t = number of flowers at the end of flowering.

Interpretation of the index values:

Index-V value	Phenophase
–1.00 – –0.51	Bud opening
–0.51 – –0.10	Beginning of mass flowering
0	Mass flowering
+0.10 – +0.50	End of mass flowering
+0.51 – +1.00	Withering of flowers (Derforescence)

Further details on the *Index-V* are given by Máthé (1977).

Results and discussion

To date, the flowering dynamics of apple varieties has been characterized mainly by registering the number of flowers in the various stages of anthesis, daily. Figure 1 shows the flowering phenogram of the variety 'Granny Smith', from 1988

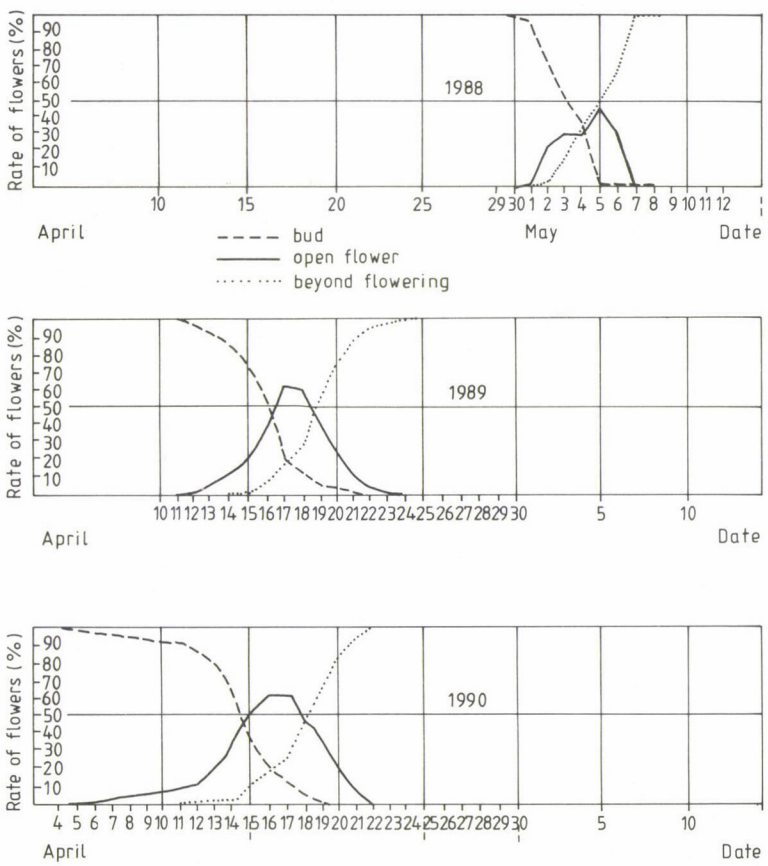

Fig.1. Flowering phenogram of the variety 'Granny Smith' (Debrecen, 1988–1990)

through 1990. It can be seen that also in apple the flowering follows the same regularities, as described for *Adonis vernalis* (Máthé, 1977). Accordingly, with the onset of anthesis, the rate of unopened flowers (buds) starts decreasing. Although in 1988 this took place at the beginning of May, in 1989 and 1990, this process started significantly earlier, in the second and first decade of April, respectively. This phenomenon is accompanied by the relatively rapid increase in the number of open flowers, which process (mass flowering) culminates between 4–7 May (in 1988), as well as 16–17 April in 1989 and 16–17 April, in 1990, respectively. In all three years of the experiment this period also sees an increase in the frequency of withered flowers (flowers over full flowering). A characteristic feature of the flowering dynamics of apple is the crossing point of the raising curve of withered flowers and that of the descending curve of buds (May 4, 1988; April 17, 1989; April 16, 1990), which coincides with the period when the number of open flowers culminates. Subsequently, the rate of open and withered flowers is negatively related and the flowering is terminated by May 6–7 (in 1988), April 24 and April 22, in 1989 and 1990, respectively.

Similar phenograms can be established also for other varieties. Thus, as another example, Fig. 2 depicts the course of flowering of the variety 'Red Winesap'

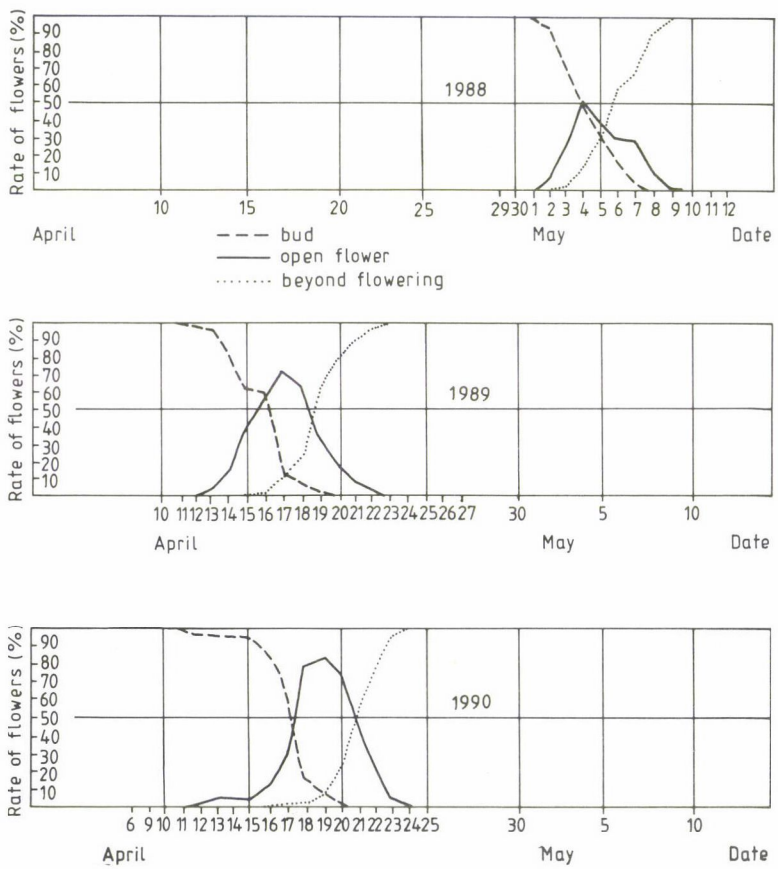

Fig. 2. Flowering phenogram of the variety 'Red Winesap' (Debrecen, 1988–1990)

in the experimental years 1988–1990. The figure demonstrates that, in 1988, flowering started with a two weeks' delay, at the beginning of May, but in 1989 and 1990, it commenced already in the second decade of April. (This can be ascribed to differences in the weather conditions of both experimental and preceding years. The analysis of this phenomenon has been subject of other studies.) Although, as illustrated by the shape of curves, the dynamics of flowering of 'Red Winesap' slightly differs from that of 'Granny Smith', it is to be seen that both varieties follow the same regularities.

For practical purposes it is most important to characterize the rate of simultaneous flowering, in order to obtain the best possible pollination with the required variety. By the traditional methods, this can be accomplished by plotting the rate of open flowers of varieties within one phenogram and by comparing the extent of areas under the curve. In the case of numerous (seven) varieties, this can be a tedious task. Moreover, this method completely omits the rate of buds and withering flowers, which also plays a significant role in characterizing the dynamics of flowering.

When applying the *Index-V* for characterizing the course of flowering of the above varieties (Fig. 3a–c) the values vary between –1 and +1. As the values between –0.5 and +0.5 indicate the range of mass flowering, it can be stated that, in 1988, it was the variety 'Granny Smith' that entered the phenophase of mass flowering on the earliest (May 2), while the variety 'Duncan Red Delicious' reached the same stage after two days' delay (May 4). It is remarkable that 'Granny Smith' retained its precocity in the entire flowering process, while the more slowly flowering 'Duncan Red Delicious' seemed to produce a less prolonged flowering, so that finally it was the variety 'Watson Jonathan' that reached the end of mass flowering at the latest date (May 8). In 1989 and 1990, 'Granny Smith' retained its precociousness (beginning of mass flowering: May 16 and May 14, respectively), while the latest flowering varieties were 'Golden Delicious 'Denmark'', 'Red Winesap', respectively.

Theoretically, it can be postulated that simultaneous flowering takes place when the values for varieties vary in the range from the 'beginning' to the 'end' of mass-flowering (–0.51 – +0.51). Thus, in 1988, for the variety 'Granny Smith' all varieties that reached the above range from May 3 until May 5 can be regarded as pollinators. Nonetheless, it is to be emphasized that, while 'Granny Smith' ends mass flowering on May 5, the varieties 'Red Winesap', 'Mutsu' and 'Golden Delicious 'Denmark'' reach mass flowering on the very same day. Consequently, the best pollination occurs in such a case when 'mass flowering' falls on the same date. Its efficacy decreases when mass flowering, as calculated by *Index-V*, does not coincide but takes place within one or two subsequent days.

Table 1 summarizes those days during which the seven varieties studied reached the phase of mass flowering (*Index-V* = 0). In 1988, the best pollination occurred between the varieties 'Redspur Delicious' 'Granny Smith' (May 4), as well as 'Golden Delicious', 'Mutsu', 'Red Winesap' (May 5). The least efficient pollination could be expected between 'Granny Smith', 'Redspur Delicious' (May 4) and

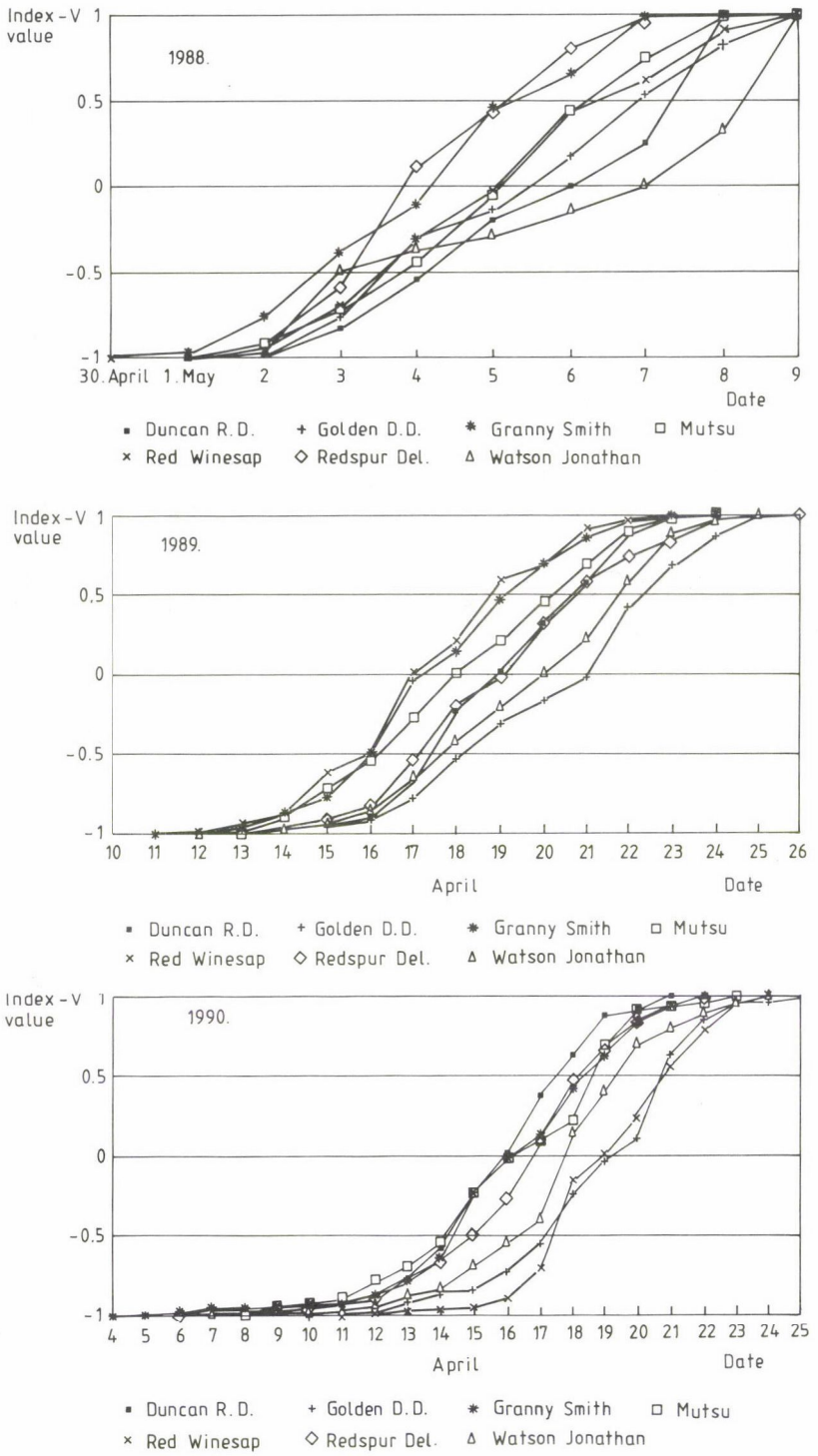

Fig. 3. The course of flowering of peach varieties as characterised by the Index-V value (1988–1990, Debrecen)

Table 1

Date of mass flowering (Index-V = 0) of apple varieties (from 1988 through 1990, in Debrecen)

Varieties	Year 1988	1989	1990
1. Duncan Red Delicious	May 6	Apr. 19	Apr. 16
2. Golden Delicious 'Denmark'	May 5	Apr. 21	Apr. 19
3. Granny Smith	May 4	Apr. 17	Apr. 16
4. Mutsu	May 5	Apr. 18	Apr. 16
5. Red Winesap	May 5	Apr. 17	Apr. 19
6. Redspur Delicious	May 4	Apr. 19	Apr. 16–17
7. Watson Jonathan	May 7	Apr. 20	Apr. 17–18

'Watson Jonathan' (May 7), with a three days' difference between their dates of mass flowering. In 1989, mass flowering was prolonged for 5 days, but in 1990, it lasted 4 days, which seems to refer to the sensitivity of the flowering process, to climatic factors. Remarkably, in spite of the similarities in the sequence of flowering, simultaneous flowering seems to occur only inconsistently, especially if it is restricted to mass flowering on the very same day. With a two days' range, it can be stated that the varieties 'Granny Smith', 'Mutsu' and 'Duncan Red Delicious', 'Mutsu' flowered simultaneously, in all three years of the experiment.

Summarizing our observations, it can be stated that the *Index-V* (Flowering index) is suitable for characterizing the course of flowering in the fruit crop, apple (*Malus domestica* L.). By appyling the *Index-V*, in contrast to previous methods (where only the number of open flowers had been considered), the number of both buds and withered flowers is considered, thus enabling a more exact expression of the dynamics of flowering.

The range of mass flowering falls within the range V = –0.51 – +0.51. (The values indicate both the beginning and the end of mass flowering, respectively.) Simultaneous flowering is most optimal when the 'mass flowering' of varieties occurs on the same day or within a one or two days' interval. As the duration of mass flowering seems to be significantly influenced by the climatic factors, the true interpretation (reliability) of this range is to be made on the basis of further studies. Still, the *Index-V*, by its numerical expression of the course of flowering, can also facilitate the analysis of long-term phenological observations.

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A STUDY ON DETECTION AND QUANTITATIVE DETERMINATION OF FLAVONOID, TANNIN, POLYPHENOL CONTENT IN *OCIMUM BASILICUM* L.

PART I

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This investigation reports on the study of detection and quantitative determination of flavonoid, tannin, and polyphenol contents in *Ocimum basilicum* L. cultivated under the Hungarian environment. The flavonoid content, glycosides and aglycons were determined in the form of Al-flavonoid complex by photometry. The determination of tannin and polyphenol contents was also carried out by spectrophotometry, according to the modified method of Ph.Hg.VII.

The above-mentioned methods were modified and optimized (optimum time of extraction, optimum quantity of crude drug, and suitable wavelength were worked out). Data were evaluated by statistical methods. Flavonoid compounds were investigated by chromatographic methods (TLC, PLC, HPLC). Rutin, isoquercitrin, caffeic and rosmarinic acids were isolated and identified.

Keywords: *Ocimum basilicum* L., flavonoid, tannin, polyphenol contents, spectroscopic, chromatographic methods

Introduction

Ocimum basilicum L. has been recommended for a long time for its high medical value. The plant is a perennial small shrub belonging to the *Fam. Lamiaceae*, and available almost everywhere in tropical zones. As is almost general in the case of the species of the *Lamiaceae* family, it has an especially strong antibiotic activity (Kurucz and Hornok, 1979).

O. basilicum has been recommended as stomachic, urine propelling, and laxative agents in the treatment of colds. Lahariya, Rao, Sawhney et al. found that the oil of *O. basilicum* showed antifungal activity against *Microsporum gypseum* and other fungi (Dikshit and Husain, 1984). In India the plant is said to be useful for treating toothache and earache, and its mixture with camphor for stopping nasal haemorrhage (Jain et al., 1972).

Thus, the chemical investigation of this plant can contribute to its correct use in therapy. Previous studies have revealed that *O. basilicum* contains 2.24–2.30% total polyphenols, flavonoid glycosides (0.6–1.10%), tannin, caffeic acid, flavonoid aglycons, essential oils (0.5–1.5%), sterols, triterpens, vitamins, and minerals (Viorica, 1987).

The essential oil of *O. basilicum* has been greatly studied in both qualitative and quantitative aspects. However, there have been few works done on the variation of flavonoid, and tannin. The main end of our work was primarily to elaborate

suitable qualitative and quantitative methods for detection and determination of flavonoids and tannins of *O. basilicum*, and to test the statistical reliability of these methods.

Our additional aim was to isolate and to identify the main flavonoids of the plant by various chromatographic methods.

Materials and methods

Ocimum basilicum was collected from Vácrátót and obtained from the trade company "Herbaria", Budapest. Drugs were ground (V) fine of sieve, according to Ph.Hg.VII., and homogenized for analysis.

I. Spectrophotometric methods

(1) The flavonoid content of *O. basilicum* was determined in the form of glycosides by spectroscopic technique according to Ph.Hg.VII. (1986) with the following modifications: 1 g of crude drug was refluxed with methanol (50 ml) by boiling in water for 1 hour, then the chlorophyll was removed with carbon tetrachloride by centrifuge. Yellow colour, having been developed by adding the reagent of 2% $AlCl_3$ $6H_2O$ in conc. acetic acid-methanol (5+95) to the extract of the drug, was measured by photometry at 425 nm against a blank sample without Al-reagent. The flavonoid content was calculated in rutin.

(2) The flavonoid content was also determined in the form of aglycon by the method of DAB 8 (Stahl and Schild, 1981).

To. 0.2 g of crude drug, 1.5 ml of 0.5% hexamethylene-tetramine solution, 20 ml of acetone and 2 ml of conc. hydrochloric acid were added. The solution was hydrolysed for 30 minutes in boiling water. The aglycons obtained were extracted with ethyl acetate saturated with distilled water. The solution was evaporated to dry and the aglycon content of the residue, solved in water-free ethyl acetate, was determined by using the colour reaction (see above). The aglycon content was calculated in hyperosid or quercetin.

(3) The tannin and polyphenol content of *O. basilicum* was determined by spectroscopic technique, according to the Ph.Hg.VII. (1986).

Water extract was analysed. Polyphenols gave blue reaction with Folin reagent (Na_2Wo_4) in 38% $NaHCO_3$ aqueous solution. The absorption was measured at 750 nm only within 1 minute (in contrast to 2 minutes proosed by Ph.Hg.VII.) against distilled water. The tannin content (polyphenol absorbed by leather powder) was measured by indirect method, also as in Ph.Hg.VII.

II. Chromatographic methods for the investigation of flavonoids

A. TLC methods

(1) TLC detection of the main flavonoid glycosides

a) Preparation of samples:

1 g of crude drug was refluxed with methanol (10 ml) in boiling water for 5 minutes. The methanolic extract was shaken with CCl_4 to remove chlorophyll, and then was concentrated to 5 ml. 10 µl of this solution was chromatographed with rutin and isoquercitrin as reference substances (5–5 µl of 0.1% methanolic solutions).

b) TLC parameters:

- Adsorbent: Kieselgel 60 F 254
- Developing systems:
  ethyl acetate-formic acid-cc.acetic acid-water (100+11+11+27)
  or ethyl acetate-formic acid-methyl ethyl ketone-water (100+11+11+27)
- Detection with "Naturstoffreagent" (N.R.) in UV 366. The flavonoid glycosides were characterized by their Rf values.

(2) TLC detection of the main flavonoid aglycons

a) Preparation of samples:

0.5 g of crude drug was hydrolysed with 3 ml of conc. HCL in 30 ml of acetone in 80 °C water for 45 minutes. The acetone extract was shaken with ethyl acetate saturated with distilled water. The ethyl acetate extract was filtered through Na_2So_4 siccum, then concentrated to 5 ml. 10 µl of this solution was separated by chromatography together with quercetin as reference substance (5 µl of 0.1% chloroform)

b) TLC parameters:

- Adsorbent: Kieselgel 60 F 254
- Developing system: toluene-ethyl acetate-formic acid (5+4+1.5)
- Detection with (N.R.) in UV 366

(3) TLC detection of main organic acid components in *O. basilicum*

a) Preparation of samples:

5 g of crude drug was boiled in distilled water (50 ml) for 5 minutes. The aqueous extract was shaken with ethyl acetate. The ethyl acetate extract was filtered through Na_2SO_4 siccum and then distilled to dryness. The residue obtained was dissolved in 5 ml of methanol; 10 µl was used directly for chromatography, besides caffeic and rosmarinic acids as reference substances.

b) TLC parameters:

- Adsorbent: Kieselgel 60 F 254
- Developing system: chlorofrom-ethyl acetate-formic acid (6+4+4)
- Detection with (N.R.) in UV 366 or with 3% $FeCl_3$ in methanol by daylight.

B. HPLC investigation of flavonoids

Parameters: HP1090 M High Performance Liquid Chromatograph, Dioder array detector, RP-18/10 Hibar 240×4 Lichrosorb column.

a) Sample 1: Methanolic extract for the investigation of flavonoid glycosides was prepared like A.I.a.

Flow: 1.000 ml/min.

Solvent system: 76% of 0.2% citric acid in bidistilled water+12% methanol+12% acetonitrile.

b) Sample 2: Ethyl acetate extract for the investigation of flavonoid aglycons was prepared like A.2.a.

Flow: 1.00 ml/min.

Solvent system: 60% of 0.2% citric acid in bidistilled water+25% methanol+15% acetonitrile.

c) Sample 3: Aqueous extract for the investigation of some main organic acids obtained like A.3.a.

Flow: 1.500 ml/min.

Solvent system: 73% of 0.2% citric acid in bidistilled water+5! methanol+22%acetonitrile.

Oven temperature: 40 °C, max. pressure 300 bar.

Stop time: 15 min.

Identification of the components was carried out by addition of standards and by relative retention factors as well as by their UV spectra.

C. Isolation

Rutin, isoquercitrin, quercetin, caffeic acid, rosmarinic acid have been isolated by preparative layer chromatography (PLC) from the crude extracts obtained as mentioned above. From the methanol extract, rutin and isoquercitrin were separated on PLC Kieselgel 60 F 254 Merck plates (20×20 cm) with a solvent mixture of ethyl acetate-formic acid-methyl ethyl ketone-water (100+11+11+27), quercetin from the ethyl acetate extract, caffeic, rosmarinic acids from the aqueous extract separated by the same plates with a solvent mixture of toluene-ethyl acetate-formic acid (5+4+1.5) and with that of chloroform-ethyl acetate-formic acid (6+4+4).

In UV light (at 366 nm) the required zones of the chromatograms were marked and removed, extracted with 30 ml nethanol for 10 minutes. The extracts were concentrated to 5 ml and used for TLC, HPLC, UV, IR spectroscopic analysis.

Results and discussion

I. Spectrophotometric methods

(1) It is remarkable that the data obtained in the course of the determination of flavonoid content of *O. basilicum* L. described in Ph.Hg.VII were lower than those seen in some previous papers. According to the method of Ph.Hg.VII. a yellow complex is formed by addition of reagent of $AlCl_3$ in methanol to the rutin or flavonoid extract in conc. acetic acid-pyridine-water. The absorption of this solution is measured at 428±1 nm.

In the method we modified the determination of flavonoid content under different conditions. First the UV-VIS spectrum of rutin-$AlCl_3$ complex was investigated by adding 1 ml solution of 2% $AlCl_3$ $6H_2O$ in conc. acetic acidic methanol (5+95) to 2 ml of 0.008% rutin in methanol. Then the spectra of flavonoid glycoside extract-$AlCl_3$, of flavonoid aglycon extract-$AlCl_3$ were produced in a similar way. (After 30 minutes their maxima were measured at 425 nm), (Figs 1, 2). To determine the optimum time of extraction the crude drug was refluxed with methanol for 0.5, 1.5, 2, 2.5, 3, 4 hours. Figure 3 shows that the optimum time of extraction was 1 hour.

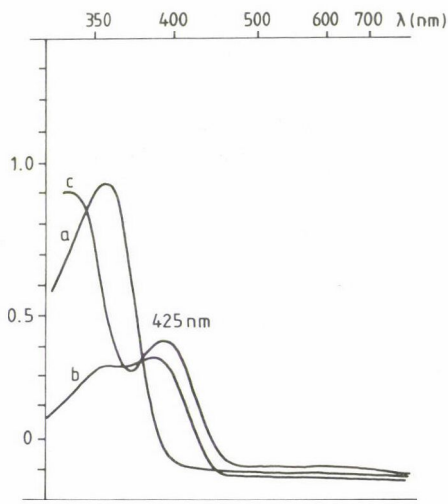

Fig. 1. Spectrum of standard rutin in methanol (a); Spectrum of rutin-$AlCl_3$ complex in conc. acetic acid-methanol (b); Spectrum of flavonoid glycoside extract-$AlCl_3$ complex in conc. acetic acid-methanol (5+95) (c)

We had carried out 10–10 parallel measurements on *O. basilicum* collected from Vácrátót and Herbária. The mean value of relative deviation was ±5.3%.

The above-mentioned method involves a sensitive colorimetric reaction using a reagent of 2% $AlCl_3$ $6H_2O$ in conc. acetic acid-methanol (5+95) to form a colour complex with the flavonoid extract that has also an absorption maximum at 425 nm. The modified method is found to be sensitive in the range of 4 to 40 $\mu g ml^{-1}$ of rutin

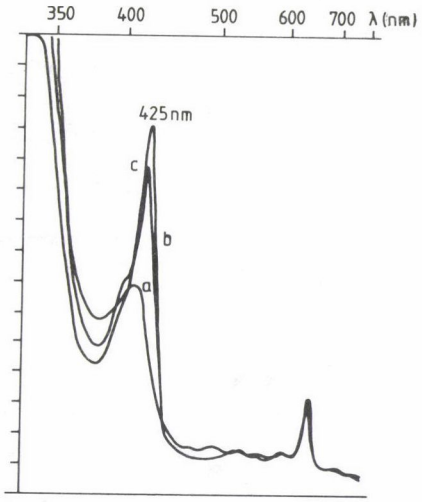

Fig. 2. Spectrum of flavonoid aglycon extract in conc. acetic acid-methanol (a). Spectrum of flavonoid glycoside extract – $AlCl_3$ complex in conc. acetic acid-methanol (b). Spectrum of aglycon extract – $AlCl_3$ complex in conc. acetic acid-methanol (5+95) (c)

Extraction times (h)	Flavonoid contents (C%)
0.5	0.16
1	0.255
1.5	0.26
2	0.26
2.5	0.26
3	0.30
4	0.32

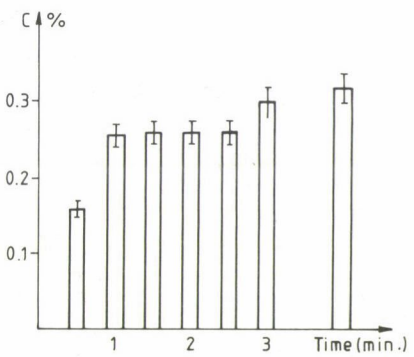

Fig. 3. The change of flavonoid content according to the different extraction times

in acetic methanol or of flavonoid glycosides in extract. We used a reagent of 2.5% $ZrOCl_2$ in methanol to form another colour complex with rutin or with the flavonoid being present in methanol extracts. It also has the maximum absorption at 425 nm, but the colorimetric reaction was only sensitive within the range of 4 to 20 $\mu g ml^{-1}$ of rutin in solution. Moreover the complex formed was unstable, so the absorption could not be measured perfectly. We found the Zr-complex less suitable for the determination of flavonoid content.

(2) The flavonoid content of *O. basilicum* was determined in the form of aglycon by DAB 8 method. In this case, all flavonoid glycosides were hydrolysed for different times (0.5, 1.5, 2 hours). The results on the flavonoid aglycon contents obtained were almost the same (0.40%, 0.39%, 0.41%, 0.40% calculated in quercetin).

The optimum time of hydrolysis was 30 minutes.

The calibration curve of standard quercetin was drawn by absorbencies of 25 ml of ethyl acetate solutions, the quercetin contents of which ranged between 0.009 mg to 0.27 mg. 10–10 parallel measurements were carried out in order to determine the error of the method. The mean value of relative deviations was ±3.32% if the flavonoid content was expressed in hyperosid, and it was ±3.51% when we calculated it in quercetin.

The modified method of DAB 8 is found to be sensitive within the range of 0.36 to 10 $\mu g\ ml^{-1}$ of quercetin in conc. acetic acid-methanol (5+95) or that range of flavonoid aglycon in extract.

(3) According to the determination of tannin content described in Ph.Hg.VII., the absorption was measured within 2 minutes after forming a blue reaction product with Folin reagent. The reaction product was, however, unstable and the solution became turbid too quickly, so that the absorption should be measured within 1 minute, just after adding it to the polyphenol extract.

To get information on the optimum quantity of starting material, we measured the tannin content of various proportions of the drug (Table 1). This experiment has revealed that 1 g of the drug proved to be the optimum quantity. The method was also evaluated statistically, as before. Its error was about ±5.54%.

Table 1

Investigation of the starting quantity of crude drug

Starting quantity of crude drug	The tannin content C%	Remarks
0.50	6.25	The solution was turbid too quickly not able to be measured
1.0	6.50	The best for measuring
1.50	5.83	The measurements show lower tannin contents
2.0	4.84	
2.5	4.87	
3.0	4.85	

II. Chromatographic investigation of flavonoid composition

In the course of TLC investigations, different developing systems have been applied for the separation of flavonoid mixtures. For the separation of flavonoid aglycons, the developing systems of chloroform-ethyl acetate (6+4) and toluene-ethylacetate-formic acid (5+4+0.5) were used, but the aglycons were not separable by them. Only the system of toluene-ethyl acetate-formic acid (5+4+1.5) was good enough for separating them. We have also found that isoquercitrin and hyperosid can be separated by the developing system of ethyl acetate-formic acid-methyl ethyl ketone-water (100+11+11+27).

Methanolic-, ethyl acetate-, aqueous extracts of *O. basilicum* have also been investigated by HPCL. The chromatograms (Figs 4, 5, 6) show the main components of *O. basilicum*. The components were isolated by PLC and have been indentified

- by TLC with reference compounds and their Rf values (Table 2),
- by HPLC with standard additions and their relative retention factors,
- on the basis of their UV-VIS, IR spectra

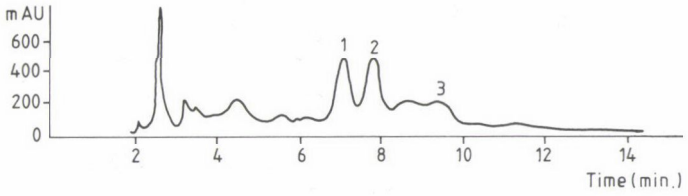

Fig. 4. HPLC chromatogram of flavonoid glycoside extract from *O. basilicum*; 1 rutin, 2 isoquercetin, 3 caffeic acid

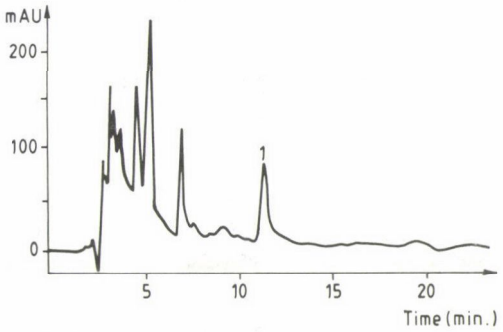

Fig. 5. HPLC chromatogram of flavonoid aglycon; 1 quercetin

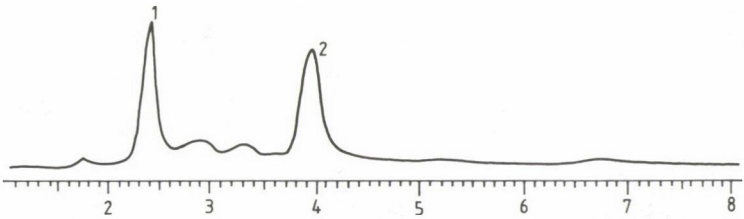

Fig. 6. HPLC chromatogram of aqueous extract from *O. basilicum*; 1 coffeic acid, 2 rosmarinic acid

Rutin, isoquercitrin, quercetin, caffeic, and rosmarinic acids proved to be the main characteristic components of the plant. In addition to these an unknown compound was isolated from the extract of the flavonoid aglycon by PLC. It has also been investigated by TLC, HPLC, and UV–VIS, IR spectroscopies. On TLC chromatograms the Rf value of the unknown compound was lower than the Rf of the caffeic acid but higher than that of the chlorogenic acid (close to isochlorogenic acid and cynarin). It is possible that the unknown molecule is built from one china acid which has two caffecyl parts, but the place of the connection is unknown.

Table 2

Thin layer chromatographic detection of some main components in Ocinum basilicum L.

Components	Developing system	Colour after treatment with naturstoff in UV_{366}	Colour after treat. 3% $FeCl_3$ in nat.light	Rf
rutin	ethylacetate-formic acid-conc.acetic acid-water	orange yellow		0.51
isoquercitrin	(100+11+11+27)	orange yellow		0.73
quercetin	toluol-ethylacetate-formic acid (5+4+1.5)	orange yellow		0.58
caffeic acid	(1) toluol-ethylacetate-conc.acetic acid			0.78
	(4+5+2)	intensive blue	greyish blue	0.72
	(2) chloroform-ethylacetate-			0.48
rosm. acid	formic acid (6+4+4)	intensive blue	greyish blue	0.49

The IR spectrum of the unknown compound was determined by SPECORD M-80 between the interval of 4000 cm^{-1}, 800 cm^{-1}.

The characteristic absorption maxima are as follows: γ OH mon: 3520 cm^{-1}; γOH ass: 3400 cm^{-1}; γ CH or O–CH_3: 2850 cm^{-1}; γ C=0: 17440 cm^{-1}; γ C=C: 1610 cm^{-1}; γ C–O: 1020 cm^{-1}.

On the basis of IR spectra, the structure of the unknown compound proved to be similar to cynarin. When this component was investigated by HPLC, it separated into two compound (compound-a and -b) (Fig. 7). The UV spectrum of compound-a was similar to the spectrum of cynarin and of caffeic acid (absorption maxima are at 326 nm, 246 nm). The other compound (compound-b) gave a very similar curve to that of compound-a, but its maximum shifted towards lower wavelengths with 15 of the two compounds.

We could successfully apply our modified methods in the evaluation of flavonoid, tannin, polyphenol content, e.g., during the study of ontogenesis of *O. basilicum* L. (See Part II.)

Acknowledgement

We are grateful to László Oláh for his help in HPLC investigation and wish to thank Szabolcs Dobson for his help in IR spectrum investigation.

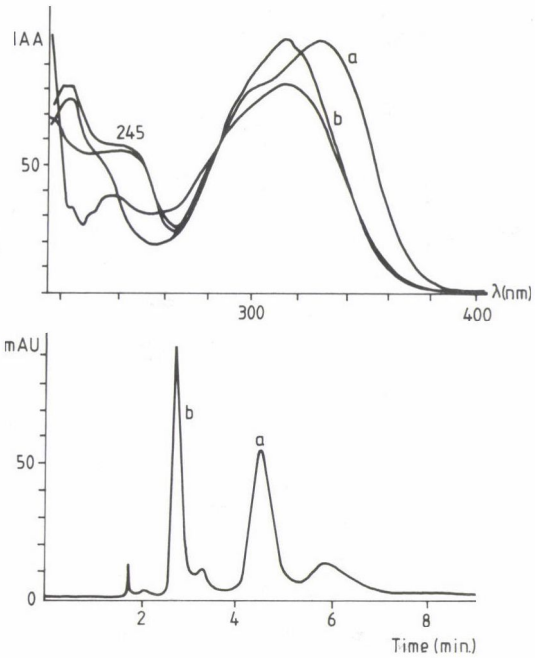

Fig. 7. a, b. The spectra of the unknown compound; HPLC chromatogram of the unknown compound

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A COMPARATIVE STUDY ON FORMATION OF FLAVONOID, TANNIN, POLYPHENOL CONTENTS IN ONTOGENESIS OF *OCIMUM BASILICUM* L.

PART II

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This paper reports on the variation of flavonoid, tannin, polyphenol contents in different periods of ontogenesis of *Ocimum basilicum* L. and a comparative study of their formation in stems, leaves, flowers of *O. basilicum* and *O. sanctum*.

The results obtained in two successive years showed that the flavonoid glycosides in herbs as well as in the organs of plants began accumulating from the budding stage and increasing during flowering. A parallel increase in concentration of both free flavonoid aglycons, tannins, and polyphenols could be observed during the vegetative stages. The variations of free flavonoid aglycons and flavonoid glycosides showed opposite tendencies: if the free aglycon concentration was low, but the flavonoid glycoside content was high. The most of these compounds were found in leaves, flowerparts, but less in stems during the flowering of plants.

There were no essential changes in composition of flavonoids, except flavonoid glycosides (rutin, isoquercitrin) which were only present from the budding stage. The other main components (quercetin, caffeic acid, rosmarinic acid) were found in all stages of growth and in all organs.

Keywords: *Ocimum basilicum* L., stems, leaves, flowers, flavonoid, tannin, polyphenol, ontogenesis

Introduction

The role of phytotherapy has been increasing significantly in recent years. The consumption of medicinal plants has been constantly growing. Among them *O. basilicum* has been recommended for its high medicinal value (Nguyen et al., 1993).

Lahariya and Rao and Sawhney et al. found that the essential oil of *O. basilicum* showed antifungal activity against *Mierosporum gypseum* and other fungi (Dikshit and Husain, 1984). The essential oil of *O. basilicum* has been much studied in both qualitative and quantitative aspects during the plant growth (Kartnig and Simon, 1986). In general, the illnesses of blood vessels and the circulatory system are of social importance in many developed countries. These cases can be treated by preparations containing the polyphenolic substances such as flavonoids. Many reports on the therapeutic effectiveness or ineffectiveness of flavonoids have been publiched. The full evidence of the effects on capillary resistance and the inflammatory action of flavonoids has been given by Gábor (1972, 1974). The main use of flavonoids has been in the treatment of pathological conditions characterized by capillary bleeding associated with increased capillary fragility. These include degenerative

vascular disease, allergic states, diabetes mellitus and various other disorders. *O. basilicum* is of therapeutic activity due to its flavonoid, tannin, polyphenol contents. The main aim of our work is to study the variation of flavonoid, tannin, polyphenol content of *O. basilicum* during the growing of the plant in order to determine the optimum time of harvest. Our further aim is to investigate the flavonoid, tannin, polyphenol contents in stems, leaves, flowers of *O. basilicum* L. Besides *O. basilicum* L., another species, i.e. *O. sanctum* L. has been similarly evaluated.

Materials and methods

We had investigated the variation of flavonoid, tannin, polyphenol contents in herbs as well as in stems, leaves, flowers of *O. basilicum* and *O. sanctum* during ontogenesis. Experiments were carried out in two successive years (1990 and 1991). Plants were collected from Vácrátót at different times of their growing stages.

In the first year the time of sowing was 26 April 1990. The collections of *O. basilicum* were carried out approximately twice a months from the end of June till the first week of October (Table 1). Herbs were collected from all shrubs, dried at room temperatue, finely ground (v) and seived according to Ph.Hg. VII (1986) and homogenized for analysis.

In the second year (1991) the sowing time was 15 May 1991. The collections of *O. basilicum* were also carried out twice a month from the second week of July till the first week of October (Table 1). The herbs of each shrubs (about 10) were collected, dried at room temperature and ground (v). The flavonoid, tannin and polyphenol contents of each sample were determined according to our modified methods (Nguyen et al., 1993). The mean values referring to 10 samples of each collection were calculated.

The stems, leaves, flowers of *O. basilicum* and *O. sanctum* obtained in the second year were regarded seperately. The moisture content of each sample was determined by the method of Ph.Hg. VII (1986).

Detection and quantitative determination of flavonoid, tannin and polyphenol contents in herbs of *O. basilicum*, and in stems, leaves and flowers of both species were carried out according to the methods described in "Part I" (Nguyen et al., 1993).

Results and discussion

During ontogenesis of *O. basilicum*, the flavonoid, tannin, and polyphenol contents changed much in quantity and only slightly in quality. The results obtained from two successive years (1990, 1991) showed that the flavonoid glycosides began accumulating from the budding stage and increasing during flowering. They reached their maximum by the late flowering or early seed-producing stages. They decreased much at the end of the generative stages (Fig. 1). The formation of free aglycons, tannins and polyphenols in *O. basilicum* began just after the time of sowing, and increased during the vegetative growth. The most of free aglycons were found in the vegetative stages. They did not change much during the generative stages, but decreased mostly when flavonoid glycosides had reached maximum (in late flowering or early seed-producing stages). Then they increased again at the end of the generative growth while the flavonoid glycosides greatly decreased (Fig. 2).

Table 1

The variation of flavonoid glycoside, free aglycon, tannin, polyphenol contents in herb of Ocimum basilicum *L. during its ontogenesis (results obtained from two successive years 1990, 1991)*

Stages		1990						1991						
		Date of harvest	1	1'	2	3	4	5	Date of harvest	1	1'	2	3	4	5
Vegetative	I	06.22. (young shoot)	–	–	0.41	0.41	4.84	9.69							
	II	07.06. (shoot)	0.08	0.04	0.66	0.62	3.45	8.55							
	III	07.20. (before budding)	0.11	0.05	0.68	0.63			07.09. (before budding)	0.07	0.03	0.58	0.55	0.68	5.44
Generative	IV	08.03. (budding)	0.16	0.08	0.61	0.53			07.19. (budding)	0.13	0.06	0.54	0.48	1.51	3.70
	V	08.17. (early flowering)	0.25	0.12	0.61	0.49	3.39	7.14	08.06. (early flowering)	0.20	0.10	0.51	0.41	2.17	3.90
	VI	08.31. (full flowering)	0.27	0.13	0.41	0.28	3.05	6.29	08.23. (full flowering)	0.30	0.15	0.53	0.38	1.93	4.28
	VII	09.14 (late flowering)	0.38	0.19	0.48	0.29	4.10	8.54	09.09. (late flowering)	0.31	0.15	0.41	0.26	2.89	4.76
	VIII	09.28. (early seed-prod.)	0.45	0.22	0.38	0.16	2.56	5.13	09.18. (early seed-prod.)	0.13	0.06	0.36	0.30	0.68	4.09
	IX	10.12.. (late seed-prod.)	0.16	0.08	0.28	0.20	2.89	4.93	10.03. (late seed-prod.)	–	–	0.13	0.13	0.67	5.07

1 Flavonoid glycosides given in rutin; 1' Bonded aglycon given in quercetin; 2 Total aglycon given in quercetin; 3 Free flavonoid aglycons given in quercetin; 4 Tannin content given in pirogallol; 5 Polyphenol content

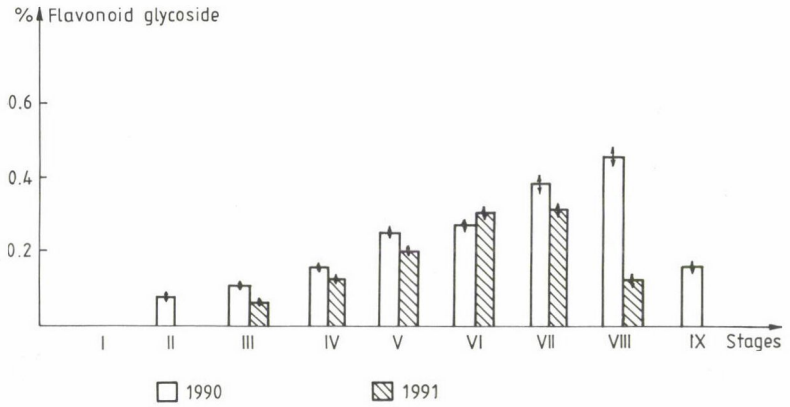

Fig. 1. The change of flavonoid glycosides in herbs of *O. basilicum* during its ontogenesis

The tannin, and polyphenol contents did not change much during the generative stages either. However, they decreased also at the end of the generative growth (Figs 3, 4).

On the basis of our TLC and HPLC investigations, we have not found essential changes in composition of flavonoids, except flavonoid glycosides (rutin, isoquercitrin) during ontogenesis of *O. basilicum*. The flavonoid glycosides accumulated only from the budding stage of the plant. Other main components (quercetin, caffeic acid, rosmarinic acid) and polyphenols were found in all growing stages of *O. basilicum* (Table 1, Figs 1–4).

In the second year we also investigated the formation of flavonoids, tannins, and polyphenols in stems, leaves, and flowers of both *O. basilicum* and *O. sanctum* during their generative growth. The comparative study revealed that no essential differences occurred in the composition of flavonoids. Differences were, however,

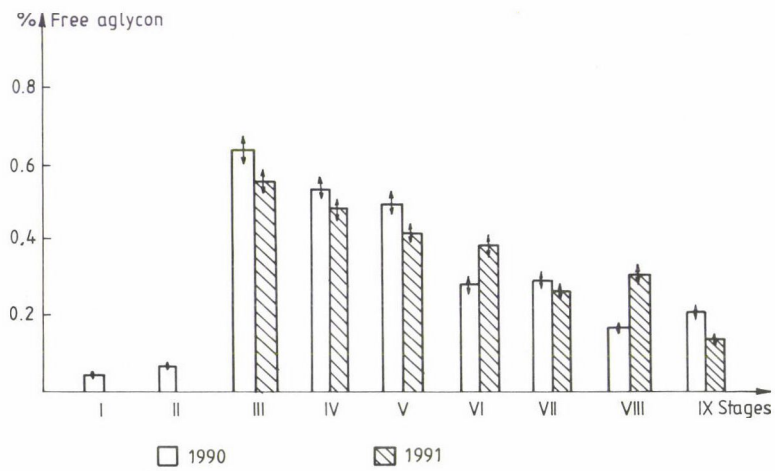

Fig. 2. The change of free flavonoid aglycons in herbs of *O. basilicum* during its ontogenesis

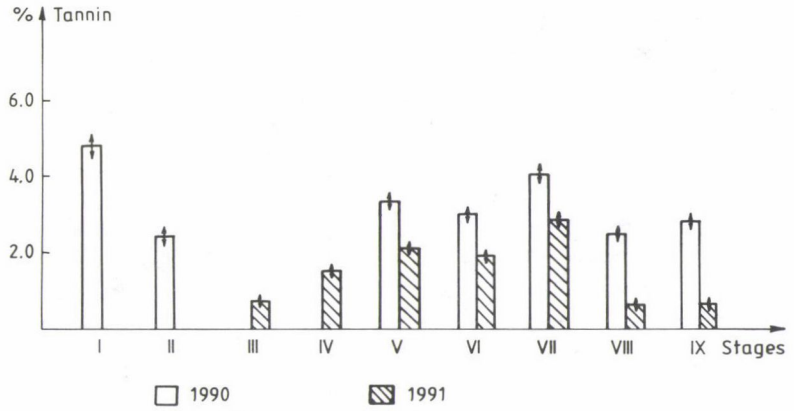

Fig. 3. The change of tannin content in herbs of *O. basilicum* during its ontogenesis

found in the flavonoid, tannin, and polyphenol contents during the generative stages (Figs 5–12). In all organs of both species, the flavonoid glycosides were increasing during flowering and reached maximum in the late flowering stage. In the seed-producing stages, they were decreasing so that they had disappeared int the stems and leaves.

As far as the flowers were concerned, these flavonoid glycosides also showed a decreasing tendency and they did not disappear even by the end of the experiments (Figs 5, 6). The free aglycon, tannin, and polyphenol contents did not change much during the generative stages, but at the end of the generative growth they decreased a little. In general these compounds were found in the largest quantities in the flowers and leaves (Figs 7–12). The free flavonoid aglycon contents of all organs decreased significantly by the late flowering stage, which the flavonoid glycosides had reached maximum. After it, their fluctuations could be observed (Figs 7, 8).

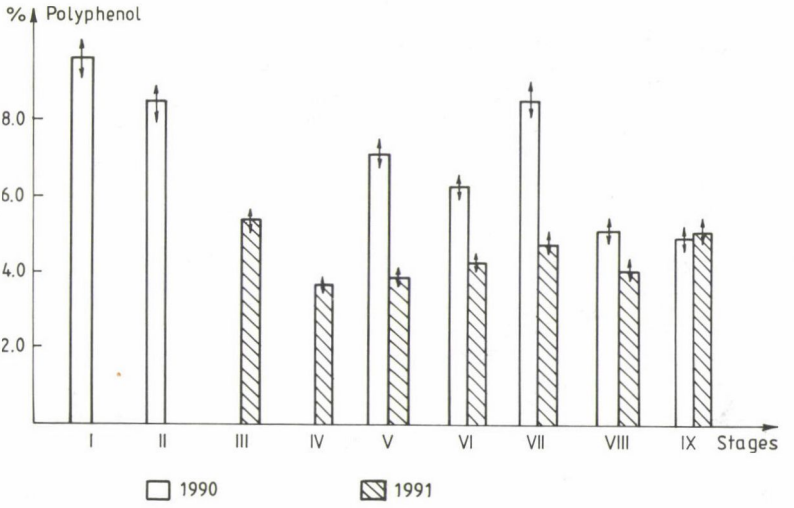

Fig. 4. The change of polyphenol content in herbs of *O. basilicum* during its ontogenesis

Finally, we found that in the flowering stages the quantity of biological active components of *O. basilicum* are generally higher than those of *O. sanctum*.

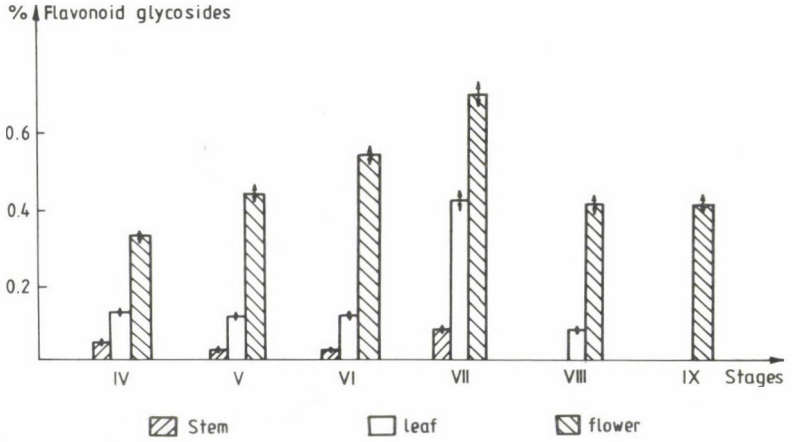

Fig. 5. The change of flavonoid glycosides in stems, leaves, flowers of *O. basilicum* during the generative stages (1991)

Our results show that both *O. basilicum* and *O. sanctum* should be collected in the late flowering or early seed-producing stages, in order to obtain the highest yield of the above-mentioned compounds.

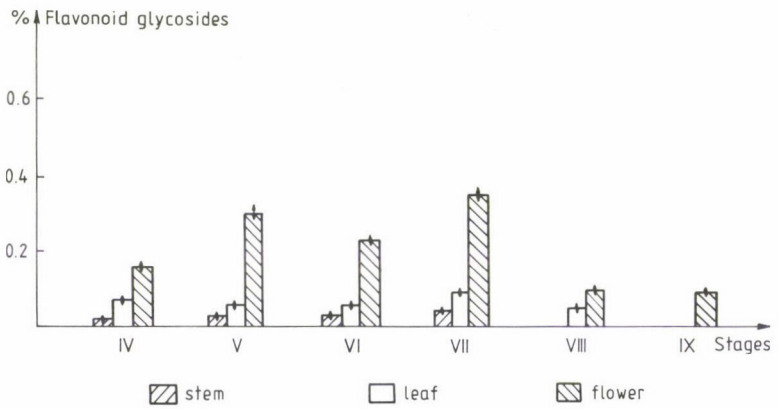

Fig. 6. The change of flavonoid glycosides in stems, leaves, flowers of *O. sanctum* during the generative stages

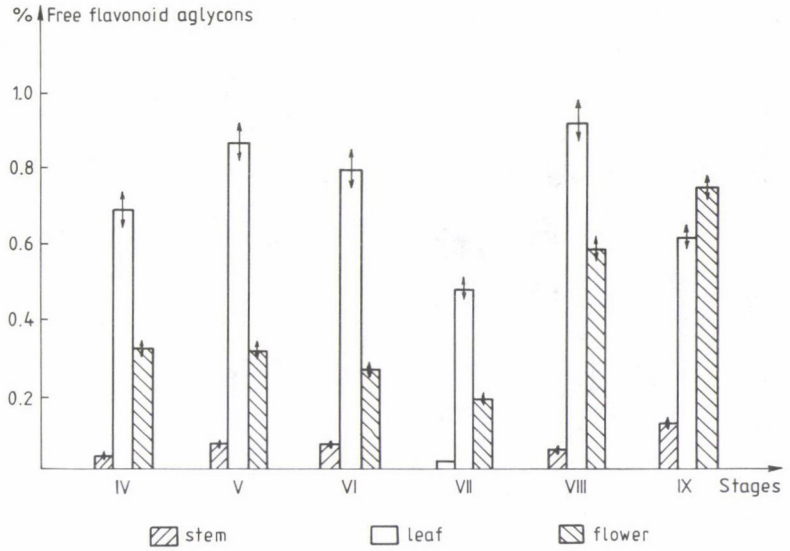

Fig. 7. The change of free flavonoid aglycons in stems, leaves, flowers of *O. basilicum* during the generative stages

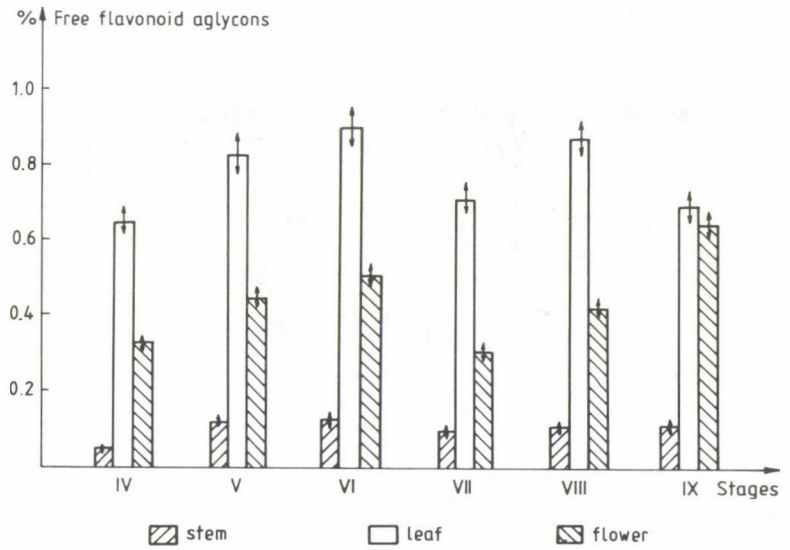

Fig. 8. The change of free flavonoid aglycons in stems, leaves, flowers of *O. sanctum* during the generative stages

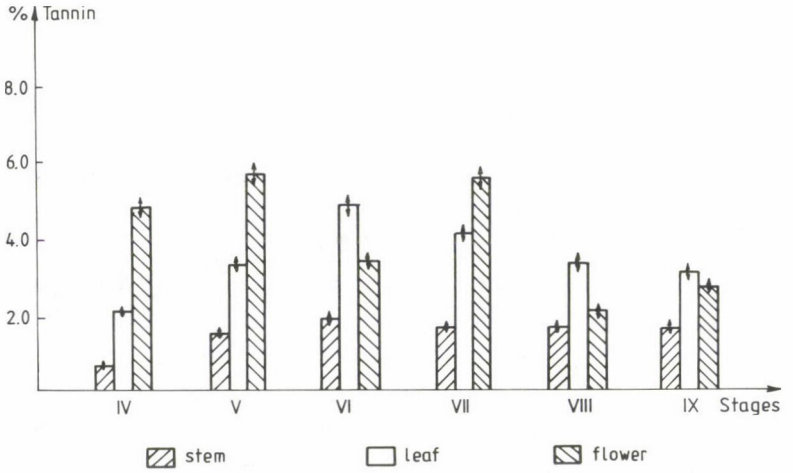

Fig. 9. The change of tannin content in stems, leaves, flowers of *O. basilicum* during the generative stages

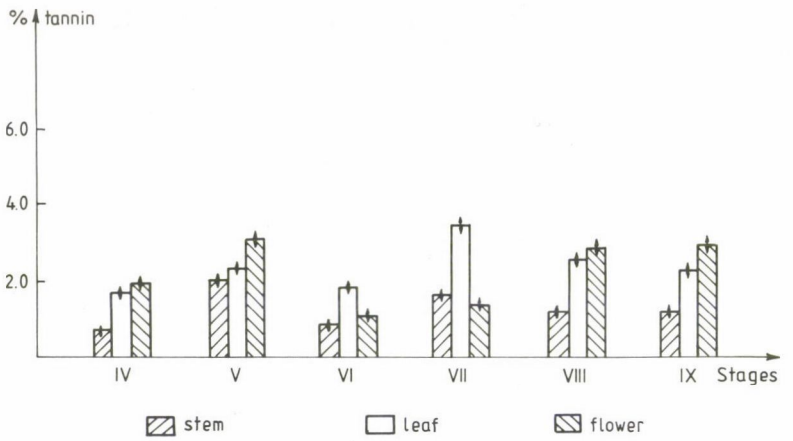

Fig. 10. The change of tannin content in stems, leaves, flowers of *O. sanctum* during the generative stages

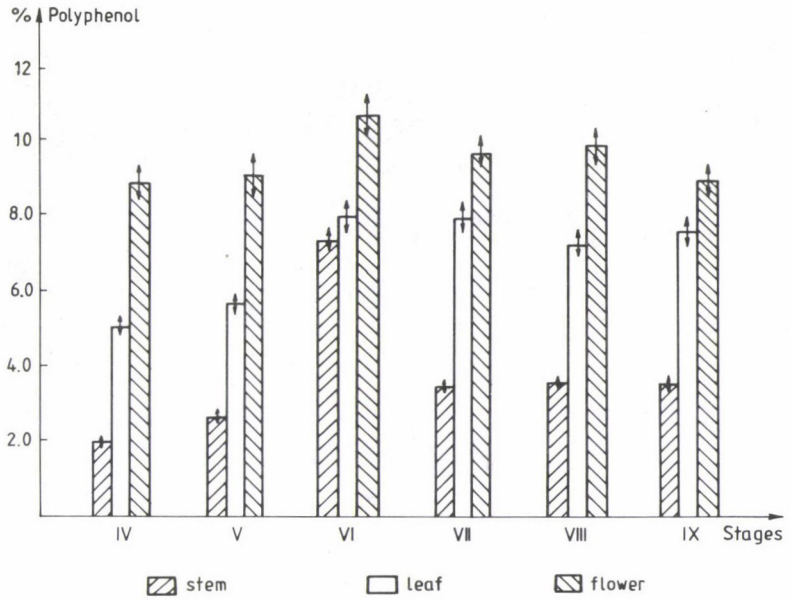

Fig. 11. The change of polyphenol content in stems, leaves, flowers of *O. basilicum* during the generative stages

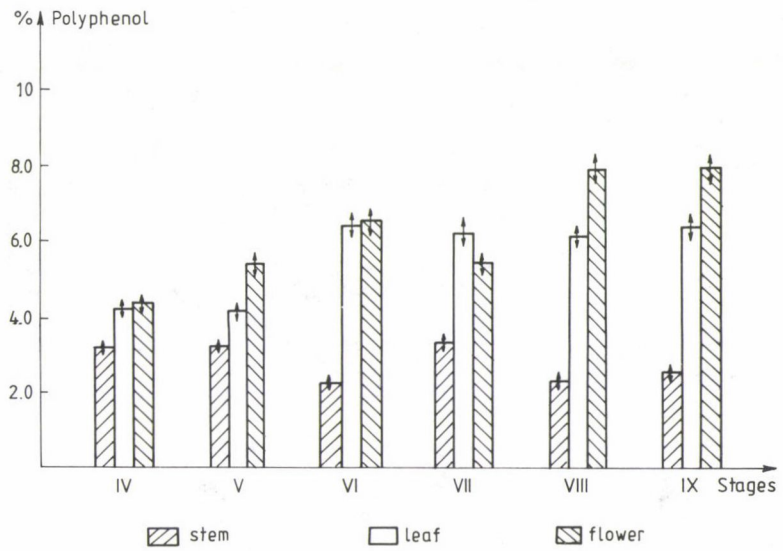

Fig. 12. The change of polyphenol content in stems, leaves, flowers of *O. sanctum* during the generative stages

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RELATIONSHIP BETWEEN SOIL MOISTURE, GROWTH, YIELDS AND NITROGEN FIXATION IN SELECTED GRAIN LEGUMES

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The effect of five regimes of soil moisture on growth, yield and nitrogen fixing ability of two important food legumes were evaluated. The species selected were cowpea (*Vigna unguiculata* Walp.) and mungbean (*Vigna radiata* (L.) Wilczek), due to their widespread cultivation in the tropics. The soil moisture regimes were field capacity and depletion of 20%, 40%, 60% and 80% of water held by the soil between field capacity and air-dried conditions.

Cowpea was more adapted to low soil moisture regimes. Mungbean plants grew better and produced higher per plant yields at higher soil moisture regimes. Depletion of soil moisture decreased all measured parameters of mungbean to a greater extent than in cowpea, although the responses differed in the two species. Per plant yields illustrated the ability of cowpea to produce a greater yield of seeds under dry conditions.

Depletion of soil moisture had an adverse effect on nodulation and nitrogen fixation of both legumes. Nodulation and nitrogen fixation of mungbean was affected to a greater extent than in cowpea. Cowpea maintained nodule activity at low soil moisture regimes. The better adaptability of cowpea to lower soil moisture condition, which is a common phenomenon and a primary determinant of grain legume productivity in the tropics, is presented.

Keywords: *Vigna unguiculata*, *V. radiata*, nitrogen fixation, soil moisture, growth, yields

Introduction

Legumes are an important component of production systems in tropical and temperate agriculture. In the temperate regions, pasture legumes play a vital role in maintaining productivity and nutritional quality of animal fodder. In contrast, food legumes are more important in the tropics, due to their ability to contribute protein to poor quality diets, their economic value in terms of producing relatively high yields and their inherent ability to utilize atmospheric nitrogen in association with rhizobia by nitrogen fixation (Okigbo, 1977; Carangal et al., 1987)

The area of food legumes cultivated under irrigation is very low in most countries, due to the prominence given to cereals and other staple food crops. Thus, legumes are generally cultivated in marginal areas under rainfed conditions or by utilizing residual moisture after a cereal crop, and yields of food legumes very widely in the tropics. This yield fluctuation can be attributed to the adverse effects of moisture stress on growth and nitrogen fixation of legumes, as studies (e.g. Muchow, 1985; Villalobos-Rodriguez and Shibles, 1985) illustrate the high susceptibility of these species to moisture deficits.

The effect of water stress on nodulation and nitrogen fixation in food legumes is less clearly defined in the tropics, where this phenomenon is vital for successful

cultivation. Reports (e.g. Eaglesham and Ayanaba, 1984) identify irreversible damage to nodules under conditions of moisture stress. However, adverse effects of water stress on nitrogen fixation of food legumes is not well quantified, and most studies (e.g. Vincent, 1982; Eaglesham and Ayabana, 1984) identify the effect of water deficits on rhizobial populations, inoculation process and nodulation.

Cowpea (*Vigna unguiculata*, Walp) and mungbean (*Vigna radiata* (L.) Wilczek) are popular food legumes of the tropics, due to their adaptability to a wide environmental range. Generally, mungbean is considered more susceptible to moisture stress than cowpea, and its cultivation is limited to relatively high rainfall areas (Rachie, 1977). However, reports do not clearly identify differential responses of these crops to dry conditions. Thus, a study was carried out and repeated under partially controlled conditions to evaluate the effect of different soil moisture regimes on establishment, growth, yields, nodulation and nitrogen fixation of these two important food legumes.

Materials and methods

The studies were performed as pot experiments at the University of Peradeniya (7° N, 81° E, 420 m above sea level). The mean environmental conditions within the plant house facility over the experimental period were – temperature 27.9 °C ± 1.34 °C, relative humidity 78.4%±3.76% and a 12–13 hour day length. The experimental design on each occasion was a complete randomized design with four replicates.

The planting medium used for the study was a mixture (1 :01) of top soil and seived river sand. Some important characteristics of the planting medium were – pH (1 : 2.5 H_2O) 6.6 ± 0.45, total N – 0.15%, total organic C – 1.24% and a CEC of 25.16 m eq/100 g soil.

Prior to the initiation of the trial, the water-holding capacities of the soil, when air dried and at field capacity, were determined. The water content held by the soil was 18.5%±0.14% by weight per kilogram of air-dried soil. The soil moisture regimes used in the study were field capacity, 20%, 40%, 60% and 80% depletion of the water content held between the field capacity and the air-dried condition. These regimes were maintained from planting up to the final harvest of both crops, by weighing the pots at 3–4 day intervals and adding the required quantity of water to bring the soil moisture up to the desired level. Each treatment consisted of five pots per species.

Plastic pots (2.5 litre capacity) were filled with 2.5 kg of air-dried soil, and the required quantities of water added to obtain the desired level of soil moisture. A basal fertilizer equivalent to 10 kg N, 62.5 kg P_2O_5 and 70 kg K_2O per ha was added to each pot. Thereafter, uniform seeds of cowpea (cv. MI 35) and mungbean (cv. MI 5) (germination 94%±1.57%) were inoculated with a broad spectrum *Rhizobium inoculum* containing *R. phaseoli* and *R. legiminosarum* and planted at a density of five seeds per pot. The soil surface was covered with aluminium foil to prevent excessive evaporation.

The measurements made on both species were germination, survival, and shoot and root growth up to 50% flowering. The latter was determined by obtaining dry weights at weekly intervals. These dry weights were used to determine growth rates by regression analysis using the equation $\text{Log}_e Y = A+BX$, where Y and X are dry weights (g) and time (days), respectively. In addition, flowering, yield components, and per plant yields were determined on three tagged plants per treatment.

At 50% flowering and pod set, three plants per treatment per replicate were carefully uprooted. The number of nodules per plant and their fresh weights were determined on two plants. The root system of the other plant was used to determine nitrogen fixation: Acetylene Reduction Assay was used for this purpose as per the method described by Hardy et al. (1968). The data were analysed for determining significant differences among treatments by methods described by Steele and Torrie (1981).

Results and discussion

Germination, survival and vegetative growth patterns of the selected legumes are presented in Table 1. In both species, germination is not significantly affected until 80% depletion of soil moisture. However, the reduction in germination is greater in mungbean. This illustrates the greater ability of cowpea to germinate under very low soil moisture conditions.

Table 1

Effect of soil water status on germination and growth of selected legumes

Soil water status	Germination (%)	Survival (%)	Shoot growth*	Root growth*
Mungbean				
Field capacity	85.65	94.51	0.1743	0.1042
20% Depletion	82.61	86.95	0.1611	0.1284
40% Depletion	86.54	85.29	0.1365	0.1412
60% Depletion	83.14	69.65	0.0966	0.0754
80% Depletion	65.87	41.37	0.0512	0.0632
L. S. D. (P=0.05)	7.54	12.66	0.0102	0.0098
Cowpea				
Field capacity	81.20	85.73	0.1956	0.1253
20% Depletion	84.39	81.40	0.1811	0.1198
40% Depletion	82.55	86.34	0.1646	0.1422
60% Depletion	79.59	74.52	0.1124	0.0986
80% Depletion	73.24	61.81	0.0727	0.0818
L. S. D. (P=0.05)	6.32	9.87	0.0056	0.0023

* Shoot and root growth rates estimated by the regression equation
$\text{Log}_e Y = A+BX$, where Y and X are dry weights (g) and time (days), respectively

The pattern of survival of germinated seedlings varies from that of germination. Mungbean illustrates a greater percentage of seedling survival at higher soil moisture, which could be due to the susceptibility of cowpea seedlings to moist conditions. In contrast, with increasing soil moisture depletion, survival of mungbean is affected to a greater extent. Thus, at a depletion level of 60%, mungbean seedling survival is reduced significantly, while cowpea shows a similar effect at 80% depletion of soil moisture. The reduction in seedling survival is also greater in mungbean (53%) than in cowpea (24%) at 80% depletion, when compared with survival at field capacity. This clearly indicates the greater adaptability of cowpea to a drier environment, as suggested by Rachie (1977) and Wood and Myers (1987).

Cowpea plants show better shoot growth rates at all soil moisture levels (Table 1). Thus, at field capacity, shoot growth rates of cowpea are 11% greater, and are affected in both species by soil moisture depletion. Thus, significant reductions are observed beyond a 40% depletion of soil moisture. This effect is greater in mungbean, and at 80% depletion, shoot growth rate is 41% less than that of cowpea.

Root growth is also affected by reductions in soil moisture, and cowpea plants show higher rates of root growth at all moisture levels. The reduction in root growth, which indicates the adaptability of the plant to dry conditions, is greater in mungbean (40%) than in cowpea (34%) when soil moisture is reduced from field capacity to 80% depletion.

The shoot growth of both species is affected to a greater extent than root growth by reduction in soil moisture. This could be attributed to a greater development of root systems under dry conditions. The greater reduction in shoot and root growth of mungbean, with decreasing soil moisture, also illustrates the greater susceptibility of this species and the greater adaptability of cowpea to dry conditions.

Cowpea is generally a longer living species, compared to mungbean, and thus flowering occurs later. However, the effect of decreasing soil moisture affects both species in a similar manner (Table 2) by reducing the time to flower by approximately 29% due to 80% depletion of soil moisture. In addition, the reduction in time to flower taken by both species becomes significant at 40% depletion. This phenomenon could be attributed to the early maturing habit of legumes under adverse ecological conditions (Turner and Kramer, 1980).

Table 2

Yield components and yield of mungbean and cowpea plants at different soil moisture levels

Soil water status	Days to flower	Flowers/plant	Pod set %	Seeds/pod	100 seed wt (g)	Yield/plant (g)
Mungbean						
Field capacity	32.30	26.50	84.50	8.75	5.89	11.26
20% Depletion	33.40	27.75	89.25	8.80	5.66	11.43
40% Depletion	29.30	19.50	78.75	7.45	5.06	6.68
60% Depletion	26.00	15.70	71.25	6.80	4.67	3.85
80% Depletion	23.30	11.65	65.00	6.19	3.85	2.94
L. S. D. (P=0.05)	2.17	1.87	4.35	0.68	0.25	1.08
Cowpea						
Field capacity	48.70	16.85	78.50	8.30	9.55	10.56
20% Depletion	45.30	17.50	80.00	8.05	9.10	10.35
40% Depletion	42.30	15.85	75.25	7.50	9.45	9.43
60% Depletion	36.00	12.85	73.00	7.15	8.70	6.14
80% Depletion	34.40	10.75	69.75	6.40	7.65	3.97
L. S. D. (P=0.05)	3.28	2.33	3.69	0.35	0.18	0.96

Mungbean generally produces a greater number of flowers than does cowpea (Table 2). However, the flower number per plant is reduced to a greater extent at a lower soil moisture depletion level (40%) in mungbean, while cowpea shows a similar effect at 60% depletion. Both species show a marginal increase in flower numbers at 20% depletion. Although this phenomenon is insignificant, and not evaluated in the present study, it could be attributed to the extension of the vegetative phase under conditions of high soil moisture. This warrants further study.

Moisture stress has a significant bearing on yield components and yield of food legumes (Muchow, 1985). This is highlighted by this study, and all measured yield components are affected by increasing soil moisture stress (Table 2). The effect of low soil moisture on percentage pod set in mungbean is greater than that observed in cowpea. Thus significant reductions occur at 60% depletion of soil moisture in both mungbean and cowpea.

Seeds per pod and seed development (measured by 100 seed weight) of both species are significantly reduced by soil moisture stress. Again, mungbean is more susceptible to low soil moisture. However, in mungbean, seed development is affected to a greater extent than is the number of seeds per pod. The reduction in 100 seed weight and number of seeds per pod when mungbean is grown at 80% depletion, in comparison to that of plants at field capacity, is 29% and 34%, respectively. In contrast, the reverse effect is seen in cowpea, where seeds per pod is reduced by 24% when compared to a 17% reduction in 100 weed weight when the crop is grown at the highest and lowest soil moisture regimes. This illustrates that responses of yield components of legumes to soil moisture stress varies according to species.

The effect of the soil moisture regimes on yields also illustrate the greater susceptibility of mungbean. Thus, while mungbean produces greater per plant yields at higher moisture levels, productivity is reduced by 75% at the lowest soil moisture regime when compared to yields at field capacity. In contrast, the yield reduction in cowpea at 80% depletion of soil moisture is 67%. A comparison of the two species also shows that at lower soil moisture levels, per plant yields of cowpea exceed that of mungbean. Thus, as these crops are generally grown at similar densities (Gunasena, 1974), cowpea has the capacity to produce greater yields per unit area under dry conditions, due to its better adaptability to lower moisture levels. The results also suggest the possibilities of per plant yields of cowpea exceeding yields of mungbean by over 100% under dry conditions. However, under adequate moisture conditions, mungbean plants produce greater yields.

The nodulation and nitrogen fixation in legumes are sensitive to changes in soil water (Ahmed and Quilt, 1980). This is associated with poor survival of *rhizobia* under conditions of low moisture (Boonkerd and Weaver, 1982). The results of this study also illustrate the adverse effect of low soil moisture on nodulation and nitrogen fixation in two important food legumes (Table 3). All measured parameters of nodulation and nodule activity decline with increasing soil moisture stress, and the rate of reduction is greater in mungbean, which is a more promiscuous nodulator. Thus, while nodulation and nitrogen fixation of cowpea are lower at both harvests, the process is less affected by soil moisture depletion. This could be attributed to the greater adaptabilitiy of this crop to the condition of low moisture, due to the close relationship between host growth and the process of nodulation and nitrogen fixation (Henzell, 1988). The process of nodulation and nitrogen fixation of both species varies with time. However, this is also affected by soil moisture. Thus, at low soil moisture levels, the rate of increase in nitrogen fixation with time is significantly lower. This shows the susceptibility of nitrogen fixation to soil moisture, and the rate of declination varies with time and species. Cowpea, the more adaptable species,

thus shows some nodule activity at the lowest soil moisture level at the second harvest, when mungbean plants show no evidence of nitrogen fixation.

Table 3

Nodulation and nitrogenase activity of mungbean and cowpea at different soil moisture levels

Soil water status	At 50% flowering			At pod set		
	nodules/plant	nodule wt (g)	nodule activity*	nodules/plant	nodule wt (g)	nodule activity*
Mungbean						
Field capacity	26.00	0.634	4.324	34.00	0.924	7.718
20% Depletion	23.50	0.596	3.411	27.00	0.821	5.112
40% Depletion	14.50	0.326	2.526	17.50	0.569	3.076
60% Depletion	10.00	0.144	1.175	12.00	0.204	2.065
80% Depletion	6.00	0.079	0.644	5.50	0.096	–
L. S. D. (P=0.05)	2.54	0.011	1.213	2.46	0.008	0.911
Cowpea						
Field capacity	21.50	0.934	3.024	28.50	1.256	5.122
20% Depletion	24.50	0.997	3.452	26.50	1.096	4.984
40% Depletion	20.00	0.865	3.241	23.00	0.974	4.178
60% Depletion	15.50	0.643	1.845	17.50	0.723	2.249
80% Depletion	11.00	0.219	0.968	10.00	0.214	0.638
L. S. D. (P=0.05)	1.98	0.032	0.857	0.76	0.035	1.298

*Nodule activity was measured by Acetylene Reduction Assay as μ mol C_2H_4/plant/hour

Conclusions

Food legumes are grown in a wide range of environments due to their importance in tropical agriculture. Their degree of adaptability varies, so the selection of a crop to a given environment must be carried out carefully in order to obtain optimum productivity. The results of this study show the response of two important food legumes to soil moisture, which is considered to a major limiting factor in tropical agriculture. The results also illustrate a greater adaptability of cowpea to soil moisture in terms of plant growth, yielding ability and nitrogen fixation. However, under conditions of adequate moisture, mungbean can produce a greater yield and utilize atmospheric nitrogen more effectively. In contrast, under conditions of low moisture, which is a common phenomenon in the tropics, cowpea, which has the ability to fix atmospheric nitrogen and produce some yield can be considered a more suitable crop.

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EFFECT OF SHADE AND FERTILIZER LEVELS AND THEIR INTERACTION ON FRUIT YIELD OF SWEET PEPPER

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The effect of fertilization under different shade levels on vegetative and fruit yield of sweet pepper during late summer season was studied. There were three fertilizer levels of 357.0, 267.8 and 178.5 kg NPK/ha, and two complete fertilizers having NPK ratios of 1 : 2 : 1 and 2 : 2 : 1 were used in the experiments under three shade levels (63, 55 and 40%), and unshaded. The plants fertilized with 357 kg NPK/ha, and grown under shade level of 63%, gave the highest fresh weight, leaves and root/plant, an increasing in plant leaf area, leaves and stem dry weight/plant. The 40% shade with highest fertilizer level had the superiority in the production of total fruit yield, average fruit weight and number of marketable fruits per square meter. The differences were significant in both seasons.

Keywords: shading-fertilizer interaction, sweet pepper

Introduction

Sweet pepper is a favourite vegetable throughout the year, as a fresh or cooked food. The average area cultivated with pepper during the period of 1987 to 1990 was about 15890 ha/year with an annual production of more than 247795 tons. More than 67% of this area was cultivated as summer and Neely crop (fall season) according to the statistical data of the Ministry of Agriculture (1988 to 1991).

Sweet pepper (*Capsicum annuum* L.) var California Wonder is generally believed to be a shade plant (Schoch, 1972). Problems of fruit quality exist during late summer season, sunscald damage especially occurring under intense solation. Therefore, ways were sought of to improve fruit quality by shading. This work also aimed at using the ventilated shading in tunnels that were covered with plastic film during the winter period, to complete the crop rotation all year around.

Many studies, carried out in different locations, proved the importance of shading for several plants. Light intensity is one of the main factors affecting yield and quality of many crops. A shade meets two plant requirements, and adequate but not excessive reduction of the solar radiation which reaches the plants, [also the transfer of heat because of its porosity between the plant mass and its exterior] (Lagier and Brun, 1988). Rylski (1986) noted that shading of sweet pepper plants increased both plant height and leaf size. Working with sweet pepper, Quagliotti (1976) established that the dry matter in shaded leaves was greater than in those under full sunlight. Also, shade increased the number of fruits/plant and the total fruit yield and improved the percentage of marketable fruits. Similar results were observed by El-Aidy et al. (1983) on tomato plants.

Terbe (1978) indicated that the growth of sweet pepper was decisively influenced by specimen nitrogen, while dry matter content in leaves increased and the dimensions of fruits were changed with abundant nitrogen. Somos (1984) showed that the highest yield of sweet pepper was obtained in soil containing 16 mg P_2O_5 and K_2O per 100 g soil.

The main objectives of this work were to evaluate the effect of some shade levels on vegetative growth, fruit yield and quality of sweet pepper grown during the late summer season, and to study the effect of fertilization under shade conditions.

Materials and methods

This work was carried out at the Experimental Farm of Faculty of Agriculture, Kafr El-Sheikh. The experiments were conducted during the late summer of 1984 and 1985 on sweet pepper, California Wonder variety. The soil-mechanical and chemical analyses are illustrated in Table 1. The seedlings were transplanting on 29th of May, 1984 and 1st June, 1985 at a density of six plants per square meter. The seed sowing date was 60 days earlier.

Table 1

Mechanical and chemical analysis of soil before conducting the experimental plots (1984 and 1985)

	Season	
	1984	1985
Mechanical analysis		
Sand %	21.03	21.52
Silt %	34.60	34.47
Clay %	44.25	44.00
Soil texture	Clay	Clay
Chemical analysis		
Soluble cations and anions in soil extract 1 : 5 (meq/100 g soil)		
Ca^{++}	0.890	0.920
Mg^{++}	0.481	0.492
Na^{+}	3.850	3.940
K^{+}	0.162	0.150
HCO_3^-	0.590	0.680
Cl^-	1.970	2.085
SO_4^{--}	2.823	2.737
pH	7.800	7.900
EC/25 °C (mmhos/cm)	0.396	0.402
Available N (mg/100 g soil)	3.610	3.703
Available P (mg/100 g soil)	5.180	4.910
Available K (mg/100 g soil)	0.351	0.366

Treatments used: Shade materials (40, 55, 63% and unshaded), were accomplished by placing shade nets on solid iron framed tunnels (7.5 m wide and 2.5 m high) provided by Tildent Co. Shade nets were fixed on 15th June and removed after three months in both seasons. Three fertilizer levels of 357.0, 267.8 and 178.5 kg NPK/ha were used. The commercial fertilizers were ammonium sulphate (20% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O). Two complete fertilizers having NPK ratios of 1 : 2 : 1 and 2 : 2: 1 were used.

The fertilizer mixture was divided into equal portions; the first was applied at 30 days after transplanting and second was added three weeks later. Surface irrigation method was used. The other culture practices were carried out as done by the local growers.

Shading levels were distributed randomly as a main plot, while, NPK ratios were randomly distributed as sub plot, and fertilizer levels were sub-sub plot. The plot area was 3.6 square meter. The split-split plot design with four replications were used. Data were tested by analyses of variance. Duncan's multiple range test was used for comparisons among treatment means.

Fruits were picked in the mature green stage. Fruit picking started at the beginning of August and continued until the end of October in both seasons.

Results and discussion

Air temperature

The weekly changes in air temperature under the three shading levels and unshaded plots at 1.6 m are given in Fig. 1. Data indicate that there were slight differences among the treatments. These results agree with those of Lagier and Brun (1988), who reported that shade had low significant effect on air temperature.

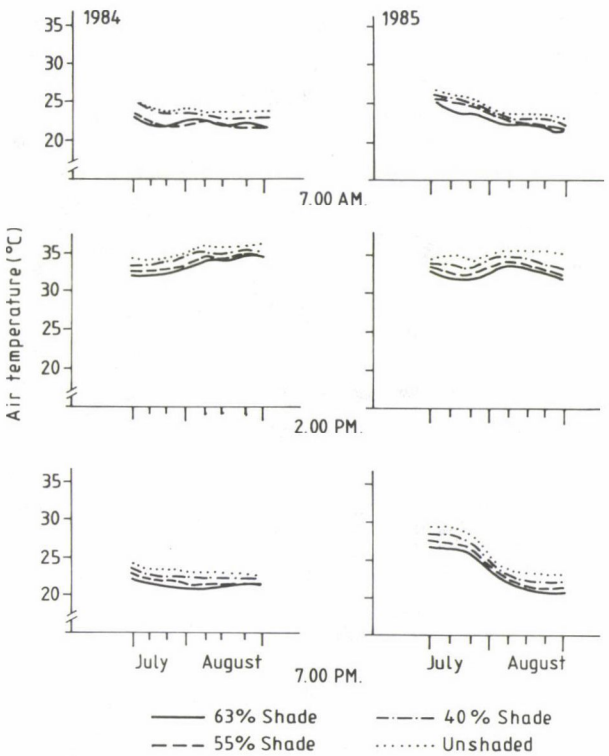

Fig. 1. Weekly changes in air temperature during July and August, as affected by shading levels several times daily (1984 and 1985)

Soil temperature

Data in Fig. 2 reveal that shading caused a reduction in soil temperature and the reduction was more evident as the shading levels increased in both seasons. Similar results were obtained by El-Aidy et al. (1983), who reported that shade reduced soil temperature. Also, Lagier and Brun (1988), reported that the warming of the soil or the substrate is limited to 2–3 °C, as compared with the control for average temperature of 27 °C.

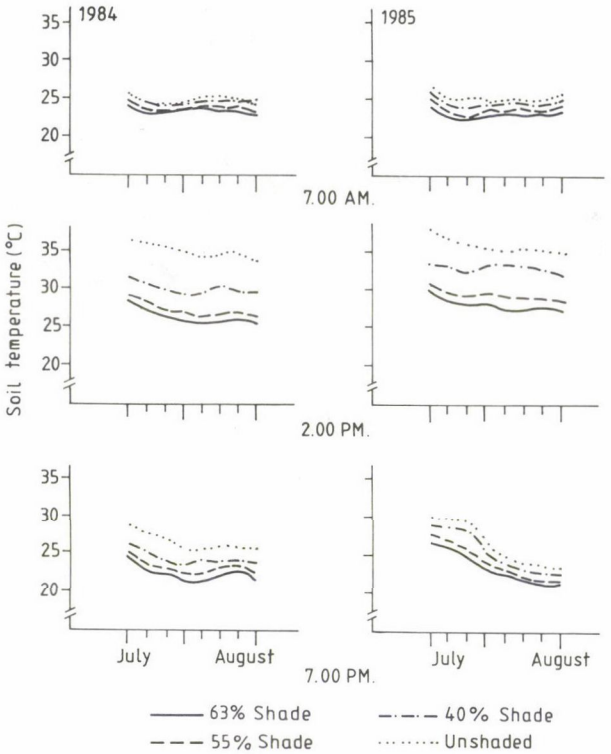

Fig. 2. Weekly changes in soil temperature at 10 cm depth during July and August, as affected by shading levels (1984 and 1985)

Treatments: T_1 – Control, T_2 – *Azolla*, T_3 – PU, T_4 – PU+*Azolla*, T_5 – CBU, T_6 – CBU+*Azolla*, T_7 – NBU, T_8 – NBU+*Azolla*, T_9 – KCl Bu, T_{10} – KCl Bu+*Azolla*, T_{11} – GBU, T_{12} – GBU+*Azolla*

Plant temperature

Data in Fig. 3 show the average temperature for the surface of the foliage and the fruits. The shading caused a reduction in the average temperature. The fruits had higher temperature than the leaves under the same conditions. These results agree those of Lagier and Brun (1988).

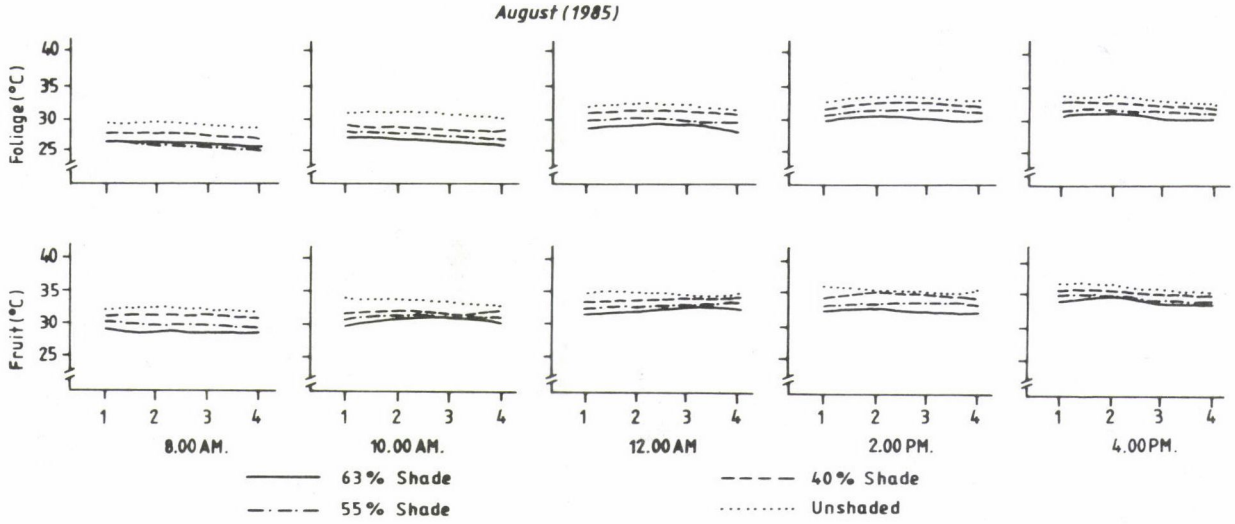

Fig. 3. Weekly average changes of foliage temperature, as affected by shading level several times daily, during August (1985)

Effect of shading

The harvesting duration lasted 81 days in 1984 and 71 days in 1985. Data presented in Table 2 show that the total fruit yield under shade decreased as shading increased. The low shade (40%) was superior and gave the highest yield in both seasons. The data also indicate that the total yield increased by 10–21% under shade, 63% by 35–47% under 55% shade and by 65–78% for 40% shade, compared with unshaded plants. The differences were significant among the treatments in both years.

Data in Table 2 indicate that the highest average fruit weight in both seasons was obtained from 55 and 40% shade. These results may be due to the favourable effect of shading on vegetative growth, as an increasing in cell division and cell expansion and hence improving the quality of fruits. The same data also reveal that the lowest number of marketable fruit yield was obtained by 63% shade in both seasons, while the highest number was produced by 40% shade in both years.

Table 2

Effect of shading levels on fruit yield and quality of sweet pepper (1984 and 1985)

Treatments		Fruit yield kg/m²	Average fruit wt (g)	No. of marketable fruits/m²	Non-marketable fruits/m²	%
			1984 season			
Shading						
	63%	2.20 *b	33.15 a	67 d	9 c	11.84 d
	55%	2.40 b	34.42 a	90 c	12 c	14.53 c
	40%	3.32 a	33.85 a	98 a	22 b	18.35 b
Unshaded		2.01 b	23.34 b	86 b	33 a	27.87 a
			1985 season			
Shading						
	63%	2.30 c	31.67 b	75 d	7 d	8.52 d
	55%	2.81 b	35.12 b	80 c	9 c	10.14 c
	40%	3.42 a	33.69 a	102 a	21 b	17.23 b
Unshaded		1.96 d	20.67 c	94 b	34 a	26.67 b

*Means separation by Duncan's multiple range test, 5% level

Effect of fertilizer level

Data in Table 3 indicate that high fertilizer levels (357 kg NPK/ha) produced the highest total fruit yield of sweet pepper, while the low fertilizer level (178.5 kg NPK/ha) gave the lowest, in both seasons. However, there was no significant differences between the high and medium fertilizer levels in the first season, while the differences among the three levels were significant in the second season.

Table 3

Effect of fertilizer levels and NPK ratios on fruit yield and quality of sweet pepper (1984 and 1985)

Treatments	Fruit yield kg/m²	Average fruit wt (g)	No. of marketable fruits/m²	Non-marketable fruits/m² %	
1984 season					
Fertilizer levels					
357.0 kg NPK	2.73 *a	32.11	87.08 a	17.57 c	16.78 a
267.8 kg NPK	2.52 a	32.01	79.42 b	18.22 b	18.92 b
178.5 kg NPK	2.19 b	30.21	74.25 c	18.65 a	18.13 c
NPK ratios					
1 : 2 : 1	2.56 a	20.86	84.75	12.21	20.00 a
2 : 2 : 1	2.40 b	32.03	75.75	18.09	18.00 b
1985 season					
Fertilizer levels					
357.0 kg NPK	2.90 a	29.78 b	97.7 a	18.54	14.86 c
267.8 kg NPK	2.66 b	31.06 a	86.0 b	17.50	15.65 b
178.5 kg NPK	2.31 c	29.36 a	79.5 c	17.21	16.41 a
NPK ratios					
1 : 2 : 1	2.73 a	30.28	91.0 a	18.25 a	15.51
2 : 2 : 1	2.52 b	29.86	84.5 b	17.25 b	15.76

*Means separation by Duncan's multiple range test, 5% level

Data in Table 3 show that there were no significant differences among the fertilizer levels in the first season, while the medium level (267.8 kg/ha) tended to give the highest average fruit weight in the second season.

Data in the same Table (3) indicate that there was a gradual increase in the number of marketable fruits with the increasing fertilizer level. The differences among the treatments were significant in both years. This response may be due to the promotive effect of high rates of N, P and K on fruit growth and quality.

Effect of NPK ratio

Data in Table 3 show that the fertilizer ratio had a significant effect on total fruit yield in both seasons. The increment of total fruit yield was produced by the plants of 1 : 2 : 1 NPK ratio in both years. The 1 : 2 : 1 NPK ratio tended to have the highest total and earliest yield.

Results in Table 3 indicate that no constant trend was obtained in both seasons, and differences were insignificant in both years. The 1 : 2 : 1 NPK ratio surpassed the number of marketable fruits per square meter in both seasons. However, no differences were significant in the second season only.

Effect of shading-fertilizer levels interaction

Data in Table 4 indicate that a generally low shade level (40%), with any fertilizer level, tended to be the best in both seasons, except with high fertilizer level in the second season. Morover, significant interactions were obtained in both seasons.

Table 4

Effect of shading level (A) and fertilizer level (B**) interaction on total fruit yield and quality of sweet pepper fruits* (1984 and 1985)

Treatments	Fruit yield kg/m²	Average fruit wt (g)	No. of marketable fruits/m²	Non-marketable fruits/m²
		1984 season		
A_1B_1	2.64 bc	36.78	71.67 f+	9.67g
A_1B_2	2.16 cd	32.32	66.83 fg	8.83 g
A_1B_3	2.08 de	33.35	62.50 g	8.50 g
A_2B_1	2.51 bc	32.36	77.50 e	12.67 f
A_2B_2	2.47 bc	36.16	68.33 fg	11.83 e
A_2B_3	2.23 cd	34.75	64.16 fg	11.50 e
A_3B_1	3.57 a	34.51	103.33 a	23.17 d
A_3B_2	3.47 a	35.62	97.50 b	21.67 d
A_3B_3	2.93 b	31.42	93.17 c	21.17 d
A_4B_1	2.37 bc	24.78	95.83 bc	34.33 c
A_4B_2	2.04 d	23.95	85.00 b	33.33 b
A_4B_3	1.64 e	21.31	77.17 c	31.33 a
		1985 season		
A_1B_1	2.48 c	29.75 d	83.33	7.67 f
A_1B_2	2.29 c	30.54 b	75.00	6.83 f
A_1B_3	2.14 cd	32.08 cd	66.67	6.50 f
A_2B_1	3.02 b	34.56 bc	87.50	9.50 ef
A_2B_2	2.88 b	36.43 ab	79.17	8.83 e
A_2B_3	2.52 bc	34.37 bc	73.33	8.83 e
A_3B_1	3.81 a	32.20 bc	118.33	21.50 d
A_3B_2	3.54 a	36.72 a	96.67	20.83 c
A_3B_3	2.93 b	32.15 bd	91.00	20.67 c
A_4B_1	2.30 c	22.61 e	101.67	35.50 b
A_4B_2	1.92 de	20.57 ef	93.33	33.50 b
A_4B_3	1.64 e	18.86 f	87.00	33.00 a

+Means separation by Duncans's multiple range test, 3% level; *Shading level A_1 = 63%; A_2 = 55%; A_3 = 40%; A_4 = unshaded. ** Fertilizer level: B_1 = 357.0; B_2 = 267.8; and B_3 = 178.5.

Data in the same table reveal that no constant trend was obtained for the effect of shading grade and fertilizer level on average fruit weight. However, the differences were significant in the second season only.

Plants grown under 40% shading tended to have the highest number of marketable fruit yield with all fertilizer levels in both seasons. However, the differences were significant in the first season only.

Conclusions

– The highest total fruit yield was obtained from plants grown under low shade (40%), and having 357 kg NPK per ha with 1 : 2 : 1 NPK ratio.

– Shaded plants tended to have the highest average fruit weight, while the fertilizer levels and NPK ratios had no constant trend.

– The plants of low shade (40%) with the highest fertilizer level (357 kg NPK per ha) with 1 : 2 : 1NPK ratio had the highest number of marketable fruits per square meter.

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EFFECT OF NITROGEN FIXING WATER FERN *AZOLLA* AND DIFFERENT FORMS OF UREA APPLICATION ON THE GROWTH, NITROGEN UPTAKE AND GRAIN YIELD OF RICE CROP

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Two field experiments were conducted during the late wet season and early wet season to study the effect of *Azolla* inoculation as dual crop with different forms of urea on rice. The different forms of urea included were prilled urea, carbofuran blended urea, neem cake blended urea, KCl blended urea and gypsum blended urea. Inoculation of *Azolla* as dual crop with carbofuran blended urea, neem cake blended urea and KCl blended urea have shown positive effect on plant height, tiller and straw yield of rice varieties IR–50 and Co–43. Prilled urea blended with carbofuran or KCl or neem cake have increased the nitrogen uptake at early stages of crop growth and also in grain and straw when applied with *Azolla* than the individual application. Individually and in combination with different forms of urea, *Azolla* inoculation also recorded more total nitrogen accumulation in soil during both seasons.

Keywords: rice, *Azolla* dual crop, nitrogen uptake, urea, neem

Introduction

Azolla is an aquatic fern which fixes atmospheric nitrogen in association with the symbiotic nitrogen fixing cyanobacterium –*Anabaena azolla* and contribute 40–60 kg N ha^{-1} (Kannaiyan, 1987b). Interest in *Azolla* – Anabaena association in relation to tropical rice production is primarily based on the ability of the association to fix nitrogen and increase the status of nitrogen in rice soil. Lumpkin and Plucknett (1982) reviewed the potential value of *Azolla* as biofertilizer for rice production. A layer of *Azolla* covering the rice field contains about 15–25 t biomass per hectare and by incorporation the nitrogen accumulated in the *Azolla* is made available to rice crops (Kannaiyan, 1987a). Incorporation of *Azolla* crops before or after transplanting rice was equivalent to split application of 30 kg fertilizer N (Roger and Watanabe, 1986). Singh and Singh (1987) reported that maximum N and P uptakes in rice were obtained by growing two crops of *Azolla* after transplanting in addition to its incorporation before transplanting. The use of *Azolla* as a dual crop coupled with the application of fertilizer nitrogen would certainly help the better exploitation of this fast-growing water fern in rice production technology. In the present investigation an attempt was made to study the effect of *Azolla* inoculation with different forms of urea on rice yield and nitrogen uptake.

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Materials and methods

Field experiments were conducted at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India during the late wet season (June–September) and early wet season (October–February) to determine the effect of *Azolla microphylla* inoculation as a dual crop and different forms of urea application on rice varieties IR–50 and Co–43, respectively. Different fields were employed in the two seasons with the total nitrogen content of 1450 kg/ha (late wet season field) and 1074 kg/ha (early wet season field). The experiments were conducted with 12 treatments and 3 replications in a randomised block design with plot size of 3 m x 5 m. The different forms of urea viz., prilled urea (PU), carbofuran blended urea (CBU), neem cake blended urea (NBU), potassium chloride blended urea (KCl BU) and gypsum blended urea (GBU) were tried at 100 kg ha^{-1} level and applied in three split doses (1/2 as basal, 1/4 at tillering and 1/4 at panicle initiation) with and without *A. microphylla* inoculation. Phosphorus and potassium fertilizers were applied at 50 kg ha^{-1} each as basal dressing.

Carbofuran, neem cake (residue obtained from the dried fruits of neem tree *(Azadirachta indica)* after oil extraction), potassium chloride and gypsum were powdered separately, sieved through 1 mm sieve and blended with prilled urea at 2% (w/w) level with the help of 2% solution of gum. *A. microphylla* was inoculated as dual crop at 200 g m^{-2} on 7th day after transplanting. Inoculated *Azolla* was allowed to grow for 3 weeks and incorporated. The left-over *A. microphylla* during first incorporation was again allowed to grow with rice crop for 3 weeks and a second incorporation was carried out.

Plant height, productive tillers per hill, number of panicles per square metre, grain yield and straw yield were recorded. The nitrogen content in the grain and straw was analysed following the standard procedure of Humphries (1956) and nitrogen uptake was calculated by multiplying the N content with the dry matter production. Soil samples were drawn at tillering, panicle initiation stages of the rice crop and after harvest and the total nitrogen content was analysed by Kjeldahl method (Bremner, 1965) using Kjeltec auto 1030 analyser.

Results

Inoculation of *Azolla* in combination with different forms of urea have increased the plant height, number of productive tillers per hill and panicle numbers per square metre more than did the individual application of the respective urea in both seasons (Table 1). The combined effect of *Azolla* and carbofuran blended urea or neem cake blended urea showed more plant height and panicle numbers. Similarly, carbofuran blended urea/neem cake blended urea/KCl blended urea with *Azolla* have registered more grain and straw yield in both rice varieties of IR–50 and Co–43 (Table 2, Figs 1 and 2). In general, the inoculation of *Azolla* as dual crop with carbofuran blended urea or neem cake blended urea or KCl blended urea have shown positive effects on the yield and yield attributes of rice varieties in both the seasons.

Combined effect of *Azolla* with different forms of urea have recorded higher nitrogen content in the leaf during the vegetative stage of the crop (Tables 3 and 4). The nitrogen content in the grain was recorded more in KCl blended urea with *Azolla* application (IR–50) and in carbofuran blended urea with *Azolla* application (Co–43). *Azolla* inoculation with neem cake blended urea, KCl blended urea and carbofuran blended urea have also shown higher N uptake than other treatments not only at early stages of crop growth but also recorded higher N uptake in grain and straw in both the seasons (Tables 3 and 4).

Table 1

Effect of different forms of urea and A. microphylla inoculation on the growth and tillers production of rice

Treatments	IR–50					Co– 43				
	Plant height (cm)		Productive tillers (No./hill)		Panicles (No./m²)	Plant height (cm)		Productive tillers (No./hill)		Panicles (No./m²)
	Tillering	Harvest	Tillering	Harvest		Tillering	Harvest	Tillering	Harvest	
Untreated control	48.67	51.23	6.67	7.83	359	64.67	68.27	5.13	6.27	304
Azolla inoculation (DC)	49.33	52.87	7.00	7.87	427	67.80	71.15	7.90	8.33	373
Prilled urea (PU)	50.67	52.90	7.97	8.20	557	74.50	77.32	6.67	7.20	452
PU+*Azolla*	52.00	53.27	8.00	8.40	556	81.33	85.45	8.90	10.10	489
Carbofuran blended urea (CBU)	51.90	53.17	7.67	8.17	509	79.17	81.00	8.13	9.57	617
CBU+*Azolla*	52.67	55.97	8.13	8.43	578	82.27	86.17	9.25	11.30	667
Neem cake blended urea (NBU)	51.00	53.97	8.00	8.53	447	75.13	77.05	8.12	10.50	642
NBU+*Azolla*	52.67	55.83	8.33	8.67	616	78.00	82.12	9.93	12.12	646
KCl blended urea (KCl BU)	52.00	53.27	7.43	8.43	529	80.67	84.25	8.60	9.27	507
KClBU+*Azolla*	53.33	55.60	7.50	8.43	532	86.67	90.13	9.90	12.03	589
Gypsum blended urea (GBU)	50.50	53.27	7.17	7.73	525	73.00	76.38	8.00	8.97	537
GBU+*Azolla*	51.33	54.53	7.67	8.47	479	76.50	80.44	8.33	10.00	559
SE_d	0.94	0.77	0.47	0.48	22.66	4.19	6.03	0.89	1.12	36.8
CD	1.95	1.60	0.98	1.00	47.00	8.70	12.50	1.85	2.32	76.4

DC: Dual cropping

Table 2

Effect of different forms of urea and A. microphylla inoculation on the yield of rice

Treatments	IR–50		Co–43	
	Grain yield (kg/ha)	Straw yield (kg/ha)	Grain yield (kg/ha)	Straw yield (kg/ha)
Untreated control	3071	6737	3442	6275
Azolla inoculation	3172	7576	4233	7667
Prilled urea (PU)	3212	7576	5717	11317
PU+*Azolla*	3374	8919	6408	11833
Carbofuran blended urea (CBU)	3444	8919	6992	13775
CBU+*Azolla*	4030	9596	7150	14333
Neem cake blended urea (NBU)	3434	8252	5983	11558
NBU+*Azolla*	3859	9263	6592	12667
KCl blended urea (KCl BU)	3434	8253	6308	11275
KCl BU+*Azolla*	3576	9424	6592	11558
Gypsum blended urea (GBU)	3263	7909	5750	8500
GBU+*Azolla*	3545	8919	5975	9475
SE_d	103	174	166	488
CD	214	360	345	1012

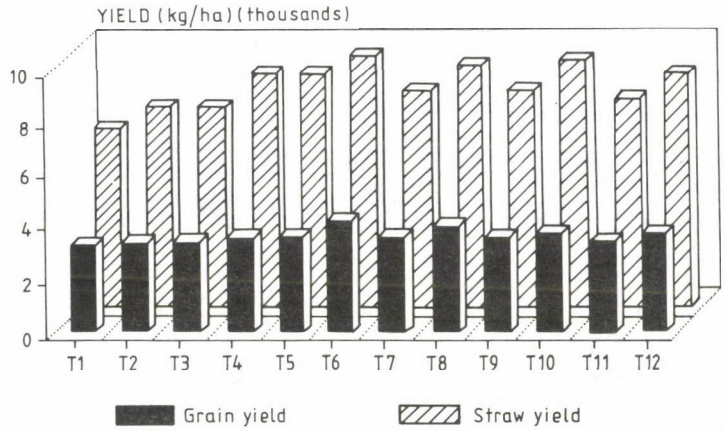

Fig. 1. Effect of *Azolla* with different forms of ures on the yield of rice (IR–50)

Inoculation of *Azolla* as dual crop recorded higher total nitrogen in soil than did the prilled urea treatment at early stages of crop growth (Table 5). Also, the inoculation of *Azolla* in combination with different forms of urea recorded more total N accumulation in soil than the individual application of respective urea during the late wet season. However, no significant variation was noticed between the different forms of urea and *Azolla* combination treatment during the early wet season. In general, the soil total nitrogen was recorded more during late wet seasons than early wet seasons.

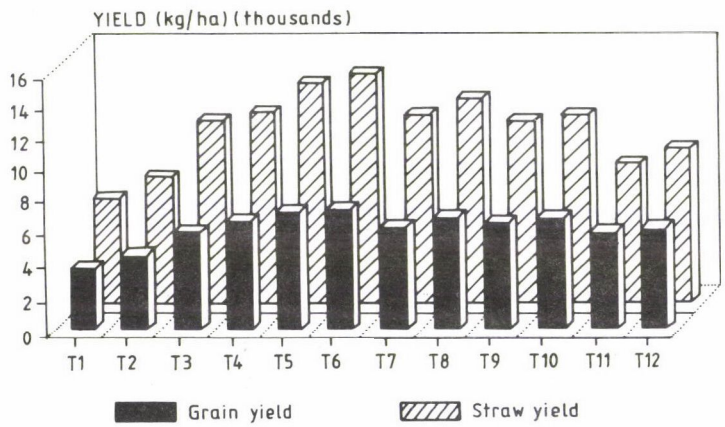

Fig. 2. Effect of *Azolla* with different forms of urea on the yield of rice (Co–43)

Table 3

Effect of different forms of urea and A. microphylla inoculation on the nitrogen accumulation and nitrogen uptake in rice (IR–50)

Treatments	N accumulation (%)				N uptake (kg/ha)			
	Tillering	Panicle initiation	Harvest		Tillering	Panicle initiation	Harvest	
			Grain	Straw			Grain	Straw
Untreated control	1.98	1.28	0.861	0.438	21.78	38.64	26.44	29.50
Azolla inoculation	2.06	1.35	0.856	0.454	22.54	52.35	27.15	34.40
Prilled urea (PU)	2.45	1.62	0.901	0.503	30.35	40.15	28.94	38.11
PU+*Azolla*	2.34	1.52	0.925	0.518	30.63	54.76	31.21	46.20
Carbofuran blended urea (CBU)	2.54	1.47	0.914	0.493	39.67	55.30	31.48	43.97
CBU+*Azolla*	2.56	1.61	0.942	0.527	32.85	62.63	37.96	50.57
Neem cake blended urea (NBU)	2.47	1.53	0.917	0.525	30.58	56.50	31.49	43.32
NBU+*Azolla*	2.39	1.54	0.985	0.517	40.43	59.58	38.01	47.89
KCl blended urea (KCl BU)	2.47	1.41	0.965	0.489	25.95	61.62	33.14	40.36
KCl BU+*Azolla*	2.51	1.33	1.015	0.556	32.63	52.74	36.30	52.40
Gypsum blended urea (GBU)	2.34	1.44	0.947	0.576	27.64	49.95	30.90	45.56
GBU+*Azolla*	2.42	1.48	0.953	0.550	26.38	50.36	33.78	49.05
SE_d	0.150	0.183	0.019	0.014	3.70	4.88	3.06	1.56
CD	0.312	NS	0.040	0.029	7.68	10.12	6.34	3.23

Table 4

Effect of different forms of urea and A. microphylla inoculation on the nitrogen accumulation and uptake of rice (Co–43)

Treatments	N accumulation (%)				N uptake (kg/ha)			
	Tillering	Panicle initiation	Harvest		Tillering	Panicle initiation	Harvest	
			Grain	Straw			Grain	Straw
Untreated control	1.28	1.05	0.750	0.376	27.92	25.50	25.82	23.59
Azolla inoculation	1.24	1.31	0.824	0.412	28.05	32.56	34.80	31.58
Prilled urea (PU)	1.39	1.25	0.815	0.454	29.56	46.78	46.59	51.38
PU+*Azolla*	1.70	1.35	0.868	0.467	36.48	53.46	55.62	55.26
Carbofuran blended urea (CBU)	1.68	1.22	0.849	0.460	36.56	55.25	59.36	63.37
CBU+*Azolla*	1.94	1.38	0.952	0.460	39.42	57.32	68.07	65.93
Neem cake blended urea (NBU)	1.56	1.25	0.869	0.475	40.56	49.48	51.99	54.90
NBU+*Azolla*	2.06	1.77	0.915	0.484	44.67	56.37	60.32	61.31
KCl blended urea (KCl BU)	1.58	1.39	0.856	0.512	42.39	51.56	54.00	57.73
KCl BU+*Azolla*	2.26	1.54	0.924	0.526	44.67	61.67	60.91	60.80
Gypsum blended urea (GBU)	1.68	1.15	0.861	0.496	39.86	50.05	49.51	42.16
GBU+*Azolla*	1.94	1.40	0.904	0.484	41.35	54.34	54.02	45.86
SE_d	0.188	0.135	0.049	0.043	3.66	3.06	4.48	3.88
CD	0.390	0.280	0.102	0.090	7.60	6.34	9.30	8.05

Table 5

Effect of different forms of urea and A. microphylla inoculation in rice on the total nitrogen content of the soil

Treatments	Soil total nitrogen (kg/ha)			Soil total nitrogen (kg/ha)		
	Tillering	Panicle initiation	Harvest	Tillering	Panicle initiation	Harvest
Untreated control	1630	1250	975	995	765	734
Azolla inoculation	1722	1864	1639	1013	1024	1120
Prilled urea (PU)	1694	1717	1924	1024	1100	924
PU+*Azolla*	1767	1828	1865	1130	1056	1016
Carbofuran blended urea (CBU)	1700	1854	1913	1094	1093	1205
CBU+*Azolla*	1854	1912	1870	1156	1060	1164
Neem cake blended urea (NBU)	1925	1907	1951	1210	1203	1050
NBU+*Azolla*	1815	1920	1865	1130	1044	1104
KCl blended urea (KCl BU)	1694	1834	1767	1096	1116	1200
KCl BU+*Azolla*	1656	1886	1817	1264	1150	1060
Gypsum blended urea (GBU)	1788	1952	1795	1120	1124	956
GBU+*Azolla*	1813	1874	1825	1200	1180	1005
SE_d	56.4	67.5	40.9	47.3	32.3	49.2
CD	117.0	140.0	85.0	98.0	67.0	102.0

Discussions

The results revealed that the inoculation of *Azolla* with carbofuran blended urea, neem cake blended urea and KCl blended urea increased the yield attributes, grain and straw yield, N accumulation and N uptake of rice varieties IR–50 and Co–43. *Azolla* was incorporated twice in the experiments and the nitrogen accumulated in *Azolla* was released after decomposition and made available for the rice crop. *Azolla* decomposed in 8–10 days after incorporation (Singh, 1977; Kannaiyan, 1987a). *Azolla* grown as a dual crop until heading stage and incorporated twice after planting was found to be equivalent to 30 kg N ha^{-1} (Kannaiyan et al., 1982). Besides the beneficial role of *Azolla*, use of different forms of urea, particularly carbofuran blended urea, neem cake blended urea and KCl blended urea, might have contributed to the increased yield and N uptake. The above forms of urea increase the efficiency of nitrogen availability for rice crops as the blending materials act as a physical barrier which generally prevents immediate dissolution of the fertilizer in soil or flood water (Parish, 1979).

Application of carbofuran to control the pest of rice under a low land system has been well known. Carbofuran is known to check the pest incidence in *Azolla* (Kannaiyan, 1987a) as the same was recommended for multiplication of *Azolla*. In addition, because of blending of carbofuran, the slow release of ammonical N from the blended urea is possible which might also be attributed to the increase in yield and yield components and nitrogen uptake.

Increased grain yield and yield components by neem cake blended urea with *Azolla* inoculation might possibly be due to the inhibitory action on the population

of Nitrosomonas which is primarily responsible for nitrification process. Such inhibitory effect of neem cake on nitrifying bacteria has been reported earlier (Khandelwal et al., 1977). Neem cake is known to induce the growth and nitrogen fixation in *Azolla* (Kannaiyan, 1978b). Also it is widely used to control pest incidence in *Azolla* (Kannaiyan et al., 1983), ostrocods in free living BGA and black rot disease of *Azolla* incited by *Rhizoctonia solani* (Kannaiyan, 1978b). These studies further strengthen the positive beneficial effects of neem cake under flooded rice soil situation, besides nitrogen use efficiency. Interestingly, KCl blended urea also performed better with *Azolla* in increasing the yield and N uptake in rice, which indicates the possible slow release of ammonia from KCl blended urea and synergistic effect of both *Azolla* and KCl blended urea in flooded rice soil system.

Total nitrogen accumulation in soil was also found to be increased by the application of *Azolla* with different forms of urea. Application and subsequent incorporation of *Azolla* in flooded rice soil release nitrogen after decomposition and mineralization which is subjected to crop uptake and to various losses. However, certain portions of the nitrogen are added into the soil, thus increasing the total N accumulation. The increase in the total nitrogen accumulation by inoculation of *Azolla* with carbofuran blended urea or neem cake blended urea or KCl blended urea might possibly be due to the cumulative contribution of biofertilizers and fertilizer nitrogen in soil. Approximately 17–20% of the applied N was recovered in the 0–5 cm layer of soil and 4% from the 10–15 cm depth (Vlek and Byrnes, 1986).

The soil total N accumulation was recorded more in the late wet season, when IR–50 rice variety was raised, than the early wet season. The experiments were conducted in different fields in these two seasons and the initial total N content of the soil of the fields also varied. In addition, the differential behaviour of the rice varieties IR–50 (short duration) and Co–43 (medium duration) in nitrogen uptake might have influenced the total N accumulation in soil.

From the results of the present investigation, it may be concluded that *Azolla* could be effectively utilized by incorporation with different forms of urea viz., neem cake blended urea, carbofuran blended urea and KCl blended urea, to increase the rice production and for enriching the soil fertility status.

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MULTIELEMENTAL ANALYSIS OF BLACKFLY AND GUMMOSIS-AFFECTED ORANGE LEAVES BY INSTRUMENTAL NEUTRON ACTIVATION

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Instrumental Neutron Activation Analysis (INAA) has been empolyed for the determination of about 15 minor and trace elements in a number of healthy, blackfly and gummosis-affected orange leaves. Marked differences in elemental concentrations of P, Zn, Mn, K, Cl and Cu have been observed. These elements may be used as an indicator of plant health. INAA technique may be conveniently used for the agricultural and mineral nutrient studies in plants.

Keywords: neutron activation analysis, orange leaves, trace elements, gummosis, mineral nutrients

Introduction

Neutron activation analysis (NAA) is a versatile technique for the determination of major, minor and trace elemental contents in geological, biological and environmental samples (Amiel, 1981; Ehmann and Vance, 1989). It has been extensively used for the analysis of different types of biological specimens (Kanias and Philianos, 1979; Awadallah et al., 1986) viz. root, stem, leaves, bark, fruit and foliage of plants (Yamaguchi et al., 1982; Vose, 1980), body fluids and tissues of animals (Versieck, 1985).

Plants are the ultimate source of energy. Hence increasing awareness is being created about their compositional studies (Ndiokwere, 1984). Some elements seem to have a tendency to accumulate in specific part and thereby increasing its importance as nutrient (Nadkarni and Chaphekar, 1977). Similarly distinct changes have been observed in the plant health status with varying minor and trace elemental supply (Fukuzaki and Moriyama, 1985). Donagi et al. (1980) used INAA for studying the decline of *Pinus helpensis* forests in the Judeg mountains of Israel, whence Mn content was found 2–3 times higher in sick needles than in healthy ones. Liu et al. (1982) have determined 17 elements in two species of fungus causing wheat bunt disease. Shuvalov (1982) reported accumulation of several radioisotopes ^{90}Sr, ^{137}Cs and ^{60}Co from radioactive fallout. Recently Aidid (1988) used INAA for trace element analysis of tropical trees and correlated the concentration of toxic elements with the age of the plants and the pollution level in the area.

Nagpur (in Central India) oranges are famous for their sweet variety throughout the country. However, in recent years this quality has been deteriorating primarily due to the attack of Black Fly mould (Raina et al., 1987). It prevents the photosynthesis,

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thereby affecting the quantitative yield and the health status. The devastating Black Fly (*Aleuro canthus* sp.) gradually sucks up the cell sap of leaves, resulting in defoliation and the fungal infection which starts from stem and affects the nutrient supply to aerial parts. We have investigated the elemental status of healthy and declining orange plant leaves, using instrumental NAA. Lemon leaves and bark samples were also analysed.

Materials and methods

Sample collection: The leaf samples were collected from three different orchards surrounding Nagpur (viz. Katol and Kalmeshwar within a distance of 20 km) and Dhamangaon (at a distance of 120 km). A total of 13 leaf samples (6 healthy and 7 declining) and one each of lemon leaves and gummosis-affected bark were analysed. Despite healthy growth conditions, a few plants fell prey to the disease. The twigs were plucked, washed with distilled water, and the normal-sized, mature leaves were separated, dried (at 80°C under IR lamp) and powdered, to prevent any contamination from dust. The bark sample was removed from an orange tree and powdered.

Irradiation and counting: 40–60 mg each of the sample and the standards such as Bowen's Kale (Bowen, 1985) and NBS SRM 1570 spinach were packed in high purity aluminium foil (Indal, India) and irradiated for 5 m, 1 h and 5 h at a thermal neutron flux of 10^{12} n cm^{-2} s^{-1} in APSARA reactor at BARC, Bombay. Hay powder, V–10 a Certified Reference Material from IAEA, Vienna was also used as comparator. The induced γ-activites due to various radionuclides were measured using a 45 cc HPGe detector (EG and G ORTEC) and 4096 multichannel analyser (TN–1700) at the Radiochemistry Division of BARC, Bombay. Short lived nuclides were further identified by their half-life plots. Nuclear characteristics of the nuclides identified or determined were adapted from the compilation of Pagden et al. (1971). The concentrations of various elements were determined by comparing the specific activites of the sample and the standards (Wankhade et al., 1986). Phosphorus was determined by short-term irradiation (2–6 h) followed by β counting, employing 28 mg cm^{-2} aluminium absorber (Weginwar et al., 1989).

Results and discussion

In Table 1 the ranges of concentrations of 15 minor and trace elements, and their mean values calculated for the orange and lemon leaves and a bark sample are given. Standard deviations were calculated from replicate analyses and different photopeaks, wherever possible. It is observed that the most elemental contents determined in standards agree well with those of certified values. Standard deviations are < 10% in most cases. Therefore, it is expected that the elemental contents of orange and lemon leaves should be reliable.

A perusal of data in Table 1 shows that the concentrations of Mn, Br, La and Ga are comparable in both the healthy and declining tree leaves. The concentrations of Na, K, Cl, Cu and Co, however, are enhanced in declining tree leaves. While comparing the elemental contents from Nagpur and Dhamangaon (140 km away), Na (0.074±0.01%) and Cu (7 ppm) contents were found to be higher in the latter case. Also it is observed that Fe, Zn and P contents are higher in healthy, compared to those of declining, tree leaves. It is well known that Fe, Zn and Mn are essential trace elements and are closely associated with photosynthetic process (Meyer and Anderson,

Table 1

Concentrations of elements in healthy and declining orange leaves and bark samples and lemon leaves

Element		Standards		Healthy		Declining		Gummosis-affected		Lemon leaves
		Bowen's Kale	Spinach SRM 1570	Range	Mean	Range	Mean	Leaves	Bark	
Na	(%)	0.267 (0.249)	1.38 (1.37)	0.028–0.034	0.030	0.033–0.080	0.060	0.016	0.025	0.09
K	(%)	2.55 (2.46)	3.61 (3.56)	1.55–1.97	1.76	1.16–3.49	2.32	1.06	0.22	1.54
Mg	(%)		0.43 (0.90)	0.25	0.25	–	–	–	–	0.44
Cl	(%)	0.43 (0.34)	0.52 (0.65)	0.043	0.043	0.062–0.092	0.08	–	–	0.051
P	(%)	0.42 (0.45)	0.49 (0.55)	0.12–0.26	0.19	0.12–0.18	0.15	–	–	0.04
Fe	(ppm)	103 (120)	337 (550)	100–380	240	132–264	200	250	222	274
Zn	(ppm)	26.4 (32.7)	66.5 (50)	71–75	73	18–24	21	–	–	52
Mn	(ppm)	11.9 (14.9)	20.4 (24)	10–20	16	8.7–29.3	19	–	–	65
Cu	(ppm)	4.91 (4.89)	11.4 (12)	3.6–4.7	4.2	5.5–6.5	6.0	8.97	8.58	3.7
Br	(ppm)	25.7 (25)	53.9 (54)	9.0–13.0	11	5.6–14.2	9.9	14.24	4.37	5.8
Cd	(ppm)	1.09 (0.85)	1.16 (1.50)	–	–	0.61–1.49	1.1	–	–	–
Co	(ppm)	–	1560 (1500)	370–460	420	380–750	570	–	–	41
La	(ppb)	–	570 (370)	250–500	375	350–480	420	460	36	356
Ga	(ppb)	–	52 (NA)	30–45	38	25–48	37	–	570	48
Sc	(ppb)	–	220 (160)	15–20	18	–	–	–	–	24

Values in parenthesis are certified or from literature

1965). In blackfly-affected trees, it is this function which is most affected due to the formation of a thick black honeyed layer of sooty mould over the leaf surface. A few exceptionally higher contents of Na, K, Mn and Cu, compared to those of the mean values for healthy plant leaves, indicate a state of infection. Similarly, even though the lower limit of phosphorus content in healthy and declining leaves is the same, its mean value is certainly lower in declining leaves.

There are only scanty reports of critical levels of micronutrients in orange leaves from India. A few such studies have indicated significant differences in elemental contents of healthy and declining leaves (Annual Report, 1972; Kathwate, 1968; Sivaraman Nair and Mukherjee, 1970). Mann et al. (1970) have observed significant differences in K, Ca and Zn contents of sweet orange trees in Punjab. In a similar study Patil and Tejam (1981) have reported Na, K, Fe, Mn, Cu and Zn in healthy and declining leaves of Nagpur oranges by radiochemical NAA but did not find any significant differences, except that Mn and Zn were depleted in declining leaves. We have also observed depletion of Zn and P in declining leaves. All these elements are considered essential for the healthy growth of plants.

The lemon plant also belongs to the same family of *Citraceae*. Most elemental contents of these lemon leaves are comparable with healthy orange leaves. However, Na and Mn contents are higher and P and Co are depleted. The gummosis-affected trees are normally treated with lime and $CuSO_4$ solution on their stems. We had taken bark and leaves samples from one such tree. A comparison of these leaves with those of healthy ones indicates enhancement in Cu content by a factor of two. No differences were observed in the elemental contents of bark and leaves. Therefore, it can be concluded that such elemental differences may be used as a criterion for distinguishing the nutrient supply, environmental conditions of growth, and the health status of plants. INAA method can be employed for the routine multielemental analysis of biological and agricultural samples.

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Plant cultivation

BIOMETRIC ANALYSIS OF CLIMATIC CONDITIONS IN HUNGARY WITH A VIEW TO THE YIELD AND QUALITY OF WINTER WHEAT

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The analysis has been carried out on the basis of 248 experimental sites for variety tests, including 15–20 varieties and the data of 33–129 meteorological measuring points during seven years. From the correlation of 54 climatic variables with main component analysis 6 climatic factors have been determined. With the coorelation of the these factors and yields climatic numbers of values have been elaborated. The values show the correlation of climatic variables and their effect on yield. Putting the values on maps climatic regions of wheat growing can be established.

The comparison of climatic number of values for quantity and quality shows that regions are different concerning the quality quantity of wheat growing. The separation of the different areas seems to be suitable.

The climatic number of values show significant correlation with the quality and quantity of yield so it can be used to measure climatic conditions. The mapping of climatic number of values can help in determining or comparing the conditions of different regions.

Keywords: winter wheat, climatic conditions, climatic parameters, climatic number of value, regional effect, wheat quality, yield quantity

Introduction

Specialists and local administrators have always been interested in the agro-ecological effects that can be effective in plant production, in the placement of the production in the effect of the site on the quantity and quality of the yield.

The first scientific works – calling themselves specially agrogeographical – appeared in the late 19th and the early 20th century. These works only contained descriptions about the production of plants and animals, with climate and soil conditions.

The development expanded in several basic directions, all which aimed to find and isolate homogenic units of the agricultural land. The selection of the methods depended on the aim of the zonation, on the methods known in the certain era, and on the quantity and quality of the information available. Thus, it is impossible and unnecessary to form permanent zones, because cultivars, production technologies, and relations of production can modify the environment-forming factors, their effect on plant production and the proportions of these factors.

Among the strategies of the zonation we would like to emphasize the zonation according to the bases of the site. We can differentiate between three main tendencies in this method.

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a) zonation according to the natural conditions – classifies the elements of the environment according to the agricultural production and takes these elements into account according to their influence on the agriculture (Papadakis, 1952; Benett, 1960; Blagovidov, 1961; Gvozdeckij and Zvonkova, 1961; Lacate, 1961),

b) zonation by suitability zones – classifies the production with the crops giving the best yield in the zone (Csákány, 1951; Visher, 1955; Rajonizace, 1960, 1963; Géczy, 1968),

c) zonation of agricultural land-use – classifies the area not according to the natural conditions but the method of tillage (Kostrowicki, 1969).

In the Hungarian wheat production this problem became urgent with the appearance of the American wheat in the European market in the 1920's (Kisléghy Nagy, 1930).

Before 1945 the formation of zones by the average yield of the crops was usual. After 1945 with the appearance of the large-scale production, because the scientific analysis of this question became more and more important, many scientific works appeared dealing with it (Aujeszky et al., 1951; Görög, 1954; Erdei et al., 1959).

In the 1960's the demand for regional production necessitated the "Regional Research Program" of the Hungarian Academy of Sciences, the results of which are summarized in Géczy's study (1968). Bulla's zonation system summarizes the geographical (soil) characteristics of the country. In the 1970's, after the publishing of the results of the "Regional Research Program", scientists started to analyse the ecological effects, as is indicated by the amount of studies that appeared on this topic (Szabó, 1970, 1973; Szániel, 1973; Nagy and Proksza, 1974), and a summarizing study (by Bernát and Enyedi, 1977) which also provided a good basis for further research.

In the late 1970's and early 1980's it became quite clear that, among the several factors effective in the agricultural production, the less influenceable are the ecological constituents, so further research had to be based on them (Láng et al., 1983).

For this reason the Hungarian Academy of Sciences in 1978 decided upon the measurement of the agroecological potential of Hungary. This posed two main questions:

– What will be the possible level of plant production determined by the agroecological characteristics around the end of this century?

– How would it be possible to increase the production and decrease the expenses by better use of our possibilities in shorter distance?

In this work the territory of Hungary was divided into 34 agroecological regions, divided by soil types, finally appearing as 205 soil-mosaics. Climatic factors were taken into account by classifying 4 climatic year-types.

The latest results of this research were published by Nagy (1981), Szász (1983), Lőrincz et al. (1983) and Ángyán et al. (1984). These essays examined the effects in each plant and gave detailed information.

In this study climatic and edaphic effects are examined separately, because there were insufficiently detailed data about climate parameters.

Our purpose was to produce complex climate numbers of value which can be placed on the map of Hungary and show correlation with the possible yield and quality of wheat.

For this reason we used a computer system based on a method of multi-variables which can:

– take many variables into account

– weigh climatic parameters according to their effect on the yield or other characteristic of the plant

– show the complex effect of climatic factors on quantity and quality.

Materials and methods

We analysed 5 climate characteristics, including 54 climatic variables and yield, gluten quantity (%), gluten extension (mm), and farinograph number of value, according to Tables 1 and 2.

On studying the climatic conditions between 1977 and 1984, the data of 33–129 meteorological measuring sites have been analysed.

We produced the climate basic-data matrix by forming 54 base-maps with the help of the seven-year records from the meteorological measuring sites. On this map we put a net-map including 287 lattice points and determined all the values of the climatic variables of these points. Finally we established a 287×54 climate basic-data matrix.

We got the yield data from the cultivar-experiments of NÖMI. We decided to take these data because the experimental sites represent the whole territory of the country and, because of the central directions, the circumstances of the setting were similar, so the ecological effects can be exactly analysed.

We collected the yields and quality parameters of 248 sites, between 1970 and 1984. In all sites we measured the annual yield of approximately 15–20 cultivars, thus acquiring a data of yields over 15 years. With these data we determined the annual progress of the yield. The average of yields – taking all sites and cultivars into account – can be characterized with $y = 3.76+0.0998\times$linear trend. The linear correlation between time and yield is at P=5% probability level significant, and is of medium strength ($r=0.5725$, $r_{5\%} = 0.4973$). We determined the progress of the yield on the basis of the trend. That was necessary because the progress is a consequence of technical and biological advance, so if we want to characterize the sites with the yields they must be cleared of these effects. Than the differences between the yield can be well explained with the different ecological characteristics.

After the trend-calculation, we standardized the yield data to the 1984 level, so we added the value of the annual progress to the yield as many times as the years before 1984 from which the data originated.

Then we attached the sites to the 287 lattice points, each site to the nearest point, and from the standardized yields we determined the yield and deviation of wheat. For further calculations we used this yield vector with 287 elements.

We determined quality parameter values for each lattice point in the same way. We used a multi-method system, tested on maize-growing (Ángyán et al., 1984). Our starting theory was that climatic parameters have no effect on the yield and quality in themselves, but in correlation with each other. (Extreme weather conditions, of course, can be decisive in themselves about the production.)

Next, we examined the groups of these variables and their effect on the yield. We made principal-component analyses on the 287×54 climate data matrix and determined the values of the principal components on each lattice point. We determined the direction and strength of the connection between the yield and quality by the binary linear correlation method. (In the case of wheat, among Hungarian climatic conditions this correlation is linear.)

Table 1

Statistical characteristics of yield and quality parameter averages by lattice point

Statistical characteristics (1)	Aspects of specification (2)			
	Yield, t/ha (3)	Gluten quantity, % (4)	Gluten extension, min (5)	Number of value of farinograph quality (6)
Arithmetical mean value (y)	4.71	30.52	4.44	66.38
Minimum (y_{min})	3.36	24.10	3.00	50.38
Maximum (y_{max})	6.80	36.50	7.00	76.50
Corrected deviation (s_y)	0.50	2.01	0.52	4.20
Corrected relative deviation (s_y%)	10.60	6.48	11.64	6.42
Class frequency (%)				
≤ 3.99	7.67			
4.00–4.49	34.40			
4.50–4.99	38.68			
≥ 5.00	19.16			
Class frequency (%)				
≤ 25		1.11		
26–30		50.19		
≥ 31		48.71		
Class frequency (%)				
≤ 4.0			16.24	
4.1–4.5			43.16	
4.6–5.0			30.34	
≥ 5.1			10.01	
Class frequency (%)				
≤ 60				13.25
61–65				36.32
66–70				46.58
≥ 71				3.85

The evaluation of principal components from the standpoint of plant production was made by multiplication of the climate factor values with the correlational co-efficient calculated according to specific points of view (yield, quality) and then summarized at each lattice point:

$$KL_{is} = \sum_{j=1}^{g} C_{ij} \times rjs$$

KL_{is} = climatic number of value counted on "I" lattice point according to "S" characteristic
C_{ij} = value of "j" principal component on "i" lattice point
r_{js} = binary, linear correlation coefficient between the values of: "j" principal comonent and "s" factor
I = number of lattice point
j = number of principal components
s = number of factors (quantity, quality) and cultivars
q = number of principal components with value higher than 1

Table 2

Surveyed climate characteristics of Hungary (1977–1984) (m=54, n=287)

Serial number (1)	Climate variables (2)	Months (3)	Average (4)	Dispersion Sx (5)
	Insolation (hour) (6)			
1		X	147.5	10.6
2		XI	04.3	8.0
3		XII	44.2	4.7
4		I	50.8	5.0
5		II	82.0	4.5
6		III	142.5	6.6
7		IV	174.0	7.3
8		V	228.5	8.2
9		VI	242.0	13.4
10		VII	253.2	16.3
11		X–VII	1430.4	68.0
	Temperature (°C) (7)			
12		X	10.6	0.6
13		XI	3.6	0.5
14		XII	0.7	0.6
15		I	–1.7	0.8
16		II	0.2	0.5
17		III	6.2	0.6
18		IV	9.7	0.5
19		V	15.3	0.6
20		VI	18.8	0.6
21		VII	19.5	0.6
22		X–XI	7.2	0.5
23		XII–II	–0.3	0.6
24		III–V	10.4	0.5
25		VI–VII	19.2	0.5
26		X–VII	8.3	0.5
	Number of frosty days (8)			
27		X	2.5	1.1
28		XI	12.8	1.6
29		XII	19.6	1.6
30		I	25.8	1.3
31		II	21.5	1.3
32		III	9.8	1.9
33		X–III	92.0	7.9
	Number of heat days (9)			
34		V	1.4	0.6
35		VI	3.4	1.3
36		VII	4.5	1.4
37		V–VII	9.3	3.1
	Precipitation (mm) (10)			
38		VIII	50.5	12.1
39		IX	39.2	7.8
40		X	34.2	9.1

Table 2 (cont'd)

Serial number (1)	Climate variables (2)	Months (3)	Average (4)	Dispercion Sx (5)
41		XI	51.3	9.8
42		XII	48.1	9.7
43		I	38.4	7.9
44		II	28.4	7.1
45		III	33.4	5.1
46		V	59.7	7.7
47		VI	80.5	13.4
48		VII	64.1	16.7
49		VIII–XI	175.2	30.4
50		XII–II	114.9	21.5
51		III–V	131.8	15.1
52		VI–VII	144.5	25.6
53		VIII–VII	566.6	77.0

Conclusions

According to the data of principal component analysis the variance of the 54 variables is contained mainly in the first six principal components (90%).

Putting them on the map, we can differentiate zones where the summarized effect of climate variables on a certain factor (yield, quality) is approximately the same. This does not mean that the factors are the same in the whole zone but their effect on the yield and quality of wheat is almost equal. These are zones where, according to the climate, we get the same yield of wheat.

Table 3

Class frequency of climate-principal component values (%)

Value (1)	Principal components (2) I	II	III	IV	V	VI
≤ (–7.0)	1.9	1.7	–	–	–	–
(–7.0)–(–5.1)	6.8	6.9	1.0	–	–	–
(–5.0)–(–3.1)	9.9	9.9	7.7	1.4	0.5	–
(–3.0)–(–1.1)	20.9	20.9	23.0	20.5	16.6	12.7
(–1.0)–(+0.9)	21.0	22.0	26.6	56.2	65.8	74.6
(+1.0)–(+2.9)	20.9	20.9	23.0	20.5	16.6	12.7
(+3.0)–(+4.9)	9.9	9.9	7.7	1.4	0.5	–
(+5.0)–(+6.9)	6.8	6.9	1.0	–	–	–
≥ (+7.0)	1.9	1.7	–	–	–	–

The different climate-number of value maps can be studied in Figs 1–5. On territories more than 300 m above the sea-level, these values are unapplicable because of significant vertical changes in the climate.

Table 4

Relations of principal components values (r) with characteristics examined

Principal components (1)	Aspects of specification (2)			
	Yield, t/ha (3)	Gluten quantity, % (4)	Gluten extension, mm (5)	Number of value of farinograph quality (6)
I	0.2666*	–0.1353	–0.0046	–0.0783
II	0.0240	–0.6688*	–0.3647*	–0.6089*
III	0.6099*	0.1643*	–0.2157*	0.0248
IV	0.0388	0.1121	–0.0976	–0.2117*
V	–0.0980	0.0756	0.1567	0.0876
VI	–0.3609*	0.2611*	0.3512*	0.2717

Note: (7): $r_{0.5\%} = 0.1638*$

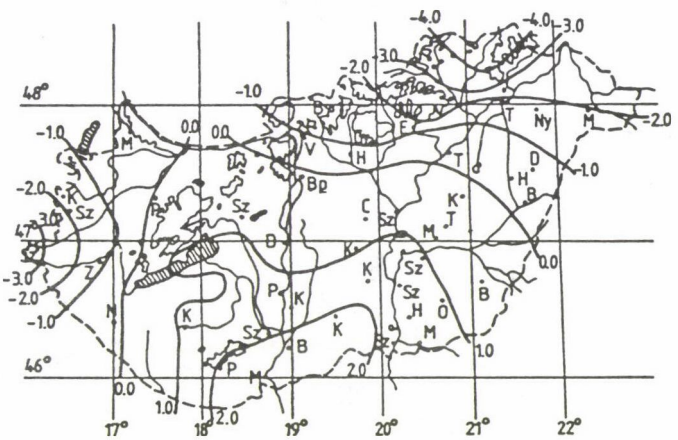

Fig. 1. Climate-number of value of wheat growing calculated on strength of yield in Hungary

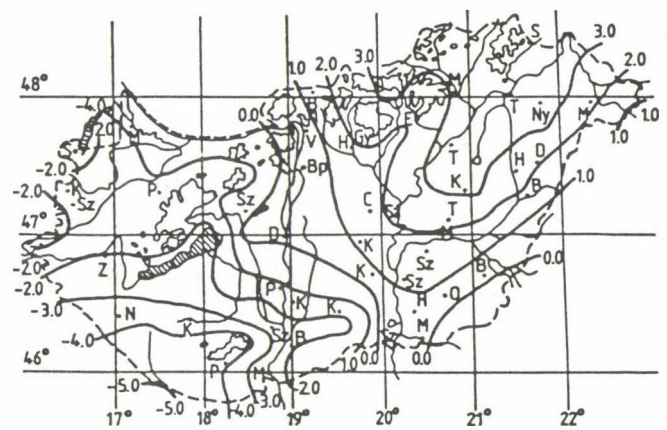

Fig. 2. Climate-number of value calculated on strength of gluten quantity (%)

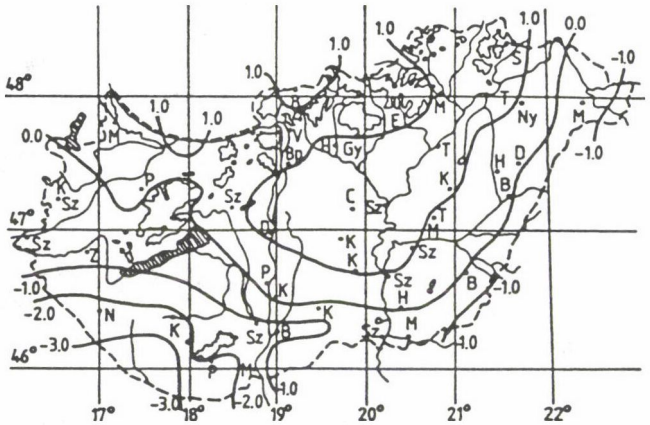

Fig. 3. Climate-number of value calculated on strength of gluten extension (mm)

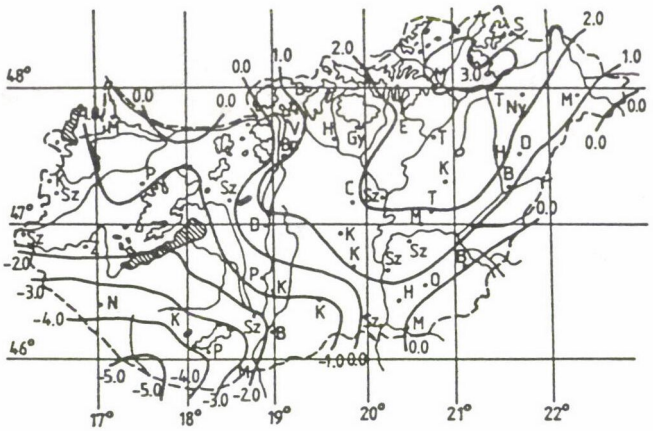

Fig. 4. Climate-number of value calculated on strength of farinograph number of value

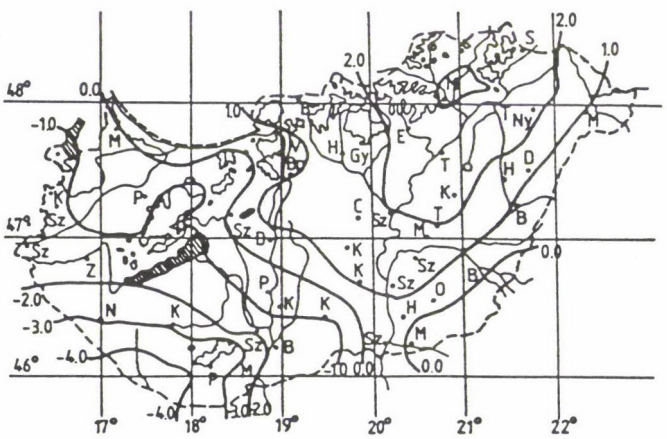

Fig. 5. Climate-number of value calculated on strength of three quality parameters

The decrease of the values on the maps refers to the lessening of climate parameters.

Where climate-number of values are negative, the areas are unfavourable for great yields and high quality of wheat. For example, quantity wheat production is possible on the right side of the Danube (Dunántúl) but quality growing is concentrated on the Great Plain. The proportions of the climate zones can be seen in Table 5.

Table 5

Categories of given conditions and their areas in % of total area

	Area (%) by aspect (2)				
Category of given condition	Number of value of climate	Yield, t/ha	Gluten quantity, %	Gluten extension, mm	Number of value of farinograph quality
(1)	(3)	(4)	(5)	(6)	(7)
Good (8)	> +1.0	20.2	40.4	26.5	42.9
Moderate (9)	−1.0+1.0	47.7	22.0	55.7	28.9
Poor (10)	< −1.0	32.1	37.6	17.8	28.2

If we summarize the quantity and quality numbers of values at each lattice point, we get the map that can be seen in Fig. 6. If we take both quantity and quality requirements, the climate conditions of the country are 14.6% good, 71.5% medium and 13.9% weak for wheat growing.

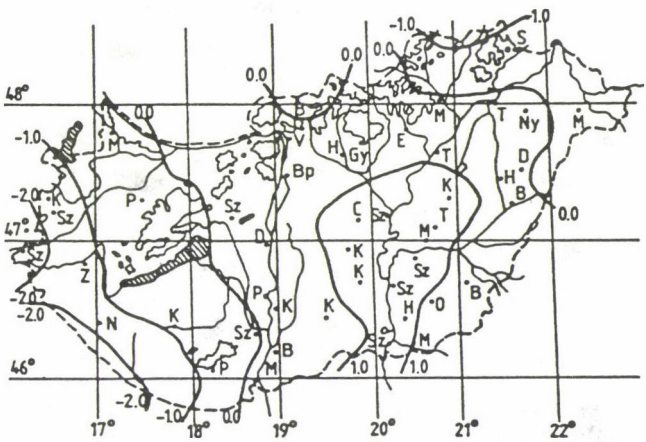

Fig. 6. Climate-number of value calculated on strength of yield and three quality parameters in Hungary

The best areas, with their values above 1.0, are in the Middle and Lower Tisza-Region, and the worst conditions occur within the territories southwest from the Sopron–Kaposvár–Siklós line and the Northern-Borsod region.

Climate-numbers of value can only be used for characterizing climatic conditions if there is a significant correlation between them and the yield and quality. Accordingly, we examined the direction and strength of this correlation.

Table 6

Binary linear correlation between weighted climate-numbers of value C'K and characteritics of wheat (n = 287, k = 1... n)

Statistical characteristics (1)	Their values by aspect (2)			
	Yield, t/ha (3)	Gluten quantity, % (4)	Gluten extension, mm (5)	Number of value of farinograph quality (6)
a	4.73	30.28	4.44	65.37
b	0.10	0.55	0.17	1.32
r	0.5100	0.6942	0.4230	0.6616
$r_{0.1\%}$	0.1638	0.1638	0.1638	0.1638

The connection is at least at medium strength between the climate numbers of value ($0.4230 < r < 0.6942$) and at P=0.1% probability level significant, so this can be well characterized with the climate numbers of value.

One unit of changing of the value means 100 kg/ha in the yield, 0.55% gluten quantity, 0.17 mm of gluten extension and 1.32 farinograph number of value changing. By these data and the differences of numbers of value on the maps 800 kg/ha yield, 5% gluten quantity, 0.9 mm gluten extension and 12 farinographic number of value difference can be explained with the different climatic conditions in Hungary. Thus, climatic conditions influence the possible quantity and quality of the yield significantly.

Our research can prove that the method we used is good for the numerization of the climatic effects.

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UTILIZATION OF NUTRIENTS BY PIGEONPEA (*CAJANUS CAJAN* L.) UNDER DIFFERENT WEED MANAGEMENT SYSTEMS

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An investigation was carried out at Agricultural College and Research Institute, Coimbatore to study the influence of manual (hand hoeing), chemical (fluchloralin, pendimethalin and oxadiazon) and biological (intercropping with sorghum and moong) methods alone and in combination with one hoeing on the utilization of nutrients by pigeonpea, using CO 4 as the test variety. The results clearly indicated that the highest N, P and K uptake were recorded under treatment receiving pendimethalin at 0.5 kg a. i. ha^{-1} along with one hand hoeing at 40 DAS, due to the least uptake of nutrients by weeds, as it efficiently suppressed the weed growth.

Keywords: *Cajanus cajan*, weed management practices, uptake of nutrients by weeds, crop, grain yield

Introduction

Nutrient uptake in crops depends largely on the growth and development of plants. Therefore, the total biological yield produced determines to a large extent the quantum of nutrient uptake. On an average, 115 kg N, 16 kg P and 53.3 kg K were removed by pigeonpea yielding 2.0 tonnes of grain and 5.4 tonnes stalks ha^{-1} on the sandy loam soil of the IARI, New Delhi (Singh, 1973). However, the utilization of nutrients by pigeonpea is affected by the competitive factor, especially of weeds with the crop. Hence, the present investigation was initiated to study the differential utilization pattern of nutrients by pigeonpea under various integrated weed management practices.

Materials and methods

Field experiments were conducted to study the influence of various weed management practices on the utilization of nutrients by pigeonpea in a clay loam soil of the Agricultural College and Research Institute, Coimbatore, during kharif and rabi seasons. The experimental soil has a pH of 7.8, with low available N (105 kg ha^{-1}) and P (7.8 kg ha^{-1}) and high available K (521 kg ha^{-1}).

Treatments consisted of three weed control methods; namely, manual (hand hoeing twice at 20 and 40 DAS), chemical (fluchloralin, pendimethalin and oxadiazon each at 0.50 and 0.75 kg a. i. ha^{-1}) supplemented with one hand weeding and biological (growing sorghum and moong with hand hoeing once), along with unweeded check (control) accounting for twelve treatments replicated thrice in randomized block design.

Pigeonpea CO 4 was grown as a test crop, adopting a spacing of 45×30 cm in ridges and furrows. The herbicides tried were pre-emergence and were sprayed as pre-treatment on the third day of sowing, using a high volume sprayer. Under the intercropping treatments, one row of moong or sorghum was grown in the intra-row spacing.

During the course of investigation, weed samples at 60 DAS pigeonpea samples at harvest were collected and estimated for nitrogen, phosphorus and potassium contents, using the procedure designed by Jackson (1973). The uptake of nutrients was computed by multiplying concentration with dry matter production.

Results and discussion

Uptake of nutrients by weeds

The data on uptake of nitrogen, phosphorus and potassium by weeds at 60 DAS during kharif and rabi seasons are given in Table 1. The results showed that the uptake of three major nutrients was significantly reduced by the weed control methods; namely, manual, chemical and biological alone, or in combinations over unweeded check (T_1). Lowest uptakes of nutrients were recorded under treatments receiving hand weeding twice; however, it was on par with the pre-emergence herbicidal spray with pendimethalin or oxadiazon 0.5 kg a. i. ha^{-1}, supplemented with hand weeding at 40 DAS. Chemical weeding requires less man days and proved to be equally effective as hand weeding twice (T_2). This might be attributed to the efficient killing of weeds by pre-emergence herbicides at early vegetative stage of the crop, and the surviving weed population would have been removed by late wedding, which resulted in least drymatter production. All three major nutrients, N, P and K, showed a similar trend in both kharif and rabi seasons. The findings confirm the findings of Ponnusamy (1982) who reported the maximum nutrient uptake under unweeded check and reduced significantly under chemical treatment supplemented with hand weeding.

Next to chemical weeding, growing the intercrops of moong or sorghum significantly, suppressed the weed growth over the unweeded check (T_1) due to a smothering effect, as reported by Shetty and Rao (1977).

Table 1

Influence of weed management practices on nutrient uptake at 60 DAS by weeds (kg ha^{-1})

	Treatments	Nitrogen		Phosphorus		Potassium	
		Kharif	Rabi	Kharif	Rabi	Kharif	Rabi
T_1	Unweeded check	49.0	45.7	11.8	11.2	48.3	40.1
T_2	Hand hoeing twice, 20 and 40 DAS	1.2	2.2	1.8	1.0	5.3	5.3
T_3	Pre-emergence fluchloralin at 0.75 kg a. i. ha^{-1}	14.2	19.7	8.3	3.3	9.2	8.8
T_4	Pre-emergence pendimethalin at 0.75 kg a. i. ha^{-1}	12.0	17.0	8.1	9.0	6.6	8.8
T_5	Pre-emergence oxadiazon at 0.75 kg a. i. ha^{-1}	16.6	21.6	8.7	9.9	12.8	10.9
T_6	Pre-emergence fluchloralin at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	2.5	3.1	2.0	2.3	2.8	2.9
T_7	Pre-emergence pendimethalin at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	1.8	2.7	1.8	1.7	2.7	2.5
T_8	Pre-emergence oxadiazon at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	2.2	2.9	1.7	1.8	2.8	2.1
T_9	Pigeonpea+Sorghum as intercrop	38.3	34.9	7.8	8.5	12.5	10.2
T_{10}	Pigeonpea+Moong as intercrop	43.6	36.5	8.4	8.2	10.3	9.9
T_{11}	Pigeonpea+Sorghum as intercrops+ one hoeing 20 DAS	9.3	13.3	8.5	7.4	11.4	5.8
T_{12}	Pigeonpea+Moong as intercrop+ one hoeing 20 DAS	10.7	16.3	7.8	6.8	10.1	4.6
L. S. D. (P = 0.05)		2.5	3.9	1.5	1.2	3.9	1.8

Utilization of nutrients by pigeonpea

The differential utilization of nutrients by pigeonpea at harvest during kharif and rabi seasons is given in Table 2. The results revealed that the uptake of nutrients had been favourably influenced by weed control methods either alone or in combination over the unweeded check (T_1). The highest uptake of nitrogen, phosphorus and potassium were registered under treatments receiving pre-emergence

Table 2

Influence of weed management practies on nutrient uptake at harvest by pigeonpea (kg ha^{-1})

	Treatments	Nitrogen		Phosphorus		Potassium		Grain yield kg ha^{-1}	
		Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi
T_1	Unweeded check	118	108	5.21	4.42	101	92	377	301
T_2	Hand hoeing twice, 20 and 40 DAS	159	158	9.00	7.24	148	131	1300	1235
T_3	Pre-emergence fluchloralin at 0.75 kg a. i. ha^{-1}	158	142	8.31	6.40	132	127	1135	1035
T_4	Pre-emergence pedimethalin at 0.75 kg a. i. ha^{-1}	154	146	7.92	6.46	124	118	1185	1100
T_5	Pre-emergence oxadiazon at 0.75 kg a. i. ha^{-1}	153	140	8.10	6.73	119	120	1100	1008
T_6	Pre-emergence fluchloralin at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	169	160	9.83	7.98	151	140	1495	1325
T_7	Pre-emergence pendimethalin at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	173	166	9.58	7.39	156	136	1560	1415
T_8	Pre-emergence oxadiazon at 0.50 kg a. i. ha^{-1}+ one hoeing 40 DAS	179	160	9.01	8.68	154	137	1405	1385
T_9	Pigeonpea+Sorghum as intercrop	158	149	8.46	6.49	118	114	570+ 1375	500+ 1055
T_{10}	Pigeonpea+Moong as intercrop	160	144	8.68	6.90	112	107	780+ 300	703+ 280
T_{11}	Pigeonpea+Sorghum as intercrops+ one hoeing 20 DAS	165	149	8.77	6.78	120	116	825+ 1545	700+ 1560
T_{12}	Pigeonpea+Moong as intercrop+ one hoeing 20 DAS	165	152	8.89	7.00	128	125	1180+ 530	945+ 470
L. S. D. (P = 0.05)		8.79	7.11	1.68	1.40	9.02	13.4	253.3	133.6

herbicide pendimethalin or oxadiazon 0.5 kg a. i. ha^{-1}, supplemented with hand weeding at 40 DAS (T_7 and T_8). It is obvious that the application of pre-emergence herbicides would have efficiently killed the weeds at an early vegetative stage and

surviving weeds would have been cleared through late manual weeding at 40 DAS, resulting in a suppressed weed population and creating more availability of nutrients for crop uptake; Metha and Khatri (1962) and Ahlawat (1980). As explained earlier, weeds are competent in utilizing the nutrients available in the soil and a weed-free environment leads to higher uptake of nutrients over the control (T_1). All three nutrient uptakes showed the similar trend in both seasons. Intercropping of either sorghum or moong decreased the uptake of the base of pigeonpea due to its competitiveness for nutrients.

Grain yield

The data on grain yield (Table 2) clearly indicated that weed control methods alone or in combination significantly increased the grain yield, registering the highest value under treatment receiving either pendimethalin or fluchloralin or oxadiazon 0.5 kg a. i. ha^{-1} herbicides supplemented with one hand weeding. However, it was on par with hand weeding twice (T_2). As explained elsewhere in the text, herbicide application significantly reduced the weed population and its dry matter, which was markedly reflected in the uptake of nutrients by weeds. Under such condition, crops take advantage of utilizing more nutrients available in the rhizophere that otherwise would have been utilized by weeds and this leads to higher grain yields. The results are in accordance with the findings of Shetty and Rao (1977).

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Plant genetics and breeding

STUDIES ON GENIC MALE STERILITY AND ITS USE IN EXPLOITATION OF HETEROSIS IN *BRASSICA CAMPESTRIS* L.

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Few spontaneous male sterile plants (GMS) were detected in a local collection of yellow *sarson* (*Brassica campestris* var. yellow *sarson* Prain). GMS plants were similar in morphological features to their fertile counterparts, except for some distinctiveness in floral parts. Compared to their fertile sibs, GMS plants had reduced sepals and petals, longer gynoecium, stamens with small, narrow, pointed and empty anthers on shorter filaments. The inheritance of male sterility was observed monogenic recessive. One double-purpose line (YSMS 8163), which ensures the supply of male sterile and fertile plants in 1 : 1 ratio, has been developed. Four single-cross hybrids were produced *in situ* using this double-purpose line. Practical application potential of this GMS in the exploitation of intervarietal heterosis is assessed.

Keywords: *Brassica campestris*, yellow *sarson*, male sterility, heterosis

Introduction

The manifestation of high amount of economic heterosis, adequate inter-transfer of pollen grains and an efficient method of pollination control are essential for commercial exploitation of heterosis in any crop. Considerable amounts of heterosis for seed yield and its components have been reported both within and between *Brassica campestris* vars. toria (TR), brown *sarson* (BS) and yellow *sarson* (YS) (Das and Rai, 1972; Patnaik and Murty, 1978; Verma et al., 1989). Genetic male sterility without genetic markers has also been reported in this crop (Chowdhury and Das, 1966; Das and Pandey, 1971; Tyagi and Verma, 1985; Chauhan and Kumar, 1986) but no concerted efforts for its commercial exploitation have hitherto been made. Earlier, the use of genetic male sterility for developing hybrids has been demonstrated as practically feasible in *Brassica napus* (Ling and Yan, 1983). The present paper deals with the study of a spontaneous male sterility identified in a local collection of yellow *sarson* and its possible use for commercial production of hybrids.

Materials and methods

Few male sterile plants were detected in a yellow *sarson* (*B. campestris* var. yellow *sarson* Prain) line collected from the Deoria District of eastern Uttar Pradesh. Observations on relative variation in floral parts of male sterile and fertile sibs were made on a random sample of 40 mature buds and freshly opened flowers. To study the inheritance of male sterility, F1, F2 and backcross generations were developed using YS 8163 and YS 8126, as male parents. The practical feasibility for use of male sterility was studied by developing a "double-purpose" line (Ling and Yan, 1983) and using it to produce experimental hybrids *in situ*.

Results and discussion

Morphology

Male sterile plants detected in the population of YS 8163 are not conspicuously distinct in morphological features from fertile counterparts, except for some variation in floral parts (Table 1). Sepals and petals were comparatively smaller in male sterile plants than in fertile sibs. Anthers of male sterile individuals were shorter, thin, pointed at the distal end and completely devoid of pollen grains. The gynoecium length was relatively more in mature buds as well as in fresh flowers of male sterile individuals than in their fertile sibs.

Table 1

Relative variation in the floral parts of male sterile (MS) and fertile (MF) sibs (mm)

Floral part	Mature buds		Flower	
	MS	MF	MS	MF
Ovary	3.83	3.90	5.12	4.55
Gynoecium	6.00	5.73	8.75	7.95
Stamens	3.42	4.36	5.95	9.07
Filament	1.57	1.81	4.05	6.50
Anther	1.85	2.55	1.90	2.57
Petal length	5.92	5.95	10.64	12.64
Petal width	3.21	3.45	5.58	6.09
Sepal length	6.67	6.25	7.19	7.32
Sepal width	2.00	2.17	2.10	2.25

Inheritance of male sterility

The segregation pattern of male sterility in different generations of two crosses is presented in Table 2. All the F1 plants were fertile, indicating thereby the dominance of fertility over sterility. The ratio of male sterile and fertile plants in F2 and BC 1 generations revealed that male sterility is governed by a single recessive gene. A monogenic recessive inheritance of male sterility has been reported earlier in *B. campestris* vars. yellow *sarson* (Chowdhury and Das, 1966), brown *sarson* (Das and Pandey, 1971) as well as in toria (Zuberi and Zuberi, 1983; Tyagi and Verma, 1985).

MAGYAR
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Table 2

Segregation pattern of male sterile and male fertile plants in different generations of two crosses

Cross/Generation	No. of plants		Ratio	χ^2	P
	Male fertile	Male sterile			
YSMS 8163×YS 8163					
F_1	91	–	1 : 0	No segregation	
F_2	326	103	3 : 1	0.23	0.70–0.50
BC_1	105	94	1 : 1	0.61	0.50–0.30
BC_2	118	–	1 : 0	No segregation	–
YSMS 8163×YS 8126					
F_1	79	–	1 : 0	No segregation	–
F_2	160	66	3 : 1	2.13	0.20–0.10
BC_1	76	68	1 : 1	0.44	0.70–0.50
BC_2	126	–	1 : 0	No segregation	–

Development and utilization of double purpose line

The YS 8163 is an agronomically acceptable strain with tetralocular siliquae. The male sterile (GMS) plants, under adequate pollen supply, show a good siliquae set with about 40 seeds per siliqua and are quite comparable to their fertile sibs in their yielding ability.

The sib line which ensures the availability of GMS and fertile plants to serve both as maintainer and GMS source has been suitably termed by Ling and Yan (1983) as a "double-purpose" line. One such a double-purpose line with tetralocular, upright bearing siliquae (YSMS 8163) has been developed through continued selection for GMS plants after four cycles of selection. This line segregates for male sterility and fertility approximately in the ratio of 1 : 1. Efforts are under way to identify a genetic marker closely linked with male sterility or fertility, to facilitate the selective elimination of fertile sibs before flowering, preferably at the seedling stage.

Even in the absence of a genetic marker, attempts to utilize this GMS for producing hybrids seems plausible. Such an attempt stems from the fact that the manifestation of heterosis ranging from 25.0–113.0% in TR (Das and Rai, 1972), 8.80–42.50% in BS (Patnaik and Murty, 1978), 2.28–23.69% in YS (Verma et al., 1989) and the still higher level of heterotic effects realised in their intervarietal crosses (Amirthadevarathinam et al., 1976) remain hitherto unexploited. The use of this GMS system will enable the commercial exploitation of heterosis occurring in such wide crosses as YS × TR and YS × BS.

Four experimental hybrids were successfully produced during the winter season of 1990–91 by sowing double purpose line and promising fertile lines in 2 : 1 ratio. Three hybrids were comprised of YS× YS and one YS× BS combination. In these combinations, the siliquae set was quite satisfactory. However, the seed set

in the siliquae formed on GMS plants was poor, ranging from 31.97 (YS × YS) to 33.47 (YS × BS) per siliqua as against 40 seeds per siliqua obtained on GMS plants in the maintenance block. This may be due to inadequate pollen supply and poor synchronization in the flowering of GMS and restorer plants. Therefore, it is imperative that (i) pollinators be planted on two dates, so as to extend the period of flowering to match the usual extended flowering span of GMS plants, and (ii) more emphasis be laid on YS × TR and YS × BS combinations. In addition, the female/male ratio in such a combination also needs to be standardized.

From the foregoing, evidently it should be possible to develop more productive hybrids in *B. campestris* L. This system would, however, exploit only about 80 to 85% of the exploitable heterosis. This is due to the fact that 15–20% of the total seed produced on GMS plants is the result of sibbing. The roguing of fertile sibs from the double-purpose line, as of present, is possible only after the start of flowering. An increased yield of hybrids could be expected to commensurate the additional cost required for hybrid seed production, using this system.

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Agricultural economics

THEORETICAL AND METHODOLOGICAL CONSIDERATIONS IN DEVELOPING THE VERTICAL RELATIONS IN HORTICULTURE AND FOOD INDUSTRY

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Considering future trends in the Hungarian agrofood sector, the relations between subsystems of the vertical food chain increasingly became a crucial factor. In the first phase of the OTKA (National Scientific Research Fund) research program, the questions of vertical integration were studied in various industries for the transition to a market economy to achieve a more efficient market performance, the effects of the world market should be continuously considered throughout the whole food chain, up to the primary producers. New approaches and methods are recommended to study and solve the economic and marketing problems of the horticultural and food industries

Keywords: vertical relations, product channel, institution system, agricultural policy, agricultural market rules, intervention funds, product councils, diversification, EEC regulations

Object and method of research

(1) In the first phase of the research program financed by the National Scientific Research Fund, we studied where and why the vertical integration would be effective in the different industries inside or outside the producing unit, in the framework of the various product marketing-, processing-, trading sectors as independent enterprises the vertical integration would be effective; in which industry it came into existence as a consequence of economic necessities or pressures: which were those industries where all phases of the channel or products could be shaped relatively freely.

Accordingly, the following industries and related vertical systems formed the subject of research:

– The apple industry under the conditions of the major forms of state farm, cooperative and small enterprise.

– Relations of red pepper and dried vegetables in the framework of the most characteristic production cooperations, corporative forms and of the "traditional" integrations of production plant.

– The fundamental questions of integration built on field crop production were represented in the study of the vegetable oil industry and, in respect to sugar beet, in the study of the sugar industry.

Extensive work started in the mentioned horticultural and field crop production, likewise regarding the analysis of the infrastructural situation characterizing agriculture, as well as the forms and solutions of the fundamental phases of vertical integration appearing independently of, and built on, each other.

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Regarding the method of research, we carried out correlation and regression analyses; in some cases (e.g. sugar industry) we also employed the computer processing method of discrimination analysis.

The study of the forms of vertical integration is primarily based on surveying enterprisal data, processing statistical data, and on exchanges of opinion with the leading experts of the firms concerned.

The present paper contains the examination of some theoretical and methodological questions.

(2) We regarded it as an essential viewpoint that the industries examined represent all the important and known momentums and forms of the product channel, reflect the basic functions of food economy, its close correlation with the supply of population and the market conditions, as well as the export relations and their effects on the vertical relations of the products concerned.

The social property- and enterprisal changes involved with the transformation of the Hungarian food economy deeply affect the industries concerned. Among others, because the collapse of the hitherto determinative "eastern" market has highlighted the structural questions of production, the emphasis made earlier on quantity has shifted to quality. Furthermore, the vertical system of industrial processing is overconcentrated in Hungary, the horticultural commercial network is underdeveloped, the infrastructure is deficient, mainly in the field of transport, road and information, the cool-stores are out-of-date, the production resources are scare, the flow of information is one-sided, and of registration character, and finally the institutional system is mostly underdeveloped.

All this became obvious at a point when the inner social processes were unable to adjust the economic and organizational structures to the most developed capitalist economic integration, or bring about some kind of synchronization.

(3) We considered the fact that, in the European Community, the institution system of the agricultural policy is successful in relation to all the participants of the vertical system in those countries where the safety of the producers was based on the balance of domestic production and import (e.g. Germany, England).

At the same time, the situation of countries with a structure similar to that of Hungary, producing considerable surpluses, is less stable and perceptibly uncertain, the support and income situation of the producers apparently insecure (e.g. Spain, Portugal, Greece etc.).

These South European countries, developing from an agricultural structure partly similar to that of Hungary, are getting closer to the Integrated Common Market. In order to achieve this:

– They introduce agricultural market rules, build up vertical and horizontal institution systems reconciling and safeguarding interests.

– They support selling-, purchasing-, processing- and packing cooperatives.

– They establish a network of up-to-date wholesale markets mainly of mixed property.

– They try to establish a relatively stable price policy by ensuring intervention funds.

– All this is carried out by the considerable contribution and support of the Common Market.

(4) In Hungary, too, there is a danger and risk of overproduction that may cause serious problems, so here also, the INTERVENTION FUND is one of the most important market-influencing means of the government. This can be ensured partly from central resources, then gradually from the contributions of the participants of the horticultural, agricultural and other industries, through their self-regulation. Namely, together with other measures of helping the balance, the intervention funds may efficiently influence the oversupply, the trend of prices, occasional restriction (development) of production, or necessary government purchases.

The development of an institutional background is also indispensable. Within this we should like to determine:

– how the equal participation of the representatives of producers, processors, traders and consumers in the STATUTORY PRODUCT COUNCILS can be ensured,

– in what system of interest can the independent participants of a vertical chain be brought to take a risk and distribute the income reasonably,

– whether such models of the information system which connect the increasing number of small producers and the remaining large farms with various members of the product channel, with the institutions of the agricultural marketing order and the traditional bases of information, respectively, can be outlined,

– whether the efficient Hungarian integrated information system and its joining to that of the common market, can be ensured.

Theoretical approaches, methods, suggestions

(1) The structure and working of the vertical chain of horticulture and food industry must be interpreted as a wholistic system. To regard it in this way or to try to do so becomes a fundamental requirement. Different organizational systems correspond to vertical integration and definitely to cooperation. Thus, horticulture may represent:

– organizationally totally independent vertical systems of each member of the product channel, which through a simple connection of products (by contract or market actions) build on each other,

– the integration of vertical systems in a single organization, private or common property,

– the horticultural (and other similar) products can be qualified "free-channel" (not identical with the term "multichannel") products capable of integration without compulsion. This explains their diversity in terms of undertakings, market and product, intellectual and material infrastructural demand and information demand,

– the complexity of the horticultural product channel is clearly seen in Fig. 1.

This indicates that at each point of connection a new situation of decision appears:

– where and in what forms we can sell our products,

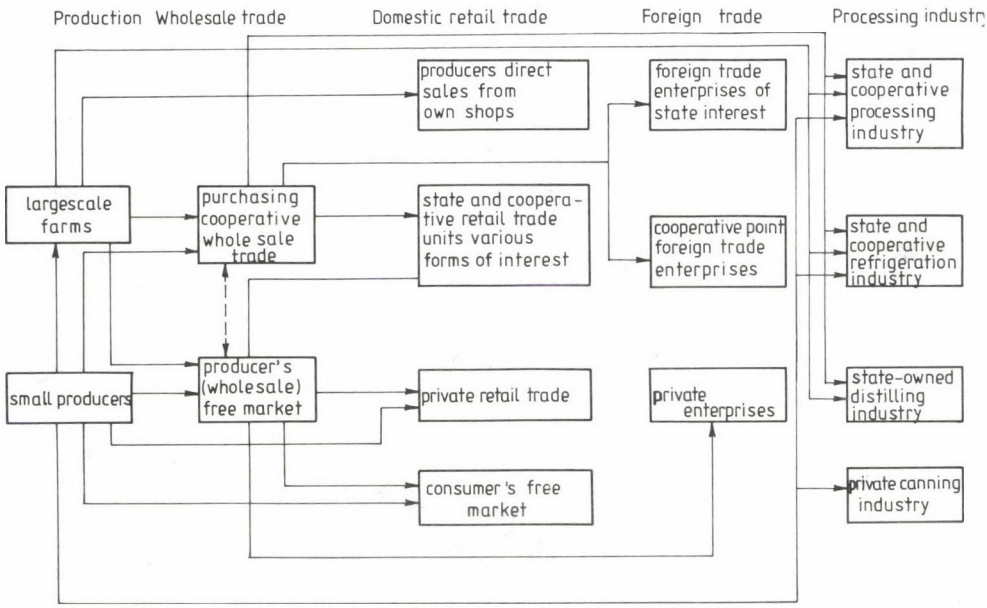

Fig. 1. Vegetable- and fruit product channels (according to Éva Borszéki and Mrs. Z. Szűcs, 1991)

– the most thoroughly possible information must be obtained about the conditions of the domestic market,

– use must be made of occasionally more favourable possibilities of the foreign market, etc.

This correlation system makes it a fundamental task for all participants of the horticultural vertical chains to elaborate a comprehensive market strategy as for "how to go on".

(2) The transformation to a market economy continually raises the necessity of reconsidering the "integrative" and "diversifying" organization of the product channel, and the concepts linked up with it in the processing sector of the food industry as well.

Under the "semi-market" conditions of the earlier economic system, the integrative and diversifying forms of appearance of the laws of economic motion were numerous but isolated initiatives of both research and actual practice.

In the system of food industry product channels, an ever-increasing emphasis has been made on marketing, or in general on the distributive (distributing, circulating) sub-systems. Renewing the concepts and economic content of integration and diversification, and laying the foundation of advancement, are also indispensable. Diversification as the opposite of integration and overspecialization may characterize the vertical chains in different measures. Its chance in the production phases is relatively small. The diversification of processing may require high surplus investments. The greatest opportunity seems to lie in widening the commercial profiles, where the circulating sector becomes independent.

In any case, in the individual vertical chains of diversification, its advantages and disadvantages are almost totally unexplored from economic, organizational and management viewpoints.

Pricing, transfer price, exchange and broker system, altogether the flow of the means of payment will be – under the concrete market conditions – critical factors of the relations both in the vertically integrated enterprise and in the sectors of the product channel having become independent.

(3) To the survey of the product channel of the vertical system of producer-oil industry the following methods can be recommended as useful:

– photographs taken of the product channel to follow up the flow and transformation of products, and of costs and returns, respectively,

– determining the organization-, direction- and supervision system of product channel management by means of the traditional and up-to-date method of programming, and logistics.

Such product channel models are needed which properly reflect:

– the outputs of the production sector, as a function of the world market prices of export–import,

– the economic estimates of variations improving the efficiency of industrial processing, the comparative analyses of domestic and foreign competitors,

– the domestic and export activities of trade (+marketing), with the demand and supply of the competing products and the world market prices taken into consideration (Fig. 2).

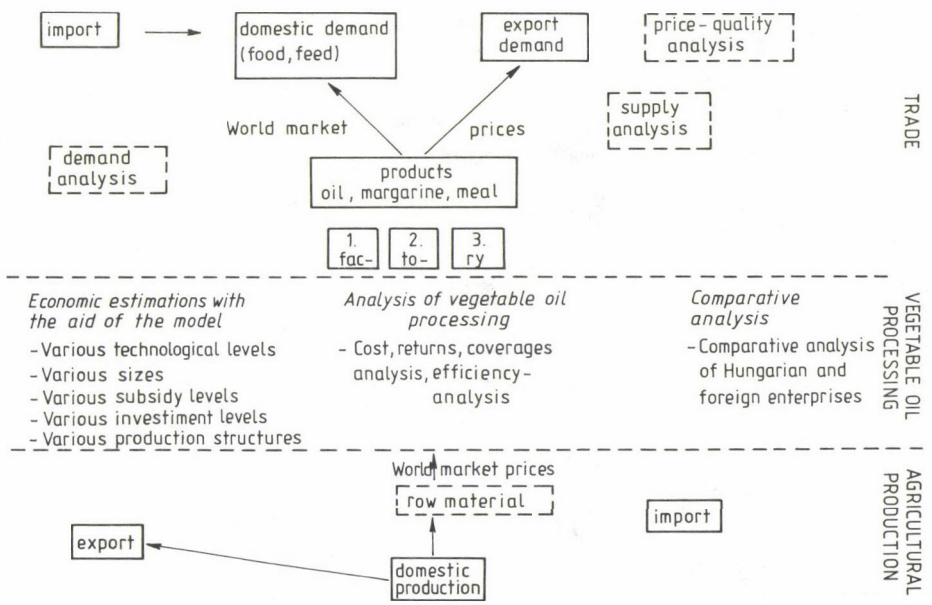

Fig. 2. Vertical economic relations in the vegetable oil industry (according to Gy. Ernyei, 1991)

These methods are suitable for studying either an economic organization that integrates the whole product channel, or management units having become independent. The potential relations and the possibilities of decision making are, naturally, best surveyed when they are broken down to the individual sectors of the product channel.

The vertical relation of vegetable oil industry reflects a peculiarly "semi-compulsion" integration. In a decisive proportion of the sunflower production, the producer's processing is not characteristic. Selling for a small degree of direct consumption, more really as crude oil export, can be an independent producer's decision. However, the participation of the multinational world company (Unilever) in the Share Holding Company certainly ensures the short transition to a market economy.

(4) The vertical relations of sugar industry provide a classical model of the "compulsion product channel".

The production of sugar-beet takes place according to contracts of the sugar factories (-industry). If there is no contract, there is no vertical relation with production.

It is also obvious in the case of forced integration that the interests of the individual vertical chains must be regulated by an "impartial" organization. This organization or institution is, in principle, the sugar market. In market economies its functioning is controlled by the quota system of the common market, backed by the "guarantees" of the countries concerned (Fig. 3).

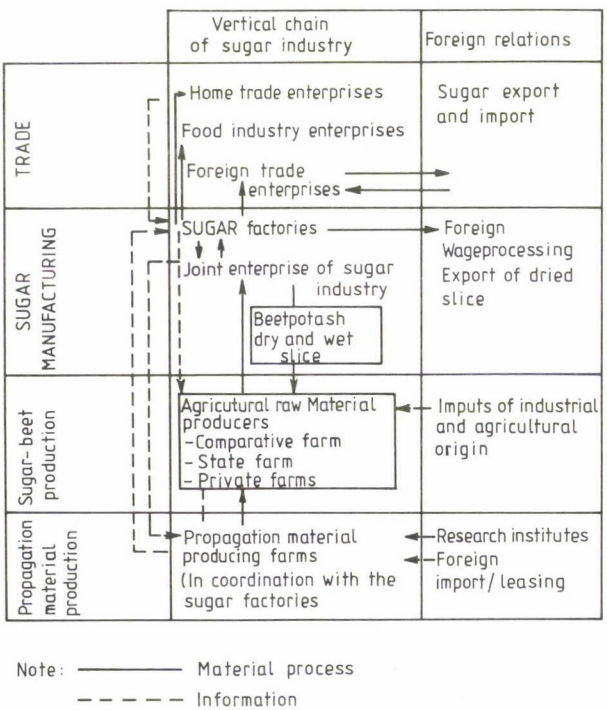

Fig. 3. **Model for the vertical chain of sugar industry (after the work of Z. Lakner 1991)** (note: —— material process, ---------- information)

The demand for sugar is determined by the requirement of population, the confectionery industry and the possibilities of export. Since sugar is a fundamental food, this demand to a certain price and income level is inelastic.

The demand of the joint industries (wine production, confectionery, distilling industry, etc.) depends on the competition of substitute products and on changes in the market demand. Thus, in the vertical relations of producer-processor, they are more of less exposed to uncertainties and fluctuations of capacity exploitation.

The export encounters difficulties owing to the stable prices that serve the protection of producers and consumers.

In Hungary – as apparently in most parts of Europe – supply can be limited exclusively by the export–import, since the capacity of the sugar factories exceeds the level of domestic demand. Quantitative increase cannot be reckoned with either in the production-, or in the processing sector. The only possibility is to improve the quality and try to harmonize the fulfilment of demands.

(5) Despite of all peculiarities and traditional technological differences, the two food industries included in the study undoubtedly show uniformity concerning the forms and possibilities of vertical relations.

Namely, the various vertical relations, the linkage of the sectors of product channel may come into existence on the basis of the necessity of "processing":

– within the plant in the form of a so-called "free integration" (fodder-, livestock keeping-, animal product processing – direct sale, etc.) almost irrespective of the size of the plant,

– through "forced integration" as e.g. sugar-beet production and sugar industry; oil crop production and the independent vegetable oil industry; the monopolized tobacco industry,

– even in the case of "free integration" each sector of the product channel may become independent, or increasingly defenceless, or fall to a "forced cooperation". This can be avoided through enterpreneurial skill and solid capital,

– according to the present state of economic progress and the tendencies of development, the forced relations become "rigid", which requires a continuous renewal of the enterpreneurial attitude and enterprisal strategies from the producer up to the consumer.

(6) Among the methods adaptable to the market relations, the elaboration of mechanisms helping and checking the economic relations of real processes (production, processing), as well as the regulation and interest systems, deserve special attention.

At the same time, further investigations are required to explore the points of impact of horizontal and vertical integration, to build an information system adapted to the individual sectors of the vertical chains, and to establish the intellectual and material infrastructure, the new institutional system and the network of up-to-date wholesale markets.

(7) Considering all this, it must also be made clear economically how the standards, and quality prescriptions of the European Economic Community will affect our products, and their control organizations, can be organically built into every sector of the product channel.

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ECONOMIC EVALUATION AND QUALIFICATION OF WINTER WHEAT VARIETIES

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The economic value of the varieties is expressed on the basis of numerous characteristics – quantity and quality of yield.

An economic model was formed to give the value of a variety in an index. The index is a comparative number of value showing the economic usefulness of given varieties. The model is based on relative production indices, input and output ratios.

In economic evaluation, the potential yield gives the highest part. Yield increase often causes problems in quality and higher costs as well. It is necessary to compare yield and quality, and their effect on input. At the time of variety tests, there are no actual cost data and price is not proportional to quality.

There is a need for a model showing the economic result on the basis of the characteristics of varieties and estimated data of the experiments. The calculation of the synthetic number of value of varieties is such a method (Tomcsányi, 1968).

Keywords: winter wheat, yield quantity, value of goods, yield, critical minimum, costs, index of variety value

Introduction

A complex economic value for the evalution of varieties was elaborated in 1955 (Tomcsányi, 1957; 1968) and called the synthetic value of varieties. Tomcsányi and Wellisch (1960) improved and adapted the method for direct human perception. Tomcsányi (1963) elaborated his method of synthetic evaluation of varieties on walnut varieties, taking into consideration the market demand and goods value.

Majoros (1967) applied this method to strawberries, Tomcsányi and Rösier (1966) evaluated almond varieties and finally Tomcsányi (1966, 1968) summarized the development of the synthetic value of varieties. Sváb (1960, 1961) reported a complex number of value for the evaluation of varieties in vegetables. He expressed in an index the different characteristics of varieties influencing yield, quality and finally their value. On this basis the method was adapted to many vegetables (cucumber, beans, green peas, tomatoes) and then flax, hemp and poppy varieties to express their value in one index (Sváb and Agócs, 1968; Bakos, 1969; Iványi and Baráth, 1968).

Materials and methods

The economic model of forming number of values for variety evaluation based on the analysis of factors influencing costs:

– plant protection and nutrient supply (disease resistance, reaction to fertilizers),

– harvest (lodging),
– transport, drying, storage.

In the comparison of wheat varieties, the different costs (constant and variable) are analysed together with yield components.

The synthetic number of value of varieties can be expressed in a complex formula:

$$F = \frac{T \times A}{K}$$

F = index of the value of varieties
T = yield
A = goods value
K = cost

Results and discussion

In this model, the complex economic value of seven winter wheat varieties are shown. The results of the calculation can be seen in Tables 1–4.

The experimental data are transformed into economic values, so yield data are given in relative number of values.

Tables 1–4 contain the results of calculations, based on experimental and field data. Quality parameters originate from these samples.

Table 1
Economic evaluation of winter wheat varieties

Variety	Yield T	Value of goods A_1	Earnings TxA_1	Cost K_1	Synthetic$_1$ $\frac{TxA_1}{K_1}$	With Fertilizer K_2	Synthetic$_2$ $\frac{TxA_1}{K_2}$	Value of goods A_2	Synthetic$_3$ TxA_2 / K_1	Synthetic$_4$ TxA_2 / K_2
Jubilejnaja	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G. K. Öthalom	1.031	0.969	0.999	0.986	1.013	1.043	0.957	0.969	1.013	0.957
Martonvásári 14	0.997	0.842	0.839	0.973	0.862	1.059	0.792	0.793	0.812	0.746
Baranjka	0.949	0.795	0.754	0.940	0.802	1.026	0.734	0.757	0.764	0.700
G. K. Zombor	1.073	0.703	0.754	0.987	0.763	1.101	0.684	0.851	0.925	0.829
Martonvásári 12	0.989	0.962	0.951	0.973	0.977	1.030	0.923	0.950	0.965	0.912
Bucsányi 20	1.055	0.848	0.894	0.983	0.909	1.040	0.859	0.816	0.785	0.827

Table 2
Yield

Variety	Experimental yield Weight 50 kg/ha	Relative	Field yield Weight 85 kg/ha	Relative	Harvest losses %	rest	Total relative Relative	T Yield
Jubilejnaja	6432.0	1.000	5264.0	1.000	3.0	0.970	1.00	1.00
G. K. Öthalom	6860.0	1.067	5403.0	1.026	4.0	0.960	0.990	1.031
Martonvásári 14	6734.0	1.047	5265.0	1.000	5.0	0.950	0.979	0.997
Baranjka	6392.0	0.994	5200.0	0.988	7.0	0.930	0.959	0.949
G. K. Zombor	7070.0	1.099	5571.0	1.058	3.0	0.970	1.000	1.073
Martonvásári 12	6574.0	1.022	5188.0	0.986	4.0	0.960	0.990	0.989
Bucsányi 20	6962.0	1.082	5465.0	1.038	3.0	0.970	1.000	1.055

Table 3
Value of goods

Variety	Milling % (flour=1.0/ bran=0.2)		Bread/100 kg flour		Quality of flour				Value of goods relative	
	%	Relative	kg	Relative	KMKE 1.1	Relative	KMKE 1.2	Relative	A_1	A_2
Jubilejnaja	73.60	1.000	146.0	1.000	1.09	1.000	1.18	1.00	1.000	1.000
G. K. Öthalom	70.50	0.969	146.0	1.000	1.09	1.000	1.18	1.00	0.969	0.969
Martonvásári 14	65.30	0.916	142.0	0.973	1.03	0.945	1.05	0.89	0.842	0.793
Baranjka	66.80	0.931	132.0	0.904	1.03	0.945	1.06	0.90	0.795	0.757
G. K. Zombor	69.90	0.962	142.0	0.973	1.03	0.945	1.07	0.91	0.703	0.851
Martonvásári 12	74.00	1.004	144.0	0.986	1.06	0.972	1.13	0.96	0.962	0.950
Bucsányi 20	68.00	0.943	139.0	0.952	1.03	0.945	1.07	0.91	0.848	0.816

Table 4
Cost

Variety	Transport	Cost of harvest		Plant protection			Fertilizer		Seed		Total proportion of cost	
	in the proportion of yield	lodging 0.05	1–5 +5	0.10	number of spraying	0.05	kg	0.2	1000 germ	0.1	without fertilizer K_1	with fertilizer K_2
Jubilejnaja	1.000	0.050	9	0.100	2	0.050	350	0.200	5.0	0.100	1.000	1.000
G. K. Öthalom	1.041	0.052	8	0.089	1	0.025	450	0.257	6.0	0.120	0.986	1.043
Martonvásári 14	1.108	0.051	7	0.078	1	0.025	500	0.286	6.0	0.120	0.973	1.059
Baranjka	0.990	0.50	6	0.067	0	0.000	500	0.286	6.2	0.124	0.940	1.026
G. K. Zombor	1.079	0.054	7	0.078	1	0.025	550	0.314	6.5	0.130	0.987	1.101
Martonvásári 12	0.999	0.050	7	0.078	1	0.025	450	0.257	6.0	0.120	0.973	1.030
Bucsányi 20	1.055	0.053	8	0.089	1	0.025	450	0.257	5.8	0.116	0.983	1.040

(1) Yield, output

The calculation (Table 2) was based on small plot experiments (average), field data and estimated loss during harvest. The average yield data of plot experiments are given in kg/ha for the relative number of values. Relative and absolute data of field trials are given at the same place. In the model, the 5-year average with 10 sites of small plot experiments can be found. The weight of these experiments is 50 and 85 for the field trials which indicates the number of data.

Harvest loss was estimated as a percentage. The corrected yield was used further.

(2) Quality

Quality is a part of the relative economic value of varieties. Table 3 shows the estimated economic values of the varieties based on quality.

Milling percentage, the quantity of bread (kg) that can be produced from 100 kg flour, glutan quantity and extension, farinograph value and the volume (cm^3) of sample bread were taken into consideration.

The economic value of milling percentage was estimated as an assumed 1 : 0.2 price ratio of flour and bran. The relative usefulness is the weighted average price.

The quantity (kg) of bread produced from 100 kg flour was taken as a significant parameter of quality. We assumed that usefulness is proportional with bread quantity. The complex economic evaluation of fluor (the important characteristics for the baking industry) was problematic. The methods developed at the Crop Production Institute (Tomcsányi, 1988) were used for this purpose. The values of KMKE number of value, correlated with critical minima, are from the following characteristics: gluten quantity and extension, farinograph number of value and volume of sample bread.

These data were weighted and drawn together, KMKE was calculated in two versions, with 1.1 and 1.2 ratio (see Tables). The complex index of goods value (multiplication of parts) is also given in two versions; A_1 and A_2 with 1.1 and 1.2 value ratio, respectively.

(3) Costs

Only the differences (between varieties) were taken into consideration. Table 4 shows the cost ratio of varieties. The other number in the table indicates the ratio from total cost.

Costs:

- transport of yield
- harvest
- plant protection
- fertilizers
- seed

The quantity of yield is proportional to the costs of transport. The differences in the cost of harvest are due to losses because of lodging. It was theoretically calculated that the time and cost of harvest doubled between the best and the worst variety. (Practically, this difference did not exist.)

The disease resistance varies with varieties. This value was estimated by the number of chemical treatments. The effect and cost of fertilizers were significant, since nutrient demand and ratio caused important differences. It was taken as 0.2, having 20% influence. Since fertilizers are an important part of costs, two versions were taken in the calculation:

K_1 without fertilization

K_2 with fertilization.

(The experimental data originated from plots with equal fertilization.)

The synthetic number of value of varieties ($F = T \times A/K$) shows the comparative usefulness of given varieties, taking unit costs. (The value of the comparative variety is 1.00.) Table 1 gives the yield goods value and the relative indices of cost, which is given in two forms (K_1 and K_2 with and without fertilizers). The goods value has two forms as well (A_1 = 1.1, A_2 = 1.2). Accordingly four synthetic indices were

formed. In A_1 – less important influence of quality and in A_2 – more important influence of quality were taken, with the combination of K_1 and K_2 nutrient supply.

Conclusions

On the basis of the results shown on the tables the following can be concluded: The qualification of varieties – based on given parameters – can be more objective.

The economic value of two or more varieties can be explained via the function of costs and yield. The results can be seen in the last columns of the tables. According to the economical model, in the future economic evaluation of varieties can be more realistic and subjective effects can be eliminated.

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Animal physiology and biochemistry

COMPOSITION CHARACTERISTICS OF HAIR, WOOL AND MUSCLE SAMPLES FROM RABBITS AND SHEEP CONSUMING CARBAMIDE FOR A LONG TIME

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The experiment involved 2 groups of 3 Hungarian Fine Wool Merino rams and 2 groups of 3 New Zealand doe-hares. The control groups were fed with urea-free fodder while the forage of experimental groups contained urea within the protein requirement. The adaptation time of fodder was 10 days and experimental time was 200 days. After this time crude protein, crude fat, crude ash and amino acid contents of wool, hair and muscle were examined in the control and experimental groups.

In the experimental groups, crude protein content of wool and hair was higher crude fat and crude ash contents of these samples were lower than in control groups.

In muscle samples no essential differences were found.

Asp, Thr, Ser, Glu, Pro, Gly, Cys, Tyr, Lys and Arg contents of wool were lower in the experimental groups.

Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg contents of hair were also lower in experimental groups.

17 amino acids of wool and hair samples were examined and there were differences in 10 amino acids of wool and 16 amino acids of hair.

Keywords: wool, hair, muscle, amino acids, urea

Introduction

Many Hungarian enterprises manufacturing feed mixtures produce widely-used fodder mixtures containing carbamide for feeding rabbits and sheep.

Acute ammonia toxicosis related with carbamide consumption has long represented a serious problem, and various solutions have been found to stop this.

There are, however, few data of studies on the biological implications of carbamide consumption.

From several viewpoints we examined the physiological effects and consequences of feeding carbamide for a long time.

The paper describes the results of determining the amino acid content of the hair and wool samples of rabbits and sheep consuming carbamide and of analysing some composition characteristics of muscles in the dead animals.

In the course of earlier examinations (Szabó, 1978) we found minor differences in the chemical composition of muscles between fattening bulls consuming carbamide and those kept on a carbamide-free diet.

The investigation of data collection character was justified by the fact that earlier observations found the hair and wool of sheep and rabbits consuming

carbamide for a long time to be less shiny and more compact than those of sheep and rabbits kept on carbamide-free diet, i.e. exclusively consuming fodder protein.

On the basis of these external, well perceptible differences it is supposed that a difference may also exist in the chemical composition.

Wool and hair are chemically keratin. According to Doehner (1964) the keratin is a protein of high cisteine content and heterogenous structure, consisting of alpha, beta and gamma fractions. Maciejewska (1965) pointed out that the fractions differed in sedimentation constant, diffusion properties, particle weight and amino acid content.

Carbamide given in the fodder as N-supplement, a part of the protein demand, may primarily cause relative deficiency of amino acids containing sulphur. Effect of this nature can be followed up by the arrangement of the experiment. A further reason for the study was the insufficiency of literary data concerning the breeds examined.

Materials and methods

The experiments included weaned rams of Hungarian combing merino breed and weaned female rabbits of white New-Zealand breed, three placed in each experimental group at the same time. At the end of the experiment the sheep were exterminated when reaching 57.3±2.1–62.0±5.3 kg body weight and the rabbits when weighing 3.0 kg.

The chemical composition of feed for the experimental rabbits and sheep is contained in Table 1, while the percentage composition and nutrient content of the fodder mixtures are shown in Table 2. The fodder mixture of the control groups contained no carbamide. The carbamide doses for the animals in the experimental rabbit group were mixed in the reduced quantity rabbit meal.

Table 1

Nutrient content of the feedstuffs consumed

Designation	Dry matter (original)	In 1000 g dry matter							
		Protein	Fat	Crude fibre	Ash	N-free extr. m.[1]	DE[2]	NE[3] m	NE[4] g
	g/kg	g						MJ[5]	
Meadow hay	880	123	27	389	91	370	7.21	5.11	2.74
Lamb meal 1	901	177	29	59	55	680	–	7.96	5.28
Lamb meal 2	899	179	29	59	51	682	–	7.88	5.21
Rabbit meal	902	146	32	109	68	645	11.95	–	–

Abbreviations: 1: Nitrogen-free extractable matter; 2: Digestible energy; 3: Net energy for maintenance; 4: Net energy for growth; 5: Megajoule

The carbamide uptake by the experimental groups was determined so that the daily N supply did not much differ from the values of the control groups. The composition and quantity of the daily feed rations can be seen in Tables 3 and 4.

The solid carbamide was mixed with the other components by means of a small cylinder feed mixer.

Table 2

Percentage composition and nutrient content of the fodder mixtures consume

Feedstuffs	Sheep meals		Rabbit meal
	1	2	
Maize %	54.6	60.60	15.00
Wheat %	21.8	22.20	20.20
Rye %	–	–	10.00
Oat %	–	–	15.00
Extr. sunflower %	21.8	14.60	–
Lucerne meal 1st class	–	–	35.00
Carbamide %	–	0.83	–
Feed lime %	0.8	0.77	–
Salt %	0.5	0.50	–
Premix %	–	–	5.00
Total%	100.00	100.00	100.00
Dry matter g/kg (original)	901.00	899.00	902.00
DE[1] MJ[4]/kg dry m.	–	–	11.95
NE[2] MJ/kg dry m.	7.96	7.88	–
NE[3] MJ/kg dry m.	5.28	5.21	–
CP[5] g/kg dry m.	177.00	179.00	146.00

Abbreviations: 1: Digestible energy; 2: Net energy for maintenance; 3: Net energy for growth; 4: Megajoule; 5: Crude protein

The animals became accustomed to carbamide in ten days through gradually increased doses of carbamide; the total period of experiment was two hundred days. In the course of the experiment the daily feed ratio was increased three times according to the demand, and the animals were weighed five times. The hair and wool samples had been taken from the *R. praescapularis* before the animals were slaughtered.

The amino acid content (17 amino acids) of the hair and wool was determined from airy-dry 0.2 g quantities of the defattened hair and wool samples by means of a Beckmann–Unichrom apparatus. In the course of the preparation the samples were hydrolized with 6 M solution of hydrochloric acid at 105 °C over twenty four hours. From the hydrolisate the solution was evaporated in a Rotadest apparatus, then the residue was dissolved in trisodium–citrate buffer solution of 2.2 pH.

The composition characteristics of the hair, wool and muscle samples were determined according to the Hungarian standard No. 6830–66.

Results and conclusions

The components of wool and hair samples from the experimental sheep and rabbit groups are to be found in Table 5. According to the values given in terms of 1000 g dry matter, the wool samples of sheep consuming carbamide contained more crude protein, but less crude fat and ash, than those of the control animals kept on a carbamide-free diet. The same applies to the rabbit hair samples. The relatively small number of data did not show significant differences; still, a tendency to an increase in the crude protein content of wool and hair samples from the sheep and rabbits consuming carbamide can be observed.

Table 3

Feed rations for the experimental sheep

13 May–19 June 1987		1 group	2 group
Meadow hay	g	500	500
Fodder mixture 1	g	920	–
Fodder mixture 2	g	–	900
Daily ration: Dry matter	g	1268.90	1249.10
NE m^2	MJ[4]	8.85	8.62
NE g^3	MJ[4]	5.58	5.42
CP[5]	g	200.80	198.90
20 June–11 September 1987		1	2
Meadow hay	g	500	500
Fodder mixture 1	g	1380	–
Fodder mixture 2	g	–	1350
Daily ration: Dry matter	g	1683.40	1653.60
NE m^2	MJ[4]	12.15	11.81
NE g^3	MJ[4]	7.77	7.53
CP[5]	g	274.20	271.40
12 September–18 November 1987		1	2
Meadow hay	g	500	500
Fodder mixture 1	g	1500	–
Fodder mixture 2	g	–	1470
Daily ration: Dry matter	g	1637.00	1609.30
NE m^2	MJ[4]	13.01	12.66
NE g^3	MJ[4]	8.34	8.09
CP[5]	g	293.32	290.62
Experiment averages:		1	2
Meadow hay g	500	500	
Fodder mixture 1	g	1328.60	–
Fodder mixture 2	g	–	1300.70
Daily ration: Dry matter	g	1637.00	1609.30
NE m^2	MJ[4]	11.78	11.46
NE g^3	MJ[4]	7.53	7.30
CP[5]	g	266.00	263.40

Abbreviations: 1: Digestible energy 2: Net energy for maintenance; 3: Net energy for growth; 4: Megajoule; 5: Crude protein

Table 4

Feeding for rabbits during the experiment

Feedstuffs	Rabbit groups	
	Control	Experimental
Rabbit meal (g)	100.00	82.60
Meadow hay (g)	100.00	100.00
Carbamide (g)	–	1.50
Daily ration:		
Dry matter (g)	178.20	163.90
DE[1] (MJ[2])	1.71	1.52
Crude protein (g)	23.99	25.83

Abbreviations: 1: Digestible energy; 2: Megajoule

Table 5

Some components of sheep wool and rabbit hair in terms of 1000 g *dry matter content*

		Crude protein	Crude fat, g	Ash
Sheep wool				
Control	1	905.34	5.80	42.60
	2	879.02	9.40	43.06
	3	852.07	7.59	42.11
	$\bar{x}$	878.81	7.60	42.59
Experimental	1	907.41	6.37	25.14
	2	921.83	7.17	27.90
	3	894.73	8.11	27.52
	$\bar{x}$	907.99	7.22	26.85
Rabbit hair				
Control	1	921.22	6.49	7.25
	2	915.00	6.25	6.30
	3	908.72	4.30	5.27
	$\bar{x}$	914.98	5.68	6.27
Experimental	1	981.40	5.07	6.14
	2	955.65	3.88	5.93
	3	974.11	4.33	6.61
	$\bar{x}$	970.39	4.43	6.23

Table 6 contains some components of the muscles of the rabbits and sheep included in the experiment.

Table 6

Components of rabbit and sheep muscles (g/kg)

	Dry matter	Crude protein	Crude fat	Crude ash		Dry matter	Crude protein	Crude fat	Crude ash
Carbamide rabbit					*Carbamide rabbit*				
1 *M. deltoideus*	267.7	220.7	42.0	12.6	1 m.brachiocephalicus	309.0	217.6	82.3	12.0
2 *M. deltoideus*	257.4	216.1	31.6	12.2	2 m.brachiocephalicus	287.1	210.3	72.3	9.9
3 *M. deltoideus*	260.9	209.1	35.8	12.1	3 m.brachiocephalicus	255.8	213.4	27.9	12.4
$\bar{x}$	265.0	215.5	36.5	12.3		284.0	213.7	60.8	11.4
Control rabbit					*Control rabbit*				
1 *M. deltoideus*	282.9	216.7	55.4	12.7	1 m.brachiocephalicus	291.4	220.9	60.1	14.4
2 *M. deltoideus*	258.7	213.8	31.2	12.5	2 m.brachiocephalicus	275.1	218.5	45.6	14.2
3 *M. deltoideus*	270.0	214.2	43.3	12.6	3 m.brachiocephalicus	266.4	219.6	52.9	14.3
$\bar{x}$	270.5	214.9	43.3	12.6		277.6	219.7	52.9	14.3
Carbamide sheep					*Carbamide sheep*				
1 *M. deltoideus*	300.4	178.7	114.4	14.7	1 m.brachiocephalicus	341.1	161.6	166.2	9.5
2 *M. deltoideus*	261.4	182.6	65.8	12.9	2 m.brachiocephalicus	297.4	183.2	105.5	9.6
3 *M. deltoideus*	260.2	176.4	70.9	9.6	3 m.brachiocephalicus	287.1	173.4	99.2	9.5
$\bar{x}$	274.0	179.2	83.7	12.4		308.5	172.7	123.6	9.5
Control sheep					*Control sheep*				
1 *M. deltoideus*	319.1	178.0	130.0	9.9	1 m.brachiocephalicus	326.2	165.3	141.4	10.8
2 *M. deltoideus*	318.6	177.9	126.8	11.1	2 m.brachiocephalicus	308.4	171.4	115.8	10.1
3 *M. deltoideus*	262.4	179.8	72.8	11.7	3 m.brachiocephalicus	334.2	162.8	162.8	10.5
$\bar{x}$	300.0	178.6	109.9	10.9		323.2	166.5	139.9	10.5

In the samples of the carbamide groups of both species – except for a single case – higher crude protein content could be detected.

The scatter of the analysis data for dry matter, crude fat and crude ash is within the limit of error of the method used.

The differences in muscle components between the sheep and rabbit groups given carbamide, and those kept on a carbamide-free diet, are negligible.

Further, from the same wool and hair samples, amino acid determination was carried out for seventeen amino acids; the results are contained in Tables 7 and 8.

According to the data of Fig. 1, the values of Asp, Thr, Ser, Glu, Pro, Gly, Cys, Tyr, Lys and Arg are characteristically lower for the carbamide group of sheep than for the control group.

The amino acid analysis of the rabbit hair samples – seen in Fig. 2 – showed lower contents of Asp, Thr, Ser, Glu, Pro, Ala, Cys, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg for the carbamide group, than for the control.

Table 7

Amino acid content of rabbit hair (g/kg)

Name of sample	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cystine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
Rabbit control																	
1	5.41	5.68	7.27	11.99	6.98	3.84	3.32	13.00	4.65	1.01	3.14	6.33	3.45	2.94	1.29	2.38	8.33
2	5.49	5.74	7.05	11.63	6.99	4.17	3.37	13.72	5.05	1.00	3.12	6.52	3.12	3.30	1.67	2.48	8.42
3	5.45	5.30	6.95	12.04	6.53	4.77	3.55	11.86	4.49	1.00	3.07	6.45	4.59	3.35	1.71	3.11	9.39
$\bar{x}$	5.45	5.57	7.09	11.89	6.83	4.26	3.41	12.86	4.73	1.00	3.11	6.43	3.97	3.20	1.56	2.66	8.71
Rabbit experimental																	
1	5.26	5.88	7.28	11.98	6.40	4.38	3.40	11.93	4.59	1.04	3.12	6.16	3.78	3.16	1.43	2.46	8.71
2	5.25	5.49	7.04	11.62	6.15	4.14	3.27	12.34	4.53	0.99	2.97	6.05	3.68	3.19	1.30	2.24	9.15
3	5.13	5.49	7.31	11.59	6.96	4.17	3.33	13.55	4.16	1.06	2.77	5.89	3.66	3.10	1.54	2.31	8.06
$\bar{x}$	5.21	5.62	7.21	11.73	6.50	4.23	3.33	12.61	4.43	1.03	2.95	6.03	3.71	3.15	1.42	2.34	8.66

Table 8

Amino acid content of sheep wool (g/kg)

Name of sample	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cystine	Valine	Methionine	Isoleu-	Leucine cine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
Sheep control																	
1	5.56	5.35	6.65	10.72	6.31	4.12	3.45	7.81	4.62	0.72	3.24	6.06	4.36	3.16	0.78	2.23	8.38
2	5.74	5.06	6.43	10.64	5.99	4.22	3.46	7.68	4.81	0.61	3.25	6.12	4.56	3.32	0.72	2.26	7.94
3	5.67	5.13	6.25	10.76	5.14	4.08	3.45	10.67	5.01	0.76	3.44	6.48	4.31	3.16	0.80	2.59	8.33
$\bar{x}$	5.66	5.18	6.44	10.71	5.81	4.14	3.45	8.72	4.81	0.70	3.31	6.22	4.41	3.21	0.77	2.36	8.22
Sheep experimental																	
1	5.69	5.35	6.43	10.92	6.93	4.15	3.49	5.54	4.63	0.73	3.32	6.00	4.03	3.36	0.73	2.13	8.77
2	5.57	5.14	6.35	11.16	6.26	3.87	3.48	9.47	4.88	0.63	3.25	6.24	3.83	3.14	0.82	2.66	9.41
3	4.81	4.40	5.45	9.69	6.23	3.88	3.48	6.41	5.05	0.60	3.44	6.91	4.23	3.37	0.97	2.96	9.60
$\bar{x}$	5.37	4.96	6.08	10.59	6.47	3.97	3.48	7.14	4.85	0.65	3.34	6.38	4.03	3.29	0.84	2.58	9.26

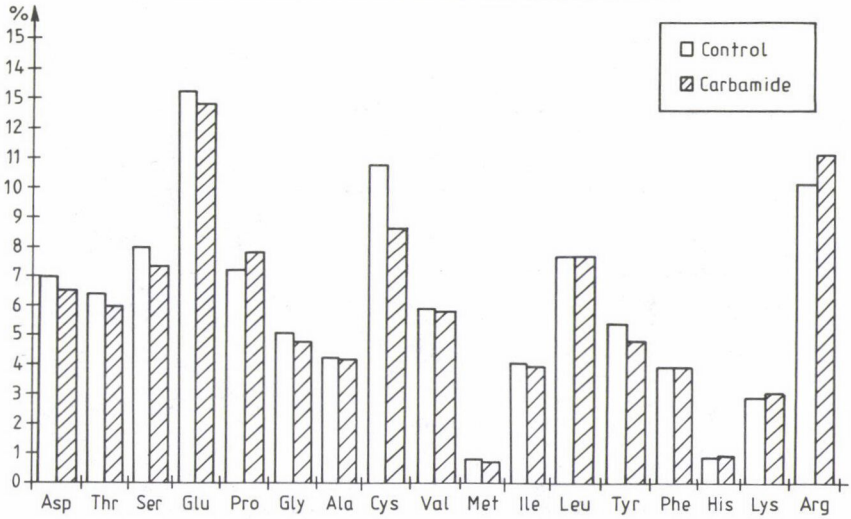

Fig. 1. Proportion of the amino acids examined in the wool samples of sheep consuming carbamide and of the control, expressed as a percentage of the protein content

The analysis of the wool samples detected differences in ten of the seventeen amino acids examined between the experimental and the control groups; while, in the case of the rabbit hair samples, sixteen amino acids showed differences, with methionine as the only exception. For the sake of comparison, data of amino acid analyses by Veress et al. (1982) are presented in Table 9, and by Orskov (1982) in Fig. 3.

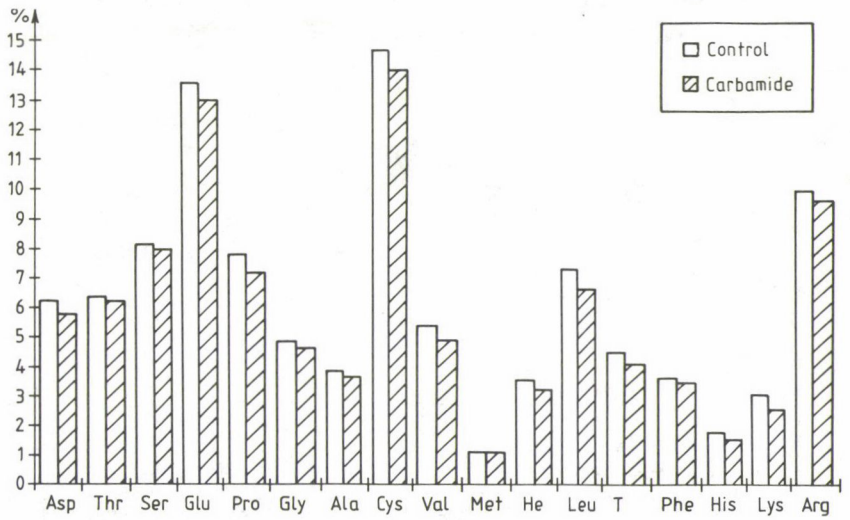

Fig. 2. Proportion of the amino acids examined in the hair samples of rabbits consuming carbamide and of the control, expressed as a percentage of the protein content

Table 9

Amino acid composition of the wool protein according to Veress et al. (1982)

Amino	Wool g amino acid/100 g protein
Arginine	8.5
Phenylalanine	4.0
Histidine	1.2
Isoleucin	3.8
Leucine ,	8.0
S-containing amino acids (methionine, cystine)	12.6
Tyrosine	5.0
Threonine	6.5
Triptophane	1.8
Valine	5.9

On the basis of our own experiments, in can be astablished that the wool and hair samples of sheep and rabbits that consume carbamide contain lower quantities of amino acids, in a higher crude protein content, compared to the control animals kept on a carbamide-free diet.

According to the data obtained, the differences between the control and the experimental samples were not significant. The mean values, however, show significant differences on 10% level for alaine, tyrosine and arginine in the wool samples of sheep consuming carbamide, and for asparagine and proline in the hair samples of rabbits given carbamide, compared with the control groups.

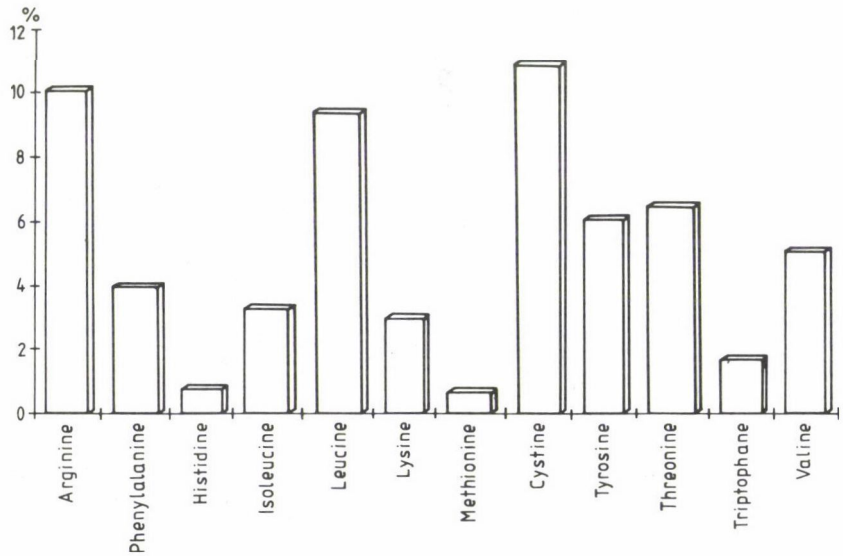

Fig. 3. Amino acid composition of sheep wool protein

Experiments with weaned rams of Hungarian combing merino breed and weaned female rabbits of white Zealand breed, three animals in each group, were then done.

The experimental groups of sheep and rabbit were given carbamide mixed in the fodder, while the control groups were kept on a carbamide-free diet.

According to earlier observations by the author, the wool and hair of sheep and rabbits, respectively, consuming carbamide for a long time are less shiny and more compact than those of animals consuming natural proteins.

During the study, not only the chemical analyses of the wool and hair samples were made, but also the components from some muscles were determined.

According to the results, the wool samples of sheep consuming carbamide contained more crude protein, but less crude fat and ash, than those of the control animals. The same results were obtained with the rabbit hair samples.

The crude protein content in wool and hair samples from sheep and rabbits consuming carbamide showed an increasing tendency. The amino acid analyses of wool samples indicated lower values of ten amino acids for the carbamide group, compared to the control; in the case of the rabbit hair samples, the same can be said of sixteen amino acids.

Altogether, the wool and hair samples of sheep and rabbits that consume carbamide contained lower amino acid quantities in a higher crude protein content, compared to the samples of the control animals.

The differences in chemical composition of muscles between rabbits consuming carbamide and those kept on a carbamide-free diet are negligible.

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PROPERTIES OF MEAT CHICKEN BREAST PATTIES AS A FUNCTION OF USING SELECTED PHOSPHATE SALTS

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Meat chicken breast patties were prepared in the presence of different ratios of phosphate salts, i.e. tetra sodium pyrophosphate (Tetra SPP), tri sodium pyrophosphate (Tri SPP) and sodium acid pyrophosphate (SAPP). Cooking weight loss, diameter reduction, cooking density, thiobarbituric acid (TBA), pH value and water holding capacity (WHC) were determined for the investigated samples. The available data proved that the applied ratios of the responsed phosphate salts improved the cookability properties of the cooked chicken breast patties as well as the WHC of the uncooked samples. On the other hand, the applied phosphate salts minimized the rate of fat oxidation (which represents as TBA values) in both the uncooked and cooked chicken patties. However, there is no noticeable variations in pH values; a trend which may be related to the buffering effect of the meat constituents. Statistical analysis assured all the previous results.

Keywords: chicken meat, phosphate salts, cooking loss

Introduction

Precooked poultry meat products entering tody's consumer market at a rapid rate are susceptible to quality changes upon short-term refrigerated storage. Treatment of processed poultry with polyphosphates, in combination with salt, has shown beneficial effects for the inhibition of oxidative changes and flavour deteriorations that occur in sausage (Ang and Young, 1987; Shahidi et al., 1987). Polyphosphates have also been used in meat formulations to increase water-binding capacity and to improve other textural qualities (Sofos, 1986). However, it has been suggested that the influence of polyphosphates on meat texture is due, in part, to their ability to increase pH and ionic strength (ISO), to chelate divalent metal cations and to interact with meat proteins (Trout and Schmidt, 1984). Among these functions, only the chelation of metal ions has been cited as a possible mechanism for the antioxidant effect of phosphates (Sofos, 1986; Younathan, 1985). On the other hand, studies have shown that adjusting pH to higher level reduced the oxidation rate in ground raw poultry meat (Chen and Waimaleongora-ek, 1981).

In recent years there has been pressure from consumer groups for legislation to reduce the amount of sodium in processed foods, due to the possible causal relationship between sodium intake and hypertension. However, in most products lowering the salt level leads to a noticeable loss of functionality, as exhibited by the increment of cooking loss and reduced textural properties (Sofos, 1983). The use of polyphosphates as a partial replacement of salt in different meat products has been studied for many years (Papper and Schmidt, 1975). Although phosphates can effectively replace salt in most meat products, their effectiveness depends on the type of phosphate salts (Shults et al., 1972) and the conditions under which they are

used (Puolanne and Terrell, 1983). From the previous viewpoints, it was intended to ascertain the effect of different types of phosphate salts, i.e. tetra sodium pyrophosphate (Tetra SPP), tri sodium pyrophosphate (Tri SPP) and sodium acid pyrophosphate (SAPP) on the cooking properties (cooking loss %, diameter reduction and cooked density).

Materials and methods

(A) Materials

Whole broilers were produced from a Middle East Company, Elkaliobia Governorate. The breast meat was removed, skined and deboned after which the broiler breast meat was held at – 20 °C overnight and then coarsely ground (chopped) through a 1.5 cm (diameter) plate. For preparing the tested treatments, each 2 kg of the ground meat were mixed with 2% salt and 200 ml of the responsed food grade phosphate solutions within the ratios given in Table 1. The previous ingredients were mixed with a food mixer for 1 min and then formed in patties of about 100 g each, using a 10 cm round plastic plate of kobba machine. The patties were weighed (raw weight) and tempered at –18 °C for 10 min to assure a uniform initial temperature and portion, were then cooked on a wire rack over aluminium tray at 150 °C for about 25 min after which they were cooled and measured for their weights, thickness and diameter. Cooked as well as uncooked samples were packaged in moisture proof bags and stored at –18 °C for further analysis.

Table 1

Ratios of food grade phosphate salts used in preparing chicken breast patties

Treat-ments	Phosphate salts		Treat-ments	Phosphate salts	
	Tetra SPP %	SAPP %		Tri SPP %	SAPP %
A	0.0	0.0	AA	0.0	0.0
B	0.5	0.0	BB	0.5	0.0
C	0.4	0.1	CC	0.4	0.1
D	0.3	0.2	DD	0.3	0.2
E	0.2	0.3	EE	0.2	0.3
F	0.1	0.4	FF	0.1	0.4
G	0.0	0.5	GG	0.0	0.5

Tetra SPP: Tetra sodium pyrophosphate. SAPP: Sodium acid pyrophosphate. Tri SPP: Tri sodium pyrophosphate.
N. B.: Treatments A to G or AA to GG were based on the research of Marcy et al. (1988)

(B) Methods of analysis

– The moisture content and pH values were carried out according to A. O. A. C. (1980).
– Cooking loss, diameter reduction and cooking density were calculated by the equations of Young et al. (1987).

$$\text{Cooking loss}\,(\%) = 100 \times \frac{\text{raw wt} - \text{cooked wt}}{\text{raw wt}}$$

$$\text{Diameter reduction}\,(\%) = 100 \times \frac{10 - \text{cooked diameter}}{10.0}$$

$$\text{Cooked density} = \frac{\text{cooked weight}}{\pi\,(\text{radius of patties})^2\,(\text{thickness of patties})}$$

Thiobarbituric acid (TBA):

Thiobarbituric acid was measured in the investigated samples as described by Vyncke (1970) and the data was given as absorbance at 538 nm.

Water holding capacity (WHC):

The water holding capacity was based on the method mentioned by Grau and Hamm method (1957) and modified by Volovinskaia and Merkoolova (1958).

Statistical analysis:

Multiple regression and analysis of variance for full regression were carried out by the SAS computer program which was applied according to Helwig (1983) using the 286 PC/AT 80286 computer; available at the Expiry Date project, Faculty of Agriculture, Ain Shams University.

Results and discussion

The meat and meat products are characterized by special cooking properties which could be summarized in the following three aspects, i.e. cooking loss, diameter reduction and cooked density. Subsequently it is important to improve these parameters in order to achieve higher quality of chicken breast patties. In such case, trials were carried out by using food grade phosphate salts and the available data are given in Tables 2–5 and Figs 1–3.

Table 2

Effect of using tetra sodium pyrophosphate+sodium acid pyrophosphate on the cookability properties of cooked chicken breast patties

Phosphate treatments	Tested parameters					
	Cooking loss		Diameter reduction		Cooked density	
	%	Improvement level	%	Improvement level	g/cm³	Improvement level
A	43.21	–	17.20	–	0.93	–
B	38.05	11.94	15.21	11.57	0.96	–3.23
C	38.07	11.87	15.20	11.63	0.95	–2.15
D	38.08	11.87	15.19	11.63	0.96	–3.23
E	38.10	11.83	15.20	11.63	0.95	–2.15
F	38.09	11.85	15.18	11.74	0.96	–3.23
G	38.07	11.90	15.19	11.69	0.96	–3.23

Table 3

Effect of using tri sodium pyrophosphate+sodium acid pyrophosphate on the cookability properties of cooked chicken breast patties

Phosphate treatments	Tested parameters					
	Cooking loss		Diameter reduction		Cooked density	
	%	Improvement level	%	Improvement level	g/cm³	Improvement level
AA	43.95	–	16.80	–	0.91	–
BB	38.84	11.63	15.05	10.42	0.95	–4.40
CC	38.80	11.72	15.15	9.82	0.94	–3.30
DD	38.89	11.51	15.12	10.00	0.95	–4.40
EE	38.85	11.60	15.10	10.12	0.95	–4.40
FF	38.84	11.63	15.08	10.24	0.94	–3.30
GG	38.87	11.56	15.13	9.94	0.95	–4.40

Table 4

Effect of using tetra sodium pyrophosphate+sodium acid pyrophosphate on some properties of cooked and uncooked chicken breast patties

Phosphate treatments	Uncooked Samples				TBA values mg onaldehyde/kg	Molsample
	Moisture	Dry-matter	pH	WHC %	Uncooked	Cooked
A	56.25	43.75	6.20	30.92	0.71	0.85
B	59.95	40.05	6.45	39.95	0.65	0.70
C	59.93	40.07	6.31	39.90	0.64	0.70
D	59.95	40.05	6.25	39.92	0.65	0.69
E	59.94	40.06	6.21	39.93	0.64	0.69
F	59.95	40.05	6.15	39.94	0.65	0.68
G	59.93	40.07	6.05	39.95	0.63	0.68

Table 5

Effect of using tri sodium pyrophosphate+sodium acid pyrophosphate on some properties of cooked and uncooked chicken breast patties

Phosphate treatments	Uncooked Samples				TBA values mg onaldehyde/kg	Molsample
	Moisture	Dry-matter	pH	WHC %	Uncooked	Cooked
AA	56.30	43.70	6.19	31.21	0.69	0.86
BB	59.99	40.01	6.17	39.80	0.64	0.69
CC	59.98	40.02	6.15	39.81	0.63	0.70
DD	59.99	40.01	6.12	39.82	0.64	0.70
EE	59.97	40.03	6.10	39.85	0.63	0.69
FF	59.98	40.02	6.05	39.84	0.65	0.69
GG	59.98	40.02	6.02	39.83	0.64	0.69

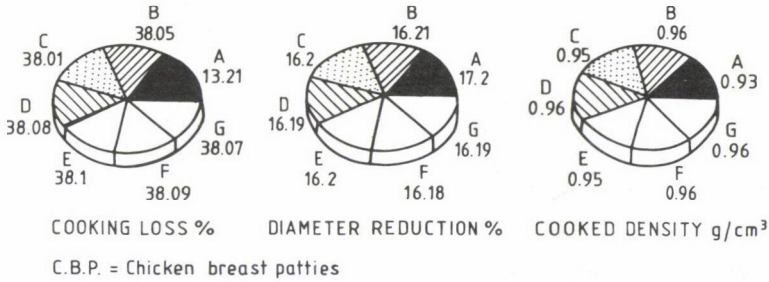

Fig. 1. Effect of Tetra SPP+SAPP on the cookability properties of cooked C.B.P.

Regarding the effect of tetra sodium pyrophosphate and sodium acid pyrophosphate on the cooking loss %, Table 1 showed the superiority of the combination effect of (Tetra SPP+SAPP) over the control sample. For instance, the cooking loss % of the control sample; "treatment A" that was 43.21% was improved and reached around 38% for the other treatments (B–G) containing phosphate salts. So, the investigated phosphate salts had minimized the loss by about 12%, as seen in the same Table.

With respect to the diameter reduction, the same Table indicates that the presence of the phosphate salts (Tetra SPP+SAPP) within the given ratios minimized the shrinkage pattern of the chicken breast meat patties as seen in Fig. 1 which showed the diameter reduction level of the tested samples in relation to the control. However, the cooked density showed a similar trend in which higher values are given for the recipes containing the previous phosphate salts.

On using other phosphate salts, such as (Tri SPP+SAPP) the cooking loss, diameter reduction and the cooked density were also improved, as seen in Table 3 and Fig. 2.

However, Hargett et al. (1980) reported that sodium acid pyrophosphate (SAPP) is currently used by the sausage industry to accelerate development of cured

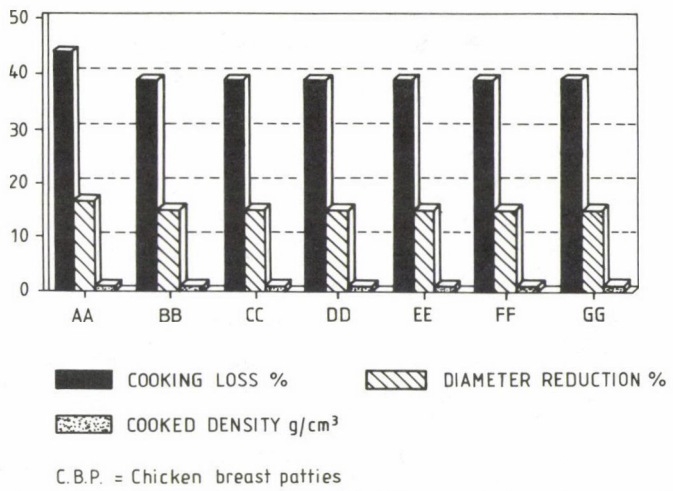

Fig. 2. Effect of Tri SPP+SAPP on the cookability properties of cooked C.B.P.

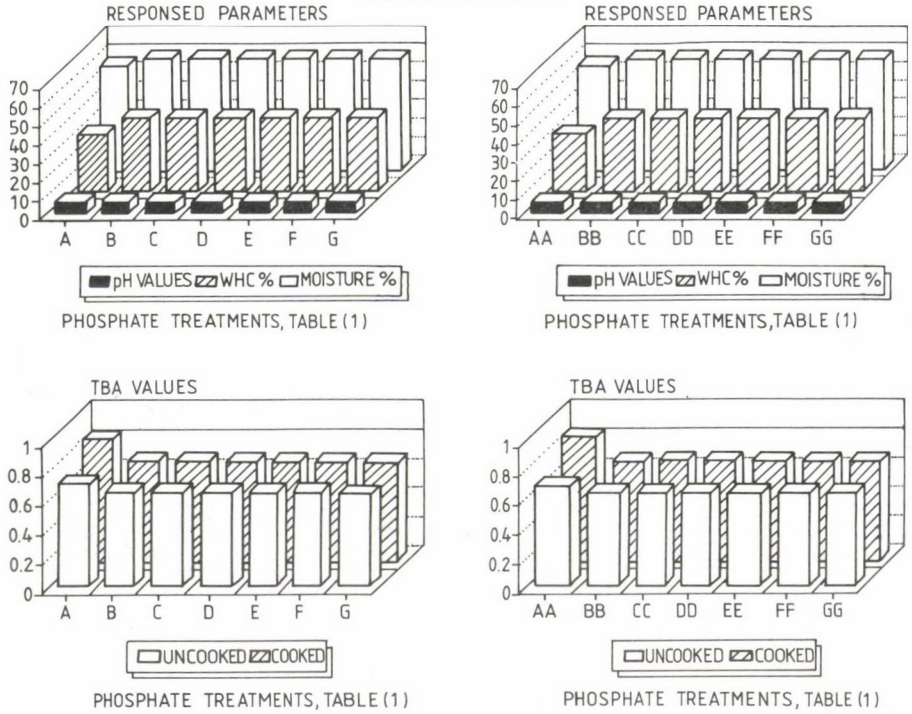

Fig. 3. Effect of using tetrasodium pyrophosphate+sodium acid pyrophosphate (A to G) and trisodium pyrophosphate+sodium acid pyrophosphate (AA to GG) on some properties of cooked and uncooked chicken breast patties

meat color. On the other hand, SAPP had no detrimental effects on texture and may have caused a slight improvement in flavor by enhancing beef, salt and seasoning flavors, as well as by diminishing fat flavor. Unlike alkaline phosphates, SAPP alone did not improve moisture retention or cook yields.

It is well accepted now that cooking loss, diameter reduction and cooking density are one group of parameters which act as a source of consumer acceptance or dissatisfaction of breast meat chicken patties. Subsequently excessive improvement in these cookability properties are of great importance, a trend which was successively achieved by using the investigated phosphate salts previously given in Table 1. Such a conclusion agrees with that of Young et al. (1987) who proved that STPP (from 0.0, 0.3 and 0.5) plus NaCl (0.0, 1.5 and 3.0) could be valuable in improving the appearance of cooked poultry meat products by reducing shrinkage. They also mentioned that the previous additives improved moisture retention and have a noticeable effect on the texture quality of the patties. In the absence of NaCl, the STPP increased the product cohesiveness, springiness and chewiness at the highest phosphate level, but, in the presence of NaCl,the phosphate tended to increase these textural attributes, especially cohesiveness and chewiness, at lower phosphate levels.

The applied phosphate salts given in Table 1 also minimized the rate of fat oxidation which represents TBA values as seen in Table 4. This means that phosphate salts could be served as an antioxidant during the cooking of the chicken

patties. These results are in accordance with Ang and Young (1989) who also added that the antioxidant function of STPP was more important during storage of cooked samples.

Other possible function of the polyphosphate salts was based on their ability to increase the pH value of meat products (Trout and Schmidt, 1983), and it is well known that higher pH slows down the oxidation process.

The pH values of the investigated samples were then measured as seen in Table 4 which indicated that there is no noticeable variation in pH values within the applied phosphate salts. Such lack of change in the pH of the chicken breast patties was due to the buffering effect of the meat constituents. A similar conclusion was given by Ang and Young (1989). On such a base, the antioxidant effect of the applied phosphate salts was due to its metal-chelating ability more than to its influence on pH.

The WHC of the control chicken patties was lower than that of the other samples containing phosphate salts. Subsequently, the applied phosphate salts were most effective in improving WHC of the chicken patties containing 2% NaCl. A similar conclusion was reached by Young et al. (1987) who mentioned that the WHC of the patties containing 2% NaCl did not differ from those containing the lower level, and so STPP was most effective in improving WHC in meat products containing less than 3% NaCl. However, Hamm (1970) has summarized the effect of phosphate on the increment of WHC and presumably other functional properties such as binding strength as being due to a real increase in both pH values and ionic strength; the ability of phosphates to bind to meat proteins, and the ability of phosphates to dissociate actomyosin into actin and myosin. A similar conclusion was given by Schmidt and Trout (1982) who stated that all the phosphates used in meat products increase both pH and ionic strength; a trend which depends upon the type and concentration of phosphate.

The multiregression analysis given in Table 6 indicated the presence of a

Table 6

Multiregression analysis of some properties of the uncooked and cooked breast chicken patties

Tested parameters of	Statistical variables			
	R-Squared		Std. error	
	I	II	I	II
Cooking loss %*	0.999953	0.999824	0.016359	0.031335
Diam. reduction %*	0.999340	0.997514	0.075590	0.039170
Cooked density g/cm^3	0.828205	0.896296	0.005648	0.005774
Moisture**	0.999960	0.999982	0.010579	0.007303
WHC %**	0.999970	0.999890	0.020260	0.013310
TBA: Uncooked	0.941609	0.903704	0.007777	0.007868
Cooked*	0.994926	0.995386	0.005255	0.005255

I: (Tetra SPP+SAPP) II: (Tri SPP+SAPP)
*: Cooked samples. **: Uncooked samples.

higher correlation coefficient between the tested parameters (cooking loss %, diameter reduction %, cooked density, moisture, WHC and TBA values) and the applied phosphate salts, i.e. (Tetra SPP+SAPP) and (Tri SPP+SAPP). However, the estimated standard error given in the same Table assures such trends.

Conclusions

The relation between using different ratios of tetra sodium pyrophosphate+sodium acid pyrophosphate and the cookability properties of the cooked chicken breast patties indicated the presence of a real improvement (minimizing) in both cooking loss and diameter reduction. A similar pattern was noticed when trisodium pyrophosphate+sodium acid pyrophosphate was applied through processing of the investigated samples. The water holding capacity of the uncooked chicken patties showed also a pronounced improvement as a function of using the applied phosphate salts. On the other hand, the possible function of the polyphosphate salts was based on their ability to increase the pH value of meat products; and it is well known that higher pH slows down the oxidation process. However, the applied phosphate salts also minimized the rate of a fat oxidation which represents TBA values. This means that phosphate salts could be served as an antioxidant during the cooking of the chicken patties.

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Lectures

STRATEGIES FOR UTILIZATION OF THE SALT-AFFECTED SOILS IN THE WORLD*

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Introduction

Among the specific land formations on the continents salt-affected lands have particular importance and significance. They are significant as formations of ecosystems on the earth affected by high concentrations of water soluble salts on the one hand, and as means of production with little value as a consequence of salinity and/or alkalinity, on the other hand.

Salt-affected lands have been giving and continue to give rise to substantial problems, not only in respect to scientific study, but also for their utilization in agri-, sylvi-, and horticulture. The strategy of their utilization poses a serious problem in many different countries worldwide, and the precondition of meeting this challenge is a better knowledge of the nature, properties, and development of all the phenomena involved.

We have quite reliable records of the global extension of salt-affected lands. They account for roughly 10% of the land surface and can be found on all continents, and in all climatic belts, at various altitudes. As a matter of course, they are distributed unevenly because they occur more frequently in arid territories than in humid conditions. They can be often seen in alluvial plains and fertile lowlands, and comparatively rarely on high mountains or in the arctic belts. Nevertheless, they also occur in such places.

As a consequence of the uneven distribution of salt-affected lands, the practical problems confronting those responsible for planning, decision-making and production vary in the different countries. In some places they have a major problem, but in other places the task is insignificant.

When speaking of salt-affected lands we always have to keep in mind the whole ecosystem. Our main concern, however, will always be the soil which composes a major part of all terrestrial ecosystems. Consequently, the main stress in the following will be put on the soil, and on soil conditions, particularly on salt-affected soils. (From now on "salt-affected lands" and "salt-affected soils" will be used synonymously.)

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Akadémiai Kiadó, Budapest

Salt-affected soils and their development

Despite the fact that the properties and attributes of salt-affected soils have long been well known, it is worthwhile to begin with a brief definition of this group of soils.

Salt-affected soils can be characterized as formations under the dominant influence of different salts in their solid or liquid phases which will then have a decisive influence on the development, characteristics, physical, chemical and biological properties and eventually the fertility of the soil. Whenever and wherever this phenomenon occurs, it produces specific formations of soils where the high electrolyte concentration and its consequences overshadow the former soil-forming processes or former soil properties and environmental conditions, often radically changing them.

High electrolyte concentration is the only common feature of all salt-affected soils. Their chemistry, morphology, reaction, and many other properties may be different, depending on the character of salinization and/or alkalization.

In spite of the fact that the high electrolyte content of salt-affected soils results in many adverse consequences, which should be mitigated or compensated, in fighting soil salinity and alkalinity by different approaches, methods, and means, we have to distinguish three main cases depending on the region and processes of these soils, as well as on the hazard of their further development.

1. Salt-affected soils developed due to natural soil forming processes
2. Man-made salt-affected soils, in other words, secondary salinization
3. Potential salinization of soils

1. The majority of salt-affected soils (accounting altogether for about 10% of the surface of the continents at present) is a product of natural processes of salt accumulation.

Evidently, the accumulation of salts in top and subsurface soil layers as well as in surface and ground waters is a result of geochemical processes. Salt accumulation often occurs not only in desert and semi-desert environments, where the leaching of salt is limited, but also in humid and semi-humid conditions due to local geochemical conditions or ground water effects. Evidently, all the described processes lead to the formation of different kinds of salt-affected soils.

2. Man-made salt accumulation, though it affects the smaller part of salt-affected soils, has a particular importance because it mostly occurs in fertile areas and causes great harm both to the environment and the economy. Since ancient times the phenomenon of man-made salinization, in other words, secondary salinization has been well known. Still the process has not been checked; on the contrary, it has an ever-growing tendency.

According to the estimates of competent international organizations, first of all, affiliated UN bodies, like the FAO, UNESCO and UNEP, more than half of all irrigated lands in the world is under the influence of this adverse process, and every year about 10 million hectares of once-fertile irrigated land must be abandoned from production because of its development.

The main causes of secondary salinization are the improper practices of irrigation and the lack, or poor condition, of drainage canals. However, other reasons, too, are to be kept in mind.

One of them is deforestation when, due to the removal of woods, the water balance of the given territory changes and, instead of leaching, the salt accumulation process becomes dominant. Often the ground water table rises lifting serious amounts of salts towards the surface. It also happens that, in the wake of deforestation, swamps develop and the accumulating salty waters salinize the surrounding soils.

Overgrazing also causes salt accumulation quite frequently as the reduction of the natural vegetation cover induces an upward movement of salt solutions in the soil profile.

Secondary salinization may also occur as a result of pollution, when the pollutants from industrial plants, sewages of communal wastes accumulate on the soil surface.

In the conditions of intensive agriculture, the overuse of mineral fertilizers, and consequently, the accumulation of residues, may also contribute to salt accumulation i.e. the secondary salinization of soils. In recent decades, serious salt accumulation was observed in the soils of greenhouses after the intensive application of fertilizers, which led to the deterioration of these soils.

3. Potential salt-affected lands are those which are neither saline nor alkaline for the time being, but where a serious hazard of salinization and/or alkalization exists if irrigation or a change in the way of cultivation occurs. It is not simple to give a more detailed definition of this phenomenon because, as it was described in point 2 above, secondary salinization may result from different causes.

It should be noted that, besides the above-described production methods which result in salt accumulation, some human activities may also have an indirect effect leading to salinization. For instance, in the latest period the increase of CO_2 concentration in the atmosphere and its consequences, the so-called greenhouse effect, lead to a number of environmental changes, including the extension of salinization. For the time being, rather little experience is available on the subject. Nevertheless, investigations should be extended in this area in the fairly near future.

It is necessary to conduct a specific survey in order to determine whether potential salinization exists in the given place and, if it does, to define its extent and conditions. It is necessary to conduct such a survey and to concretize the hazard of the processes, because neglecting preliminary studies may result serious harm in the potentially salt-affected areas.

Main aspects of strategy in fighting salinization and alkalization

1. No account is available on the acreage of ameliorated salt-affected soils developed through the natural processes of salt accumulation, despite the fact that great territories have been reclaimed in the different parts of the world for a fairly long time, perhaps as long as several centuries. However, reclaimed territories do not account for even 1% of all existing salt-affected soils. The reason for this is partly

the vast territories of saline areas and partly the high cost and labour requirements of improving salt-affected soils, the majority of which can be found in desert areas and barren lands.

It is most probable that the overwhelming majority of existing salt-affected soils will remain as they are in the foreseeable future. Some parts of these areas can be conserved as national parks or protected territories. There are plenty of examples of such cases, as in the environment of the Yellowstone Park in the Rocky Mountains of the US, the Ambroseli National Park in Kenya, the Hortobágy region in Hungary, and many others.

Accordingly it is not only a lesson of history but also a current fact that there are no ambitious prospects for the amelioration of most salt-affected areas in the world. Amelioration projects can be planned or initiated mainly in cases of the following conditions:

a) If the salt-affected land can be ameliorated comparatively easily, i.e. either the degree of salinity is not too high or the removal of excess salts is easily possible by reasonable irrigation and drainage systems, or by other means.

b) If there are serious reasons for the introduction of intensive production systems which can be expected to yield fair returns on the investments in the reclamation.

There are plenty of good examples of both cases, mainly in countries with moderate climate where the degree of salinity and alkalinity is comparatively low, the reclamation of soils is possible with the application of proper agrotechnics, low amounts of chemical amendments, and the right crop rotation.

Such methods have been introduced in the agricultural utilization of solonetz soils in Russia, Hungary, Canada, China, and other countries.

There are also many examples of the utilization of saline soils in arid areas, where the introduction of proper irrigation and drainage techniques ensures good returns on the investment by reliable and steady yields. Such methods are well known and widely used in Egypt, India and the western states of the US.

Parallel with the practice of the mentioned methods of amelioration, due attention should be paid to the maintenance of the irrigation and drainage systems and to the control of the processes of salinization in short and long term.

2. In contrast to the situation of the salt-affected soils created by nature, without or with little human interference, the combat against salinization and alkalization goes on for the majority of man-made salt-affected soils. There are two different cases here.

a) To control or mitigate the processes of salinization, in other words, to keep the salt balance at its recent level; and

b) to reduce the processes of salinization, in other words, to remove part of the salts from the soil layers.

The proper strategy of this kind of combat against salinization depends on the local circumstances, techniques of irrigation and drainage, and on the requirements of production level.

In irrigated areas, or in areas to be irrigated, the study and control of alkalinity and/or salinity has to be started well before putting the irrigation and drainage systems into operation; even before making plans for their construction. Such an approach is necessary because, by proper survey, it can be decided whether the territory is suitable for reasonable irrigation. By sizing up in time the salinity problems of the areas where irrigated farming is envisaged, a lot of money and worry can be saved. During the preliminary survey not only the soils but also the environmental conditions, as for example climate, hydrology, etc. as well as the possible sources of irrigation and the quality of both ground and irrigation water, should also be studied.

In irrigated areas it is essential to construct and employ up-to-date monitoring systems to observe and control salinity conditions and their changes and to enable the anticipation of possible consequences.

In Table 1 a scheme of the methods recommended for the control of salinity and alkalinity in irrigated areas is demonstrated.

Table 1 shows that a comprehensive survey and monitoring system is necessary both before and during irrigation.

Table 1

Scheme of methods recommended for the control of salinity and alkalinity in irrigated areas

(A) Before construction of irrigation system

Preliminary survey	
Landscape	Planned irrigation
climate	available irrigation water (quality and quantity)
hydrology	ground water depth and quality
hydrogeology	technology of irrigation
geomorphology	cropping pattern tolerance

(B) During irrigation

Monitoring
salinity and alkalinity of soil and ground water table
chemical composition of ground water
chemical composition of irrigation water
water filtration
physical soil properties
toxic elements, if any, in soil and water

As the worldwide increase of the territory of irrigated lands is envisaged, and consequently, the increase of secondary salinization/alkalization can also be expected, it is highly recommended to take such measures in every case.

3. The approaches and methods, described above, also relate to potential salt-affected lands with the only remark that, apart from irrigation, other possible changes, either in natural conditions or in the conditions of production (e.g. deforestation, changes in hydrological conditions, overgrazing, pollution, overfertilization, etc.) should also be taken into consideration.

The second part of Table 1 covers the main aspects of the strategy of resisting the salinization of potential salt-affected areas.

As it also follows from the foregoing, not only environmental aspects but also socioeconomic aspects have very great importance in the course of making the decision on the methods to be used against salinization processes.

Generally, we can agree that the strategy of fighting salinization and for the utilization of such areas has both technical and political aspects, including cost benefit analysis, specific studies of the environmental conditions, economic and social consequences, influence on the landscape, and urbanization, etc.

It is evident, apart from the very important but few general considerations partly described above, that the local conditions should determine the way, schedule, and means of the combat against salinization.

In spite of the fact that feasibility studies and operation projects should be elaborated locally, international collaboration and exchange of experiences have paramount importance not only because the adaptation of good experiences and methods can promote the elaboration of proper plans and save a lot of work and money, but also in the consequence of that fact that salt accumulation is not only regional but also a global problem.

Parallel with the sharp increase of irrigated territories, the exploitation of available world resources of fresh water grows more intensive. Both the shortage of water and the extension of salinization are phenomena that overpass national borderlines and can influence the areas and watersheds of neighbouring regions or countries.

The interested international organizations should take a larger share also in the elaboration of scientific and technological methods of the world combat against the further extension of the adverse processes of salt accumulation on the continents.

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Reviews

EVAPOTRANSPIRATION: EVAPORATION+TRANSPIRATION (+INTERACTION)

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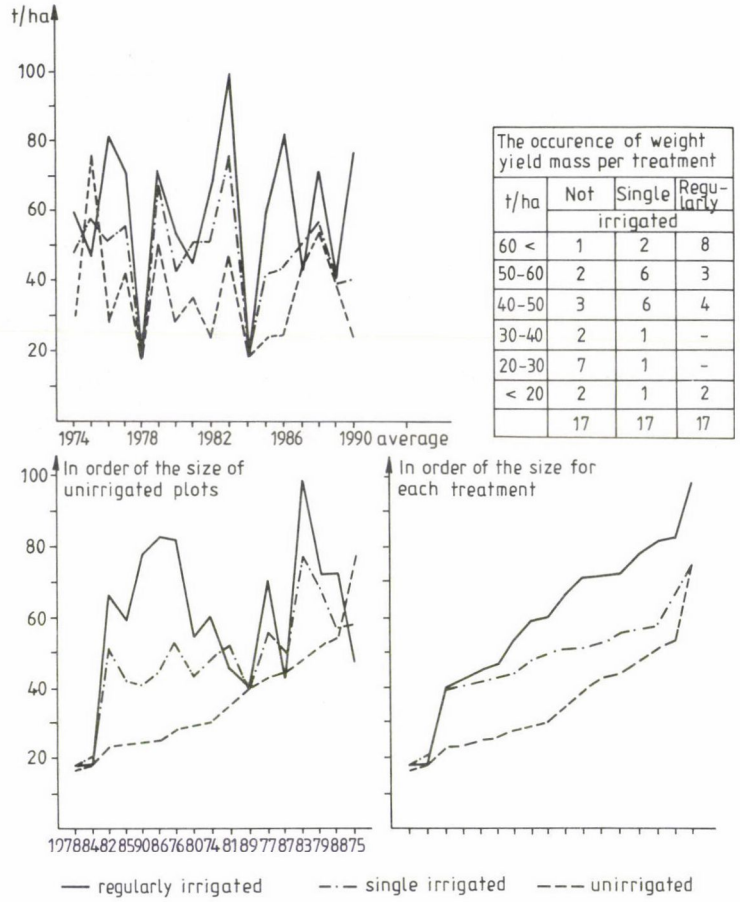
The water turnover operating within the system of relations between the plant and its environment is discussed by most of the specialist literature as one phenomenon called evapotranspiration (ET). However, this consists of evaporation (E) and transpiration (T), and their interaction, depending on the life stage of the plant stand and on the actual conditions. In this way the handling of these two processes as one, within the complex effect, conceals the roles of the part effects, restrains considering them consciously and the demand for their formation. Accordingly, it is desirable to judge the expected influence of evaporation and transpiration separately in the production under given conditions. So we should consciously reckon, in the course of production on the basis of precipitation, and when irrigating respecting the objectives of complementary water supply, with the soil moisture supplementary and refreshening-conditional effect of rain and/or irrigation water.

Keywords: evapotranspiration, evaporation, transpiration, interaction, water supplementary irrigation, refreshening-conditional irrigation

The productivity of plant production is strongly affected by the variable weather – mainly the changing of precipitation year by year and during the year – in Hungary. This condition means that the production of yield mainly depends on, and the varying temperature and humidity closely relates to, the amount of precipitation. Thus, the science of soil cultivation in Hungary considers proper management of precipitation use and the conservation of water to be essential for the plants (Gyárfás, 1925; Kemenesy, 1972; Cselőtei, 1964, 1971). The construction of cultivation technologies, the variety, the plant density, the soil cultivation, etc. are submitted to this.

To overcome the deficiency in water arising in certain periods, irrigation has developed significantly during the recent 3–4 decades. Accordingly, the complementary water supply, the time and dose of irrigation, especially the soil moisture content, were taken into consideration in the first stages. We have also constructed our treatments on the basis of small plot irrigation experiments over long periods, sometimes decades, with vegetables at the Department of Horticulture of the Gödöllő University of Agriculture. Beside the unirrigated plots as "second control" we applied plots the soil moisture content of which we kept between field water capacity and its rate % (50–60–70%) determined from the demands of the variety applied in the experiment. Practically, this means that irrigation is applied only after consuming 40–50–60% of the disposable water. On the basis of different

theories (different soil moisture level, number of irrigation, dose of irrigation, etc.) we applied further treatments between the two levels of water supply. As an example of the data obtained in this way, we list in Fig. 1 the results of three treatments in the period of 1974–1990, from the second half of our series of experiments with tomato, launched in 1961.



The occurence of weight yield mass per treatment

t/ha	Not irrigated	Single irrigated	Regularly irrigated
60 <	1	2	8
50-60	2	6	3
40-50	3	6	4
30-40	2	1	-
20-30	7	1	-
< 20	2	1	2
	17	17	17

Fig. 1. The effect of irrigation on the weight yield of tomato. Variety: Kecskeméti Jubileum, Gödöllő, 1974–1990

It is clearly reflected from the figure that keeping the soil moisture content at an optimum level resulted in a very variable extra yield, comparing the treatments without or with a single irrigation. Furthermore, in one case a depression of yield resulted. Analysing this, we can conclude that the reason lies in the different extent and rate of part effects within the complexity of irrigation, that are related to the weather before and during, but mainly after the irrigation. This, partly not depending on water supplement, mainly with air humidity increase and the cooling effect of evaporation, favorably or unfavorably influences the vital functions of the plant. Furthermore, the moisture conditions of the plant and of its environment can have

a different influences on the living organisms (diseases, pests) competing on the plant. These are reasonably taken into consideration already when choosing irrigation method (sprinkling, dripping, subsurface) and deciding the dose of irrigation.

The cooling effect of irrigation on plants and their environment – depending on the weather – lasts for days, although to a decreasing extent. In the course of our investigations of this type, depending on whether we moisturized the whole area or only a part of it (sprinkling, and furrow irrigation), we experienced significant differences (Cselőtei, 1968, 1991).

This is also supported by our irrigation dose experiments with early variety cabbage, where depending on the weather we observed substantial differences in the results. In these series of experiments in certain years the highest yields were obtained from applying irrigation several times, but with a smaller total amount of water (Table 1).

Table 1

The effect of irrigation on the yield of cabbage. Variety: Szentesi korai, Gödöllő

Year	Date of planting and harvesting	No. and dose of irrigations, mm	Quantity of irrigation water, mm	Leaves weight, t/ha	Yield weight, t/ha	Rate of head formation %
	14.04.	–	–	31.6	9.0	23.4
1980	26.06.	4×20	80	32.9	48.0	80.0
		3×40	120	33.7	35.8	58.3
	11.04.	–	–	35.2	46.8	87.5
1985	15.06.	4×20	80	42.0	61.4	86.7
		2×40	80	32.1	54.0	90.8
	09.04.	–	–	27.7	27.1	61.7
1987	29.06.	4×20	80	28.4	44.6	77.5
		2×40	80	29.8	49.5	80.8
	11.04.	–	–	31.0	52.6	91.1
1989	15.06.	2×20	40	39.5	65.6	93.3
		1×40	40	37.0	65.6	91.1
Average of	08.04.	–	–	33.5	31.6	61.1
nine experi-		3.2×20	64	37.3	58.1	84.8
mental years	17.06.	2.2×40	88	35.4	56.0	83.5

These and our other experiments (Cselőtei, 1965) since the beginning of our research activity also suggest that *among the complex effect of irrigation, the so-called refreshening-conditioning effects supplementing the moisture of the soil and influencing the moisture conditions and temperature of the parts of the plant above the surface and its surrounding should be observed rationally.* It has been realized that the development of the yield is indirectly related to the amount of water consumed by the plant (Fig. 2). First of all, it depends on transpiration, influencing

in this case the life activity of the plant through its temperature. There is an even less linear relation between yield and precipitation, and precipitation+the quantity of irrigation water consumed. The total effect of these depends on how the supplement of soil moisture deficiency and their refreshening–conditioning effect influences the life activity of the plant.

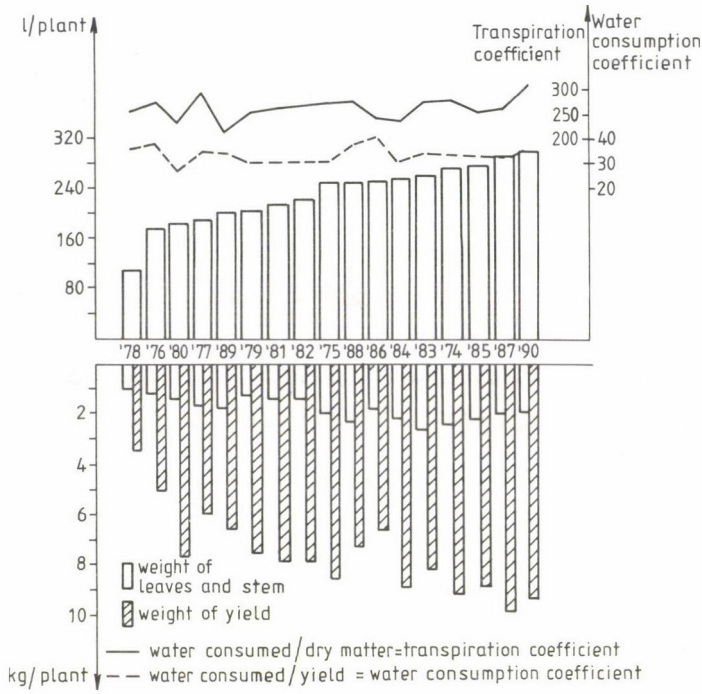


Fig. 2. The water consumption and yield of tomato depending on the year of growth in propagation dish. Variety: Kecskeméti Jubileum, Gödöllő, 1974–1990

Almost all of the references (Cserkaszov, 1952; Füri et al. 1974; Petrasovits, 1988) obviously show that, throughout the life activity of the plant, the dynamics of either evaporation or transpiration also develops differently. While the first changes around a certain level depending on the weather, the latter depends on the development of the leaves and, later, the ageing of the plant shows a consistently increasing then, after reaching the peak, a decreasing curve. This is completely obvious because the so-called evapotranspiration appears only as evaporation in the stage of sowing-emergence, where the continuous presence of sometimes only a few liters of water required for the expansion of the seed can only be supplied by the necessary emergence irrigation of several ten thousand or hundred thousand times as much an amount (50–100 m^3) of water, or by even more. The situation is the same after emergence, when the soil coverage is 1–2% and the plant develops only slowly and gradually, where the transpiration within evapotranspiration is minimal in the initiating phase and is only slowly overtaken by the transpiration. It is also obvious that the precipitation, as well as the irrigation water, differently influences the plant

and its environment, even in the phase of total coverage depending on the weather conditions and on the dose of irrigation water and on the method of irrigation.

The transpiration can decrease and the evaporation can increase again when the plant ages, and the stock undergoes the so-called "opening stage".

Conclusions

On the basis of our previously presented experiments and our other research, we think that within the complex effect of irrigation its part effects – although they prevail together, in the same time and are impossible or very difficult to separate experimentally – are reasonable to be considered separately. By conscious understanding and formation of the part effects, efforts should be made to achieve optimum influence on the life activity of the plant according to the conditions, objectives and methods of production (Cselőtei, 1964, 1971, 1991). Naturally, there can be obstacles deriving from irrigation technology and quantity of irrigation water. Finally, the method, time and the quantity of interference depend on the economic efficiency of irrigation. Besides the sensitivity of the plant produced under given conditions, this also depends significantly on how the expected extra production enables and requires satisfying the demands of the plant. The more and more widespread hydroponic production (e.g. vegetable propagation), because of different reasons in propagation investments, is a good example for this. Here with controlling water supply we influence mainly the temperature of the plant. In this course we practically exclude evaporation and influence individually the air humidity according to the demand of the plant. When affecting the moisture conditions of the plant and its environment we also pay attention to the fact of whether the suppression of competitor organisme (dieseases, pests) competing on the plant allows us to moisturize the plant when controlling the air humidity. It is a disturbing approach that, as a bad habit of propagation literature, sometimes this is referred to even in this case as evapotranspiration.

Summary

The relationship between plant and water, and indirectly related to this, the plant and heat (radiation) relationship, play a fairly significant role in plant and environment interaction. In this course *evaporation* (E) – affected by biological effects – is *physical, transpiration* (T) is physical environment dependent, *biological process* interacting each other. These two phenomena are discussed as one process, *evapotranspiration* (ET), by several authors. However, according to the growth-development of the plant, the quantity and conditions of natural precipitation or irrigation, these processes develops differently, largely contrary to each other. The more and more prevailing tendency to refer these two processes together as ET conceals *the role of part effects* of natural precipitation as well as irrigation *within the complex influence, and conceals their purposeful consideration, along with the demand for affecting them.* In this way, we can mention evapotranspiration several times, when coming to hydroponic propagation in propagation equipments. Although in that case we practically exclude evaporation, we control the humidity of plant-, water-environment separately. This is the way that precipitation and irrigation water can be taken as synonyms when analysing the water supply of a plant in field conditions.

For these purposes it is desirable to judge the expected effect of evaporation and transpiration one by one within the altering conditions in the course of production. According to the objectives of production – when irrigating the purposes of supplementary watering – we should purposefully reckon with the soil moisture supplementary and refreshening-conditioning effects of rain and/or irrigation water. In this way *normally our ambition is to create optimal transpiration and minimum evaporation in field conditions.* Furthermore, in propagation equipment – and even in highly intensive field production – *we should distinguish the water supply adjustment of the soil (root holding media) to optimum level from the control of air humidity.*

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Book reviews

ANNIE CHARTIER: *Glossaire de genetique molecular et genie genetique.* INRA Editions, Route de Saint Cyr, 78026 Versailles, Cedex, France.

The Terminological Committee of the French Ministry of Agriculture and Forestry charged Madame Annie Chartier, documentator of the INRA, with the task of compiling this dictionary. She completed the work with the assistance of four researchers: Madame Francine Casse–Delbart (CNRS/INRA) engaged in the molecular biology of bacteria, Monsieur Alain Deshayes (INRA) in the genetics of cultivated plants, Monsieur Claude Gaillardin (INA–PG/INRA) in the molecular genetics, and biology of yeasts and Monsieur Louis-Marie-Houdebine (INRA) in the genetics of animals. Their activities were mutually complementary.

This timely dictionary will be highly suitable to prevent the excessive use of English, or even "Franglais" (French English) scientific technical terms, if they can be expressed in acceptable French.

The dictionary undertakes to solve two tasks. It gives a brief, concise explanation of each of the 500 terms chosen. These explanations are excellent definitions of the subjects from a scientific standpoint, and the authors deserve credit for it.

I have the following problems: the genetic dictionaries known contain 4–5000 entries. Was it absolutely necessary to reduce the number of definitions so much? The other problem: among the authors there are prominent plant and animal genetists, but the entries show hardly any evidence of this. Such fundamental genetic concepts as polyploidy, cytoplasmic sterility, chromosome aberrations, etc. are not included in the dictionary. At least 95% of the subjects deal practically with molecular biology and genetic concepts only.

The second part of the dictionary summarizes the French versions of the English terms. An objection is raised against the practice that the French research workers use the English terms, even when there are equivalent French terms. For example: [the French term in brackets] linkage of group [pour groupe de liaison], screening [pour criblage], mapping Sl [pour cartographie Sl], gene cluster [pour clonage de genes], primer [pour amorce], shotgun [pour clonage en aveugle], nick translation [pour translation de coupure] ... In many cases there is no way to change the internationally accepted terms: at the most the orthography, and the endings of words of Latin–Greek origin, became French-like. I made a statistical analysis. Out of 130 terms, 24 could be replaced by French words. Sometimes this could be done only with one of the two words of the expression.

The English expressions have the advantage that often they are shorter than the French ones (nick, coupure simple brin: tailing, extension homopolymérique; ...).

This does not, however, mean that the linguistic laxity should not be opposed in order to protect the purity of language.

In Hungary, the Plant Breeding Committee of the Hungarian Academy of Sciences has a work-group engaged in improving the professional language. Several terms suggested by this work-group have been placed among the generally used professional terms, which is why I highly esteem the similar French endeavours.

A. BÁLINT

A. COMEAU and K. M. MAKKOUKK (Eds): *Barley yellow dwarf virus in West Asia and North Africa.* Proceedings of workshop, Rabat, Morocco, 19–21 November 1989. Published by ICARDA, 1992. Edited and designed by: Sayce Publishing, Exeter, United Kingdom.

This 239-page book deals with the complex problems raised by barley yellow dwarf virus (BYDV), which is perhaps the most important viral disease affecting cereals. In the Preface, the two editors reflect that "the choice of Rabat, Morocco as the venue for this meeting

was very appropriate, as this part of North Africa is one area where the BYDV problem is now known to have economic importance".

In the Introduction (pp. 1–30) it is stated that plant virus epidemics are induced and sustained through ecological associations linking environments with host plants, viruses and vectors; these associations are enhanced through the specific interactions between host plants and viruses, viruses and their vectors, and vectors and their host plants. When each of these interactive elements is, in itself, multifaceted, ecological complexity is greatly increased.

The five further sections of the book cover experimental results and the problems and challenges facing virus research.

The first part, "International and national programs on barley yellow dwarf virus" (pp. 31–67), contains 4 papers on research carried out within the framework of CIMMYT and ICARDA, and in national research programmes in Chile and Canada. The aspects of this work are germplasm screening, yield loss assessment, aphid trapping studies, identifying BYDV isolates, integrated control and resistance breeding.

Part 2, entitled "Reports from the West Asia/North Africa region" (pp. 69–111), consists of 6 papers providing detailed accounts of BYDV research in Morocco, Ethiopia, Jordan, Egypt and Kenya. The reader is presented with information on epidemiology, host range and strain identification, yield losses and the economic importance of BYDV.

The 4 papers in Part 3 (Breeding for resistance to barley yellow dwarf virus, pp. 113–152) discuss aphid infestation and damage, host plant resistance to aphids, breeding for resistance, and developing host plant resistance to BYDV.

Part 4, the longest section (pp. 153–206), covers "Methodologies for research on barley yellow dwarf virus" and includes 6 papers. It has been found that the symptoms of BYDV are inappropriate as a diagonistic tool, except in severe epidemic situations, so visual scoring must be followed by the ELISA test. The use of artificial inoculation with viruliferous aphids is a great help in achieving infection and selecting resistant or tolerant genotypes. Interesting data are presented on the purification of BYDV from dried leaf tissue and the production of antisera. Biotechnology is regarded as a "new weapon" against barley yellow dwarf virus.

Part 5 is entitled "Multiple stress resistance" (pp. 207–233) and contains 3 papers on the relationship between virus resistance and drought, the effect of BYDV on the root system of barley and the interactions between virus, aphids, plants and fungal diseases.

The book also contains a foreward, a list of contributors and workshop participants, and concluding remarks. There is a substantial quantity of literary references.

This will be of great use to virologists, plant protection experts, plant breeders and growers. It can also be recommended as a supplementary source of information to university lecturers and students.

L. SZUNICS

ADAM DALE and JAMES J. LUBY (Eds.): *The strawberry into the 21st Century.* Timber Press, Portland, Oregon, USA

This respresentative and excellently edited book of 292 pp with 7 colour plates, 22 black and white illustrations, and numerous line drawings, contains the *Proceedings of the Third North American Strawberry Conference* held in Houston, Texas, in 1990. At the Conference, marked as the most important forum organized so far in this field, scientists, growers and others from 35 U.S. states, 8 Canadian provinces and 13 other countries surveyed the recent achievements in research and in production of the strawberry along with the future developments and innovations which will continue into the next decade.

Strawberry is certainly one of the most popular fruits in the world, and its production has been growing dynamically during the last five decades. USA is the largest strawberry producer and California is the "Mecca" for strawberry-growers. These facts give a clear explanation why the North American Conference became the most reputable meeting of strawberry-people from all over the World.

Participants discussed the present state and defined the questions and tasks of strawberry-research for the next decade in four sections as follows:

1. Breeding, germplasm and biotechnology.

2. Physiology, production and post-harvest handling.

3. Biology and management of diseases and pests.

4. Systems approaches to managing strawberry production.

The above sections covered practically all fields of strawberry-research, and reflected the great changes that took place since the last Conference held 10 years ago. There were quite

a number of new aspects in research and the results were also more profound than earlier.

The papers were focused mainly on the following topics:

1. Improvement of the strawberry sortiment by a more effective inclusion of genetically important wild species into the breeding programme and by the use of sophisticated methods of biotechnology (gene-transformation, protoplast-fusion etc.). The rotation of strawberry cultivars is faster than that of any other fruit – it is about 10 years now. The most important tasks of present and future breeding are: increase in productivity, resistance against pests, diseases and environmental stresses and better adaptation abilities to various climatic conditions.

2. Improvement of production technologies.

3. Possibilities for improvement of plant protection taking into account the interest of consumers as well. Research is aimed at minimalization of pesticides by utilizing the results of resistance-breeding and the new possibilities for biological control. In this respect, central topics were the diseases Phytophtora, Botrytis and Colletotrichum.

4. Improvement of the quality of strawberry fruits grown for fresh consumption.

In conclusion, let me repeat that the book *The strawberry into the 21st century* is a treasure of valuable information for all scientists and growers dealing with the strawberry, and also for the people working in related fields of biology, trade and food technology. After reading it, we can only share the opinion of participants that conferences of this type should be organized more frequently and more internationally in the future.

J. PAPP

HORNOK, L. (Ed.): *Cultivation and Processing of Medicinal Plants.* Akadémiai Kiadó, Budapest, 1992. pp. 338. (Joint edition with John Wiley & Sons. Chichester, New York, Brisbane, Toronto, Singapore)

Throughout the last century, Hungary was a recognized supplier of medicinal and aromatic plants. Her rich traditions, combined with active and prosperous research activity, have resulted in a treasury of information on the large-scale production of this special group of crop plants, and this has now been finally published also for English language readers.

The present volume is the collective work of a team (eleven) of academicians and specialists in the large-scale production of medicinal and aromatic plants. The main aim of this English version is to provide a guide both for the growers and users of medicinal plants.

The volume, containing 114 illustrations (drawings, diagrams and photos) and 18 tables is divided into three main sections.

Part I deals with the following topics: General aspects of medicinal plants, Biological aspects, Technical and technological conditions for Medicinal Plant Cultivation, Primary processing of Medicinal Plants.

Part II provides detailed information on the cultivation of 35 medicinal plants grown in Hungary. Each plant is discussed according to a unified concept. Following a short introduction on the history of the crop and its geographic distribution, a detailed botanical description is given of the main species and its relatives under the subtitle "Characterization". The subchapter "Environmental requirements" surveys the main edaphic and climatic ecological factors that bear importance from the viewpoint of production. The subchapter "Cultivation" provides a detailed description of nutrient supply, soil preparation, sowing (multiplication), the care of plants, and harvesting.

Part III deals with another 23 species, which are mainly collected in their wild state. Wherever these are processed (e.g. *Achillea millefolium* L.) the production technology is also des-cribed.

The volume is concluded by two comprehensive tables (Tables 17 and 18) both of which are valuable for summarizing the main data of essential oil plants and the main growing data of medicinal plants, respectively. The great merit of this work is that it is the first comprehensive English summary of the vast scientific information available in Hungary on medicinal and aromatic plant cultivation and processing. The book presents this in a straightforward and clearly understandable style. Consequently, it can be recommended literature for both the specialist and those taking an interest in medicinal and aromatic plants.

Á. MÁTHÉ

FOSTER, ST. and DUKE, J. A.: *Eastern/Central American Medicinal Plants.* Peterson Field Guides Series, Houghton Mifflin Co., Boston, 1990.

The volume in hand is the first field guide survey of medicinal plants of the eastern and

central North America. Based on a practical approach, the book provides a tool for the identification of nearly 500 medicinal species.

The book starts by giving some brief, though necessary information on its general organization for plant identification, then describes the medicinal uses of each plant discussed. It is of special value that the authors have not forgotten to stress the importance of plant conservation, and, in close context with this, the proper methods of harvesting. Of not lesser importance are the words of caution given on the hazards of medicinal plant usage.

No matter how important the introductory chapters are, the main body of the book deals with the identification of medicinal plants. Should anyone be able to determine either the flower colour of the plant or its "life form" (shrub, trees, etc.) one cannot any more escape the success in locating the species in the book and obtaining some farther useful information on its morphological characters, habitats and uses.

It is a special value of the book that the tedious job of plant determination is facilitated by the accompanying line-drawings and the 48 colour plates of plant photos taken during the field trips.

The book concludes with the glossary of medicinal and botanical terms, as well as indexes to the plants and medical topics, all of them making the book very handy.

All in all, the reviewer has the impression that Peterson Field Guide Series has gained a lot by the publication of this latest volume, though it is doubtless that the main winners will be those using this book to their complete satisfaction.

Á. MÁTHÉ

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Acta Agronomica Hungarica, Vol. 42 (3–4), pp. 155–161 (1993)

Soil science and agrochemistry

EFFECT OF TRAFFIC AND URBAN-INDUSTRIAL LOAD ON SOIL

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The only sound way of acquiring really comprehensive reference data on trace-element levels is to analyse a statistically adequate number of samples for the elements in question. This has been done earlier for rural soils, field crops and all kind of mineral fertilizers used in Hungary. Several hundreds of samples were analysed for 27 elements using ICP technics (Kádár, 1991). This work is dealing mainly with soil heavy metal contamination caused by traffic, urban and industrial load.

The general picture of this survey showed that soils in urban areas are contaminated with a wide range of elements. This contamination is so marked that it over-rides differences based on soil parent material. Elements whose concentrations are shown to be generally enhanced in urban soils are: boron, cadmium, cooper, lead and zinc. Among macronutrients: Na, P, S.

It is now possible to identify a soil as having an urban or rural origin from its composition with respect to the elements which are common urban contaminants. It is so, even when its element composition is different as a result of geochemical factors.

There are two main trends affecting the rural and urban soil environments in very different ways. In the rural areas the main trend is slow depletion of the essential trace-element reserves in the soil. In urban and industrial areas, however, it is quite rapid contamination of the soil with both essential and nonessential elements. Soil found here are strikingly contaminated with Zn, Pb, Cu and Cd. In urban areas, effect of industrial pollution on soil are frequently superimposed on an elevated background level of conurbation. We are producing new soil environments with element composition quite unlike anything in natural soils.

Keywords: trace metal pollution, heavy metal load, soil, traffic

Introduction

While geological and biological changes on Earth are relatively slow, the cumulative effect of human activity grows exponentially and may result in undesirable changes in the environment. The accumulation of potentially dangerous chemical elements, of toxic heavy metals in particular, has a decisive sanitary and ecological influence on the environment (Purves, 1985).

To get a reliable picture of the accumulation of elements, a statistically sufficient number of samples has to be analysed. Such analyses of hundreds of soil, plant and fertilizer samples were carried out earlier, using the ICP technique (Kádár, 1991). The present paper intends to supply data on soil contamination caused by

traffic, settlement and industry. We do not wish to discuss a literary data in detail, although the subject has a vast literature both in Hungary and abroad.

The results reported in the literature often represent different circumstances, as the authors employed different methods or may have pursued different purposes. Not long ago, Hargitai (1990), for example, gave an account of the results of a 3-year work, in the course of which 97 soil profiles were exposed and examined for heavy metals in more contaminated regions of Hungary. To characterize the tolerable load of the soil the author attempts to determine the so-called environment protection capacity mainly on the basis of humus status and humus quality.

In the framework of an overall national survey, Regius (1990, 1991) analysed a large number of fodder crops for Mn, Zn, Mo, Ni, Cd content. The analysis of crops collected at different growing sites was completed by the analysis of hair samples and organs taken from animals at the same sites. The author found correlations between the element contents of soils, crops and animals. Crops near power plants and highways, as well as animals in the vicinity, were examined for Pb and Zn content in the same way. In the extensive investigations the aspects of feeding were dominant.

Materials and methods

Our investigations took place along the highway "M-7" and over the area of the capital. Separate samples were taken in the sparsely populated (Buda), the densely populated (Pest) and the industrial quarters of Budapest. To be able to judge the background contamination, we give the element concentrations at the Institute's experiment stations (arable soils in rural areas):

Nyírlugos – acid sandy soil (Nyírség, North-East Hungary),
Őrbottyán – calcareous sandy soil (Duna–Tisza Interfluve),
Nagyhörcsök – calcareous loamy chernozem (Mezőföld, South-Transdanubia),
Martonvásár – degraded calcareous loamy chernozem (Transdanubia).

Soil samples were taken from the upper 10 centimetres; 15–20 samples represented an average sample. With the effect of the prevailing north-western winds taken into consideration, sampling took place along the south-eastern side of the highway, at distances of 1, 5, 10, 30, 100 m from the road. At the same time plant samples were also taken, mainly from fields where the soil was undisturbed and uniformly covered by grass. Sampling was repeated 5 times while moving towards Budapest, in order to be able to chek statistically the effect of environment contamination. The plants were not washed before analysis, because sampling was preceded by several days of rainy weather, and the samples were free of dust (Kádár, 1992).

Sampling took place late in autumn, between 31 October and 2 November 1991, so that on the ageing plants the cumulative effect of contaminations could be better detected. It is well known that in periods of frequent spring rains and rapid plant growth the plants are less contaminated. Furthermore, the effect of dilution makes it difficult to demonstrate the accumulation of elements. The available element content of the soils was determined by the ammonium acetate + EDTA method (Lakanen and Erviö, 1971), and by the conventional KCL + EDTA extraction. Since the two methods were of equal tendency in reflecting the changes, and only the absolute concentrations differed, only the results obtained by the ammonium acetate + EDTA method will be given below.

Results and conclusions

As seen from the data of Table 1, Mn occurs in the largest quantities in the soils, on an average. It does not indicate contamination. With the heavier soil texture, the concentration of the available Mn increased in the soils of cultivated arables. In the immediate vicinity of the highway (on the grass-covered shoulder of the road), the lower concentration of Mn was also in connection with the looser soil texture. The available Na and P contents of the shoulder exceeded many times those of the soils found at some distance from the road, and by nearly one order of magnitude the Na and P supplies of the uncontaminated rural arables. The Na may originate from salting and the P from washing the roads.

Table 1

Available element-content of the upper 10 cm soil along the highway "M-7" (Sampling: 31 Oct. 1991)

Distance from the road, in m	NH_4-OAc+EDTA soluble, ppm						
	Mn	Pb	Zn	Cu	Ni	Co	Cd
1	161	411	412	25	2.0	1.1	0.58
5	225	38	14	10	2.1	1.3	0.19
10	302	22	33	13	3.1	2.0	0.17
30	292	24	55	10	3.2	2.0	0.17
100	292	15	14	11	3.4	1.9	0.16
Mean	254	102	102	14	2.7	1.7	0.26
L.S.D.$_{5\%}$	136	175	289	12	1.8	1.1	0.11
Cultivated rural soils as background contamination							
Nyírlugos	64	1	2	2	0.3	0.3	0.03
Őrbottyán	147	2	3	2	1.4	0.7	0.09
Nagy-hörcsök	410	4	3	5	3.5	2.1	0.15
Marton-vásár	462	7	4	6	5.4	3.5	0.15
Mean	271	4	3	4	3	2	0.10

The amounts of Ni and Co (and of the B elements not discussed here) increased with the heavier structure of the cultivated soils, but their concentrations did not practically change nearer the highway. Among the elements examined Pb, Zn, Cu and Cd accumulated in the soils along the highway; the accumulation was statistically demonstrable and represented changes in order of magnitude compared to the topsoil of the rural arable. Out of the heavy metals, Pb and Cd also showed an increasing tendency in the cultivated land, in the function of the soil structure.

Out of the analysis data of the grass cover, only those for Zn, Pb, Cu and Cd, which reflect contamination, are given in Table 2. The accumulation of all the four

elements – examined as a function of the distance from the highway – is significant and statistically demonstrable. Accumulation on the shoulder of the road suggests that the contaminants partly fall directly on the road from where they are carried with the dust or washed by the rainwater onto the shoulder.

Table 2

Zn, Pb, Cu and Cd content of the grass along the highway "M-7" (Sampling: 31 Oct. 1991)

Distance from the road, in m	Element content in mg/kg DM			
	Zn	Pb	Cu	Cd
1	111	77	11	0.2
5	31	22	5	0.1
10	33	22	6	0.1
30	30	16	6	0.1
100	30	17	6	0.1
Mean	47	31	7	0.1
$L.S.D._{5\%}$	25	18	2	0.05
Uncontaminated*	30	1–10	5	0.1

* Based on literature (Kádár, 1991)

Another part of the contaminants of the atmosphere may remain for some time in the air and be carried by the prevailing winds to more distant areas. Although the traffic on the highway is heavy, the contamination of the grass appears to be moderate compared to the limit concentrations in the literature, probably because the highway has been used for only 3 decades.

In Table 3, data on soil contamination showing the effect of urbanization and industrial activity (certain quarters of Budapest) and of traffic compared to the less contaminated cultivated soils of the country are summarized.

Out of the ammonium acetate + EDTA soluble elements, S was present in the largest quantities; its value was 35 ppm in the cultivated rural fields, 52 ppm along the highway, 70 ppm in Budapest and 140–160 ppm in the heavily contaminated industrial districts of the capital (Nagytétény, Csepel). In towns and industrial regions, the source of S can be attributed, to the burning of brown coal, a predominant energy carrier since the turn of the century. The changes in B content (not given in the table) are also related to the use of brown coal; while its concentration in the rural soils is about 1 ppm, it has increased to 2–3 ppm in soils around the capital.

Zn, as a contaminant has increased to 3 ppm in the rural soils, to 60 ppm in Budapest and 102 ppm along the highway, which is 20–30-fold on the average. Pb shows a similar picture. Lead contamination was found to be extremely high at road junctions in Budapest and in certain industrial districts, even exceeding 100 ppm. Cu has increased 3–4-fold along the highway and 6-8-fold in Budapest, compared to the rural soils. While Ni and Co cannot be regarded as contaminants, Cd shows a 5-fold accumulation.

Table 3

Available element-content of the upper 10 cm soil as affected by urban, industrial and traffic activity in Hungary

Sampling sites	NH_4-OAc+EDTA soluble, ppm								
	S	Zn	Pb	Cu	B	Ni	Co	Cd	Na
Sparsely populated areas (Buda), (n = 14)									
Rózsadomb (TAKI)	71	37	30	21	2.0	1.8	0.9	0.4	–
Városmajor	55	33	44	20	2.7	1.7	0.6	0.4	–
Vérmező	65	42	38	66	3.9	1.9	0.7	0.4	1
Széna tér	93	65	101	27	2.8	2.1	0.6	0.6	8
Mean	69	43	52	35	2.9	1.9	0.7	0.4	2
CV %	31	34	65	125	30	12	22	21	325
Densely populated areas (Pest), (n = 18)									
Andrássy út	75	51	79	16	2.4	1.7	0.6	0.5	59
Városliget	49	38	39	20	2.9	1.8	0.6	0.6	–
Népliget	31	69	52	27	1.8	1.6	0.5	0.6	–
Mátyásföld	83	50	108	6	1.0	1.3	0.6	0.4	28
Mean	70	61	69	18	2.4	1.6	0.6	0.5	19
CV %	70	68	71	68	75	30	34	56	210
Industrial areas (Pest), (n = 20)									
Kőbánya	38	37	19	12	2.8	2.7	1.4	0.5	–
Pestlőrinc	34	22	20	24	0.8	1.0	0.8	0.2	3
Csepel	140	131	55	52	3.0	1.6	0.6	0.6	–
Nagytétény	160	96	151	36	3.5	1.8	0.7	0.7	3
Mean	93	72	61	31	2.5	1.8	0.9	0.5	2
CV %	124	92	105	74	56	44	44	53	152
Budapest (n = 52)									
Mean	79	60	61	28	2.6	1.8	0.7	0.8	8
CV %	98	81	84	101	55	33	41	49	323
Highway "M-7" (n = 25)									
Mean	52	102	102	14	2.0	2.7	1.7	0.3	172
CV %	78	213	128	66	36	48	49	32	98
Rural plowland (Research stations' fields), (n = 8)									
Mean	35	3	4	4	1.1	3.7	1.9	0.1	2
CV %	37	49	76	45	94	74	70	42	133

Ultimately, it can be said that both the soils and the plants indicate the accumulation of the contaminants resulting from the load on the environment. A sudden increase in the available quantities of Pb, Cd, and partly of Zn, gives cause for serious alarm. Comprehensive studies will be required to acquire a better knowledge of the rate of contamination of urban gardens that ensure the vegetable supply of the capital, and of the soils and horticultural crops around the towns. It is not known to what extent this supplying area of 30–40 km radius contributes to the lead and cadmium load of the 2–

2,5 million inhabitants. By a simple analysis, urban soils can already be differentiated from rural ones on the basis of the accumulation of some heavy metals, which reflects anthropogenic effects.

Conclusions

The mobile toxic element content determined by the ammonium acetate + EDTA method in the soils of the "M-7" highway and of Budapest was examined in 1991 with the ICP technique. Separate samples were taken in the sparsely populated (Buda), the densely populated (Pest) and the industrial quarters of Budapest. For judging the background contamination, ploughed soils of the Institute's experiment stations served as a basis: acid sandy soil (Nyírlugos), calcareous sandy soil (Őrbottyán), calcareous loamy chernozem (Nagyhörcsök) and degraded chernozem (Martonvásár). The samples were taken from the upper 10 cm soil layer, 15–20 samples representing an average examination. The major conclusions are summarized below:

(1) Out of the elements examined, Mn, Ni and Co did not represent contamination. Supplies of these three elements showed positive correlation with the soil structure.
(2) The available Na and P contents of the road shoulder were manifold compared to those in normal soils. Na may originate from salting, and P from washing the roads. Out of the macroelements, S accumulated 3–4-fold in the soil of the capital's industrial quarters, while the microelement B 2–3-fold. Their presence is due to the burning of brown coals, a predominant energy carrier from the beginning of the century.
(3) Among the heavy metals, the accumulation of Zn, Pb, Cu and Cd demonstrable both in the soils and in the plants, may cause concern. Soils reflecting anthropogenic effects can today be differentiated from the rural arable soils by a simple analysis. While the cultivated rural soils become poorer in essential microelements over a longer period, in the anthropogenic soils a rapid accumulation of some essential and nonessential elements can be observed.
(4) Comprehensive studies will be required to acquire a better knowledge of the rate of contamination in the soils and horticultural crops of suburban gardens that supply the vegetable diet of the town inhabitants, to be able to work out interventions preventing the toxic elements from entering the nutrition chain.

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RELATIONSHIP BETWEEN SOIL MOISTURE, GROWTH, YIELDS AND NITROGEN FIXATION IN SELECTED GRAIN LEGUMES

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(Received: 28 May, 1991; accepted: 8 January, 1993)

The effects of five regimes of soil moisture on growth, yield and nitrogen fixing ability of two important food legumes were evaluated. The species selected were cowpea *(Vigna unguiculata Walp.)* and mungbean (*Vigna radiata* (L.) Wilczek), due to their widespread cultivation in the tropics. The soil moisture regimes were field capacity, with depletions of 20%, 40%, 60% and 80% of water held by the soil between field capacity and air-dried conditions.

Cowpea was more adapted to low soil moisture regimes. Mungbean plants grew better and produced higher per plant yields at higher soil moisture regimes. Depletion of soil moisture decreased all measured parameters of mungbean to a greater extent than in cowpea, although the responses differed in the two species. Per plant yields illustrated the ability of cowpea to produce a greater yield of seeds under dry conditons.

Depletion of soil moisture had an adverse effect on nodulation and nitrogen fixation of both legumes. Nodulation and nitrogen fixation of mungbean were affected to a greater extent than in cowpea. Cowpea maintained nodule activity at low soil moisture regimes. The better adaptability of cowpea to lower soil moisture conditions, which is a common phenomenon and a primary determinant of grain legume productivity in the tropics, is presented.

Keywords: *Vigna unguiculata, Vigna radiata,* soil moisture, nitrogen fixation, yield analysis

Introduction

Legumes are an important component of production systems in tropical and temperate agriculture. In the temperate world, pasture legumes play a vital role in maintaining productivity and nutritional quality of animal fodder. In contrast, food legumes are more important in the tropical world, due to their ability to provide a source of protein to poor quality diets, their economic value in terms of producing relatively high yields and their inherent ability to utilize atmospheric nitrogen in association with rhizobia by nitrogen fixation (Okigbo, 1977; Carangal et al., 1987).

The area of food legumes cultivated under irrigation is very low in most countries, due to the prominence given to cereals and other staple food crops.

Thus, legumes are generally cultivated in marginal areas under rainfed conditions or by utilizing residual moisture after a cereal crop, and yields of food legumes vary widely in the tropics. This yield fluctuation can be attributed to the adverse effects of moisture stress on growth and nitrogen fixation of legumes, as studies (e.g. Muchow, 1985; Villalobos-Rodriguez and Shibles, 1985) illustrate the high susceptibility of these species to moisture deficits.

The effect of water stress on nodulation and nitrogen fixation in food legumes is less clearly defined in the tropics, where this phenomenon is vital for successful cultivation. Reports (e.g. Eaglesham and Ayanaba, 1984) identify irreversible

damage to nodules under conditions of moisture stress. However, adverse effects of water stress on nitrogen fixation of food legumes is not well quantified, and most studies (e.g. Vincent, 1982; Eaglesham and Ayabana, 1984) identify the effect of water deficits on rhizobial populations, inoculation process and nodulation.

Cowpea *(Vigna unguiculata,* Walp.*)* and mungbean *(Vigna radiata* (L.) Wilczek) are popular food legumes of the tropics, due to their adaptability to a wide range of environments. Generally, mungbean is considered more susceptible to moisture stress than cowpea and its cultivation is limited to relatively high rainfall areas (Rachie, 1977). However, reports do not clearly identify differential responses of these crops to dry conditions. Thus, a study was carried out and repeated under partially controlled conditions to evaluate the effect of different soil moisture regimes on the establishment, growth, yields, nodulation and nitrogen fixation of these two important food legumes.

Materials and methods

The studies were carried out as pot experiments at the University of Peradeniya (7 °N, 81 °E, 420 m above sea level). The mean environmental conditions within the plant house facility over the experimental period were: temperature 27.9 °C ±1.34°C; relative humidity 78.4% ±3.76, and a 12–13 hour day length. The experimental design on each occasion was a complete randomized design with four replicates.

The planting medium used for the study was a mixture (1:1) of top soil and seived river sand. Some important characteristics of the planting medium were pH (1:2.5 H_2O) 6.6 ±0.45; total N 0.15%; total organic C 1.24% and a CEC of 25.16 meq/100 g soil.

Prior to the initiation of the trial, the water holding capacities of the soil when air dried and at field capacity were determined. The water content held by the soil was 18.5 ±0.14% by weight per kilogram of air-dried soil. The soil moisture regimes used in the study were field capacity, 20%, 40%, 60% and 80% depletion of the water content held between field capacity and air-dried conditions. These regimes were maintained from planting up to the final harvest of both crops, by weighing the pots at 3–4 day intervals and adding the required quantity of water to bring the soil moisture up to the desired level. Each treatment consisted of five pots per species.

Plastic pots (2.5 litre capacity) were filled with 2.5 kg of air-dried soil, and the required quantities of water added to obtain the desired level of soil moisture. A basal fertilizer equivalent to 10 kg N, 62.5 kg P_2O_5 and 70 kg K_2O per ha was added to each pot. Thereafter, uniform seeds of cowpea (cv. MI 35) and mungbean (cv. MI 5) (germination 94% ±1.57%) were inoculated with a broad spectrum *Rhizobium inoculum* containing *R. phaseoli* and *R. leguminosarum* and planted at a density of five seeds per pot. The soil surface was covered with aluminium foil to prevent excessive evaporation.

The measurements made on both species were germination, survival and shoot and root growth up to 50% flowering. The latter was determined by obtaining dry weights at weekly intervals. These dry weights were used to determine growth rates by regression analysis using the equation $\text{Log}_e Y = A + BX$, where Y and X are dry weights (g) and time (days) respectively. In addition, flowering, yield components and per plant yields were determined on three tagged plants per treatment.

At 50% flowering and pod set, three plants per treatment per replicate were carefully uprooted. The number of nodules per plant and their fresh weights were determined on two plants. The root system of the other plant was used to determine nitrogen fixation. Acetylene Reduction Assay was used for this purpose as per method described by Hardy et al. (1968). The data was analysed for determining significant differences among treatments by methods described by Steele and Torrie (1981).

Results and discussion

Germination, survival and vegetative growth patterns of the selected legumes are presented in Table 1. In both species, germination is not significantly affected until 80% depletion of soil moisture. However, the reduction in germination is greater in mungbean. This illustrates the greater ability of cowpea to germinate under very low soil moisture conditions.

The pattern of survival of germinated seedlings varies from that of germination. The mungbean has a greater percentage of seedling survival at higher soil moisture, which could be due to the susceptibility of cowpea seedlings to moist conditions. In contrast, with increasing soil moisture depletion, survival of mungbean is affected to a greater extent. Thus, at a depletion level of 60%, mungbean seedling survival is reduced significantly, while cowpea shows a similar effect at 80% depletion of soil moisture. The reduction in seedling survival is also greater in mungbean (53%) than in cowpea (24%) at 80% depletion when compared with survival at field capacity. This clearly indicates the greater adaptability of cowpea to drier environment as suggested by Rachie (1977) and Wood and Myers (1987).

Table 1

Effect of soil water status on germination and growth of selected legumes

Soil water status	Germination (%)	Survival (%)	Shoot growth*	Root growth*
MUNGBEAN				
Field capacity	85.65	94.51	0.1743	0.1042
20% Depletion	82.61	86.95	0.1611	0.1284
40% Depletion	86.54	85.29	0.1365	0.1412
60% Depletion	83.14	69.65	0.0966	0.0754
80% Depletion	65.87	41.37	0.0512	0.0632
L.S.D. (P=0.05)	7.45	12.66	0.0102	0.0098
COWPEA				
Field capacity	81.20	85.73	0.1956	0.1253
20% Depletion	84.39	81.40	0.1811	0.1198
40% Depletion	82.55	86.34	0.1646	0.1422
60% Depletion	79.59	74.52	0.1124	0.0986
80% Depletion	73.24	61.81	0.0727	0.0818
L.S.D. (P=0.05)	6.32	9.87	0.0056	0.0023

* Shoot and root growth rates estimated by the regression equation $\text{Log}_e Y = A + Bx$, where Y and x are dry weights (g) and time (days) respectively.

Cowpea plants show better shoot growth rates at all soil moisture levels (Table 1). Thus, at field capacity, shoot growth rates of cowpea is 11% greater. Shoot growth rates of both species are affected by soil moisture depletion. Thus, significant reductions are observed beyond a 40% depletion of soil moisture. This effect is greater in mungbean and, at 80% depletion, shoot growth rate is 41% less than that of cowpea.

Root growth is also affected by reductions in soil moisture and cowpea plants show higher rates of root growth at all moisture levels. The reduction in root growth, which indicates the adaptability in root to dry conditions, is greater in mungbean (40%) than in cowpea (34%) when soil moisture is reduced from field capacity to 80% depletion.

Shoot growth of both species is affected to a greater extent than root growth by reduction in soil moisture. This could be attributed to the greater development of root systems under dry conditions. The greater reduction in shoot and root growth of mungbean with decreasing soil moisture also illustrates the greater susceptibility of this species and the greater adaptability of cowpea to dry conditions.

Table 2

Yield components and yield of mungbean and cowpea plants at different soil moisture levels

Soil water status	Days to flower	Flowers/plant	% Pod set	Seeds/pod	100 Seed wt (g)	Yield/plant (g)
MUNGBEAN						
Field capacity	32.30	26.50	84.50	8.75	5.89	11.26
20% Depletion	33.40	27.75	89.25	8.80	5.66	11.43
40% Depletion	29.30	19.50	78.75	7.45	5.06	6.68
60% Depletion	26.00	15.70	71.25	6.80	4.67	3.85
80% Depletion	23.30	11.65	65.00	6.19	3.85	2.94
L.S.D. (P=0.05)	2.17	1.87	4.35	0.68	0.25	1.08
COWPEA						
Field capacity	48.70	16.85	78.50	8.30	9.55	10.56
20% Depletion	45.30	17.50	80.00	8.05	9.10	10.35
40% Depletion	42.30	15.85	75.25	7.50	9.45	9.43
60% Depletion	36.00	12.85	73.00	7.15	8.70	6.14
80% Depletion	34.40	10.75	69.75	6.40	7.65	3.97
L.S.D. (P=0.05)	3.28	2.33	3.69	0.35	0.18	0.96

Cowpea is generally a longer living species when compared to mungbean, and thus flowering occurs later. However, the effect of decreasing soil moisture affects both species in a similar manner (Table 2) by reducing the time to flower by approximately 29%, due to 80% depletion of soil moisture. In addition, the reduction in time taken by both species to flower becomes significant at 40% depletion. This phenomenon could be attributed to the early maturing habit of legumes under adverse ecological conditions (Turner and Kramer, 1980).

Mungbean generally produces a greater number of flowers than cowpea (Table 2). However, the flower number per plant is reduced to a greater extent at a lower soil moisture depletion level (40%) in mungbean, while cowpea shows a similar effect at 60% depletion. Both species show a marginal increase in flower numbers at 20% depletion. Although this phenomenon is not significant and not evaluatad in the present study, it could be attributed to the extension of the vegetative phase under conditions of high soil moisture. This warrents further study.

Moisture stress has a significant bearing on yield components and yield of food legumes (Muchow, 1985). This is highlighted by this study, and all measured yield components are affected by increasing soil moisture stress (Table 3). The effect of low soil moisture on percentage pod set in mungbean is greater than that observed in cowpea. Thus, significant reductions occur at 60% depletion of soil moisture in both mungbean and cowpea.

Table 3

Nodulation and nitrogenase activity of mungbean and cowpea at different soil moisture levels

	At 50% flowering		*At pod set*			
Soil water status	Nodules/plant	Nodule wt (g)	Nodule activity*	Nodules /plant	Nodule wt (g)	Nodule activity*
MUNGBEAN						
Field capacity	26.00	0.634	4.324	34.00	0.924	7.718
20% Depletion	23.50	0.596	3.411	27.00	0.821	5.112
40% Depletion	14.50	0.326	2.526	17.50	0.569	3.076
60% Depletion	10.00	0.144	1.175	12.00	0.204	2.065
80% Depletion	6.00	0.079	0.644	5.50	0.096	-
L.S.D. (P=0.05)	2.54	0.011	1.213	2.46	0.008	0.991
COWPEA						
Field capacity	21.50	0.934	3.024	28.50	1.256	5.122
20% Depletion	24.50	0.997	3.452	26.50	1.096	4.984
40% Depletion	20.00	0.865	3.241	23.00	0.974	4.178
60% Depletion	15.50	0.643	1.845	17.50	0.723	2.249
80% Depletion	11.00	0.219	0.968	10.00	0.214	0.638
L.S.D. (P=0.05)	1.98	0.032	0.857	0.76	0.035	1.298

* Nodule activity was measured by Acetylene Reduction Assay as μmol C_2H_4/plant/hour

Seeds per pod and seed development (measured by 100 seed weight) of both species are significantly reduced by soil moisture stress. Again, mungbean is more susceptible to low soil moisture. However, in mungbean the seed development is affected to a greater extent than is the number of seeds per pod. The reduction is 100 seed weight and number of seeds per pod, when mungbean is grown at 80% depletion, in comparison to that of plants at field capacity, is 29% and 34%

respectively. In contrast, the reverse effect is seen in cowpea, where the seeds per pod are reduced by 24%, when compared to a 17% reduction in 100 seed weight when the crop is grown at the highest and lowest soil moisture regimes. This indicates that responses of yield components of legumes to soil moisture stress varies with species.

The effect of the soil moisture regimes on yields also illustrate the greater susceptibility of mungbean. Thus, while mungbean produces greater per plant yields at higher moisture levels, productivity is reduced by 75% at the lowest soil moisture regime, when compared to yields at field capacity. In contrast, the yield reduction in cowpea at 80% depletion of soil moisture is 67%. A comparison of the two species also show that, at lower soil moisture levels, per plant yields of cowpea exceed that of mungbean. Thus, as these crops are generally grown at similar densities (Gunasena, 1974), cowpea has the capacity to produce greater yields per unit area under dry conditions, due to its better adaptability to lower moisture levels. The results also suggest the possibilities of per plant yields of cowpea exceeding yields of mungbean by over 100%, under dry conditions. However, under adequate moisture conditions, mungbean plants produce greater yields.

Nodulation and nitrogen fixation in legumes are sensitive to changes in soil water (Ahmed and Quilt, 1980). This is associated with the poor survival of rhizobia under conditions of low moisture (Boonkerd and Weaver, 1982). The results of this study also illustrate the adverse effect of low soil moisture on nodulation and nitrogen fixation in two important food legumes. All measured parameters of nodulation and nodule activity decline with increasing soil moisture stress, and the rate of reduction is greater in mungbean, which is a more promiscuous nodulator. Thus, while nodulation and nitrogen fixation of cowpea are lower at both harvests, the process is less affected by soil moisture depletion. This could be attributed to the greater adaptability of this crop to the condition of low moisture, due to the close relationship between host growth and the process of nodulation and nitrogen fixation (Henzell, 1988).

The process of nodulation and nitrogen fixation of both species varies with time. However, this is also affected by soil moisture. Thus, at low soil moisture levels, the rate of increase in nitrogen fixation with time is significantly lower. This shows the susceptibility of nitrogen fixation to soil moisture, and the rate of declination varies with time and species. Also, the more adaptable species, cowpea shows some nodule activity at the lowest soil moisture level at the second harvest, when mungbean plants do not show any sign of nitrogen fixation.

Conclusions

Food legumes are grown in a wide range of environments due to their importance in tropical agriculture. Their degree of adaptability varies, and thus, selection of a crop to a given environment must be carried out carefully in order to

obtain optimum productivity. The results of this study show the responses of two important food legumes to soil moisture, which is considered to be a major limiting factor to tropical agriculture. The results indicate a greater adaptability of cowpea to soil moisture in terms of plant growth, yielding ability and nitrogen fixation. However, under conditions of adequate moisture, mungbean can produce a greater yield and utilize atmospheric nitrogen more effectively. In contrast, under conditions of low moisture, which is a common phenomenon in the tropics, cowpea, which has the ability to fix atmospheric nitrogen and produce some yield, can be considered a more suitable crop.

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HEAVY METAL CONTENT IN CEREALS IN INDUSTRIAL REGIONS

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In industrial regions loaded with heavy metals in different degrees larger quantities of heavy metals are accumulated by wheat and barley than by maize.

The intensive root system of all three monocotyledonous crops has a higher capacity for heavy metal accumulation and a higher transfer factor of heavy metals than that of dicotyledonous weed plants in the same habitat.

Where the quantities of Cd, Cu, Pb and Zn exceeded the tolerable values determined for the soils, the heavy metal content in the grains of barley and maize did not exceed the so-called limit values of consumption and feeding, respectively.

The industrial regions loaded with heavy metals are suitable first of all for cereal production (provided the heavy metal content in the soils is not increasing).

Keywords: heavy metals, *Hordeum distichon, Triticum vulgare, Zea mays, Convolvulus arvensis, Ambrosia elatior, Cirsium arvense, Amaranthus retroflexus, Atriplex tatarica*

Introduction

In consequence of an industrialization during recent decades, the heavy metal content in the soils has increased in Hungary, as it has worldwide. A part of the agricultural areas of Hungary is situated in industrial regions (thermal plants, oil-refineries, foundries etc.) more or less loaded with heavy metals.

The ever-rising amount of heavy metal load originating from background contamination must also be taken into consideration (cf. Mészáros et al., 1988; Bozó and Horváth, 1992; Bozó et al., 1992).

In Hungary the estimated value of Cd and Pb deposition is 0.63 and 6.68 mg/m²/year, respectively.

The heavy metals accumulate in the soil and some plants take up larger quantities of them. They are translocated into the vegetative and generative organs in various degrees. The physiological processes of plants and their quality are influenced by the heavy metals. Through the food chain they may be introduced into human and animal organisms.

Since in certain compounds and concentrations several heavy metals can be toxic (e.g. Cd, Pb etc.), it is necessary to determine their quantities in the various organs of plants used for human or animal consumption.

Materials and methods

Our investigations were carried out in the following industrial regions (the loading plants are indicated):
Ajka (thermal plant, aluminium factory, glass factory)
Százhalombatta (thermal plant, oil-refinery)
Dunaújváros (ironworks)
Nagytétény (lead-works).

The soil and plant samples were taken from 5 sites of each region, in an area of 500 m radius around the respective emitters, in the direction of the prevailing wind.

The plants examined were: wheat (*Triticum vulgare* – Ajka, Százhalombatta, Dunaújváros), barley (*Hordeum distichon* – Nagytétény), maize (*Zea mays* – Ajka and Nagytétény).

At two sampling sites (Dunaújváros, Nagytétény) the heavy metal contents of some more frequent weed species among the cultivated plants were also determined.

The soil samples were taken from the top 0–20 cm layer.

The soil samples, as well as the plants, which had been previously washed in distilled water and separated into organs, were treated with HNO_3 (in teflon bombs), then examined by ICP-AES spectrometry in the Atomspectroscope Laboratory of the University of Horticulture and Food Industry.

Results

The heavy metal contents of soils

The heavy metals detectable in the soils, and their quantities are determined by the geochemical environment and the emission of the local industry.

In the regions concerned, the loading heavy metals depending on the emitters are: Ajka: Mn, Ni, V (out of the other elements Al was also taken into account here); Dunaújváros: Cr, Fe, Zn; Százhalombatta: –, Nagytétény: Cd, Cu, Pb, V, Zn.

Boldis (cit. in Kádár, 1991) determined the average heavy metal content for 10 soil types. These average values are exceeded by the Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn contents in the soils of the industrial regions examined by us.

Only in soils of Nagytétény were Cd, Cu, Pb and Zn found in amounts exceeding the tolerable values established for the soils (Table 1).

Heavy metal contents in cereals

The largest quantities of heavy metals were found to be accumulated in the roots of wheat (Table 2). The chemical composition of the roots also indicates the heavy metal content of the soil.

In the region of Dunaújváros the Fe load is indicated by the high Fe content in the roots and leaves. In comparison with the samples from Ajka and Százhalombatta, the quantities of Zn and V are also the largest. The extent of translocation is well indicated by the Fe content in the stalk and leaf.

Table 1

Heavy metal content in soils (μg/g dry weight)

Elements	Ajka		Dunaújváros	Százhalombatta		Nagytétény		1	2
	average	extreme values		average	extreme values	average	extreme values		
As	0	0	0	0	0	0.16	0–1.0		
Cd	0.6	0.1–1.5	1.7	1.4	1.0–1.8	6.0	3.0–11.3	1	1–3
Co	7.1	5.6–13.2	5.7	5.5	3.7–7.4	4.4	4.0–4.7		
Cr	14.6	11.7–20.9	11.4	9.4	5.8–13.1	13.0	9.9–16.5	50	
Cu	8.9	7.4–13.6	17.2	18.7	7.2–30.3	277	116–426	50	50–140
Fe	11000	9213–14023	9625	6799	4566–9033	7476	6768–7794		
Mn	700	444–1837	459	420	260–581	233	193–280		
Ni	16.1	13.4–25.8	17.0	15.5	9.4–21.6	16.6	14.0–22.1	40	30–750
Se	0	0	0	0	0	0	0		
V	15.7	11.6–24.2	9.9	8.9	6.1–11.8	24.9	12.2–67.9		
Zn	37.8	35.6–45.1	55.4	30.1	20.3–39.9	784	282–1515	150	150–300
pH		6.4–7.2	7.4–7.7		7.2–7.4		7.0–7.4		

Values tolerable by the soil: 1. Eikmann – Kloke, 1988
2. EC limit values (Crössmann, 1990)

Table 2

Heavy metal content in the organs of wheat (Triticum vulgare) in industrial regions (μg/g dry matter)

Element	Root		Stalk		Leaf		Grain	
	average	extreme value	average	extreme value	average	extreme value	average	extreme value
Ajka								
Cd	0.4	0–1.2	0.28	0–0.6	0.2	0–0.05	0.05	0–0.2
Co	3.0	1.6–6.8	0.25	0–1.5	0.07	0–0.3	0	0
Cr	20.9	7.7–45.8	1.2	0–5.4	1.82	1.1–3.1	0.2	0–0.9
Cu	7.5	5.5–14.6	2.6	1.2–4.1	4.73	3.8–7.6	3	2.6–5.6
Fe	5492	3460–9691	614	141–2591	547	399–678	73.8	42.3–182
Mn	277	139–675	48.5	17.1–175	84	56.8–109	37.5	31.3–43.8
Mo	0	0	0.92	0–2.4	2.35	0.3–5.3	0.8	0.4–1.6
Ni	14.7	5.8–29.5	1.23	0.3–4.8	2.07	1.1–4.6	0.4	0–1.3
Pb	6.2	3.9–11.6	0.38	0–2.2	6.08	3.9–9.4	0	0
V	7.7	4–15.4	0.67	0–3.3	1.03	0.7–1.4	0.03	0–0.2
Zn	24.7	18.4–37.3	11.75	6.8–19.7	14.3	12.3–17.4	28.25	20.7–37.1
Dunaújváros								
Cd	1.3	0–1.9	0	0	0.6	0.48–0.7	0	0
Co	3.2	0–4.8	0	0	0.55	0.32–0.9	0	0
Cr	8.3	0–12.6	0.42	0–0.8	2.76	1.9–3.7	0	0
Cu	13.5	4.41–18.3	4.47	1.85–7.1	9.64	6.25–11.7	6.23	2.37–11.2
Fe	14073	13971–14176	234	145–324	3347	2788–3781	137	104–203
Mn	276	28.2–406	23.4	22.2–24.5	103	82.6–121	27.9	19.1–34.9
Mo	0	0	0	0	0	0	0.3	0–0.4
Ni	11.0	0.5–16.6	1.6	0.68–2.5	3.41	2.7–3.9	2.92	0.43–5.8
Pb	8.9	0–14.0	0	0	7.31	6.31–8.6	0	0
V	5.9	0–9.0	0	0	1.73	0–2.5	0	0
Zn	62.9	45.8–75.3	18.4	14.6–22.7	32.2	29.8–34.9	41.6	27.07–52.9
Százhalombatta								
Cd	0.5	0.50–0.5	0	0	0.2	0.15–0.2	0.09	0–0.2
Co	2.1	2.03–2.2	0	0	0.3	0–0.5	0.2	0–0.4
Cr	1.7	1.18–2.3	0	0	0	0	0	0
Cu	11.8	8.62–15.0	2.1	1.9–2.3	6.8	5.75–7.8	5.1	4.63–5.6
Fe	3500	3479–3521	113	85.4–142	504	319–689	34.0	33.4–34.6
Mn	122	88.1–157	22.3	15.75–28.9	96.8	66.53–127	30.4	29.7–31.1
Ni	5.3	5.1–5.5	1.1	0–2.2	0.8	0.43–1.1	0.2	0–0.4
Pb	4.4	4.28–4.5	0	0	5.6	4.96–6.3	0	0
V	4.7	4.63–4.8	0.1	0–0.3	1.0	0.54–1.5	0.1	0–0.2
Zn	21.9	21.14–22.8	12.45	10.69–14.2	33.0	25.16–40.9	59.7	33.7–85.7

The heavy metals concentrated in the roots of wheat remain in the soil even after the vegetation period. As regards the decreasing quantity of heavy metals, the order of the plant organs is: leaf, stalk, grain.

Kádár (1991) measured the heavy metal content in the straw and grain of wheat grown on various soil types (in regions not loaded with heavy metals). Our values for Cd, Cu, Fe, Ni, V, and Zn in stalk and leaf, while higher than those obtained in the unloaded regions, did not exceed the limit values for food consumption.*

The extent of the elements' translocation is shown by the transfer factor** calculable for the plants and plant organs (Table 3).

Table 3

Transfer factors for the organs of wheat (Triticum vulgare) in industrial regions

Elements	Root	Stalk	Leaf	Grain
AJKA				
Cd	0.66	0.46	0.33	0.08
Co	0.42	0.03	0.009	0
Cr	1.43	0.08	0.12	0.01
Cu	0.84	0.29	0.53	0.33
Fe	0.49	0.05	0.04	0.006
Mn	0.39	0.06	0.12	0.09
Ni	0.91	0.07	0.12	0.02
Pb	0.47	0.02	0.46	0
V	0.49	0.04	0.06	0.001
Zn	0.65	0.31	0.37	0.74
DUNAÚJVÁROS				
Cd	0.76	0	0.35	0
Co	0.56	0	0.09	0
Cr	0.72	0.03	0.24	0
Cu	0.78	0.25	0.56	0.36
Fe	1.46	0.02	0.34	0.01
Mn	0.60	0.05	0.22	0.06
Ni	0.64	0.09	0.20	0.17
Pb	0.48	0	0.39	0
V	0.59	0	0.17	0
Zn	1.13	0.33	0.58	0.75
SZÁZHALOMBATTA				
Cd	0.35	0	0.11	0.06
Co	0.38	0	0.03	0.03
Cr	0.38	0	0	0
Cu	0.63	0.23	0.36	0.27
Fe	0.51	0.01	0.07	0.005
Mn	0.29	0.05	0.20	0.07
Ni	0.34	0.07	0.05	0.01
Pb	0.36	0	0.32	0
V	0.52	0.01	0.11	0.01
Zn	0.72	0.41	1.09	1.98

* Limit values (Hungary and GFR):
Cd: 0.1–0.5 μg/g, Cu: 15–30 μg/g, Pb: 3–5 μg/g, Mo: 2–5 μg/g
ZEBS limit values for wheat grain (Delschen – Werner, 1989):
Cd: 0.12 μg/g, Pb: 0.35 μg/g

** transfer factor = $\frac{\text{quantity of element in the plant}}{\text{quantity of element in the soil}}$

On the basis of the transfer-factor (Sauerbeck and Lübben, 1991; Lübben and Sauerbeck, 1991) or accumulation index (Kickens and Camerlynck, 1982) the specific differences of elements, plants and plant organs can be determined. It is an index of the concentration of heavy metals taken up by the plant from the soil (Lehn and Bopp, 1987).

The value of the transfer-factor depends on the concentration of the element (ion) present in the soil, and considerably changes with the increasing heavy metal content of the soil.

The value of the transfer-factor is highest in the roots (Lübben and Sauerbeck, 1991).

In the caryopsides, the relatively high transfer-factor value of Cu and Zn indicates the higher translocation of the two elements.

Besides translocation from the roots, the quantity of elements deposited on the leaves as well as entering through the stomata and then incorporated in the leaves also contributes to the element content of the leaves.

In the caryopsides, some heavy metals only appear in very small amounts, if at all, Cu, Fe and Zn can be detected here in relatively larger amounts. As, Cd, Cr, Mo, Ni and V occur in smaller amounts. The amount of heavy metals does not exceed the limit value for food consumption.

The quantity of heavy metals in the vegetative organs of plants (referring to data by Sauerbeck, 1986*) does not exceed the critical value (at which the growth of particularly sensitive plants is inhibited).

In the Dunaújváros area, the potential heavy metal accumulation capacity of each species can be determined on the basis of the maximum values of wheat and its more frequent weed species (Table 4).

Each of the examined heavy metals appeared in the intensive root-system of the wheat in a larger quantity than in the extensive root-system of the dicotyledonous weed plants.

Besides translocation, foliar absorption also plays a role in the heavy metal accumulation capacity of leaves. In the large leaves of *Matricaria maritima* Cu, Mn, Pb, and Zn can be detected in larger amounts.

In the area of Nagytétény the Cd, Cu, Pb and Zn content of barley roots is indicative of the heavy metal load of the soil (Table 5).

The translocation of heavy metals to the caryopsides of barley is of greater extent that in the case of wheat or maize, but does not exceed the limit value for food consumption. As in the caryopsides of wheat, a larger amount of Mn – compared to maize – can also be detected.

In the region less loaded with heavy metals (Ajka), the roots of wheat contained larger amounts of heavy metals than did those of maize (Table 6).

* Limit values of heavy metals critical for sensitive plants:

Cd: 5–10 μg/g	Ni: 20–30 μg/g
Co: 10–20 μg/g	Pb: 10–20 μg/g
Cu: 15–20 μg/g	Zn:180–200 μg/g

Table 4

Potential heavy metal accumulation capacity of wheat and its weed plants (μg/g dry weight) in Dunaújváros (On the basis of the maximum values measured)

		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Triticum vulgare	root	1.9	4.8	12.6	18.3	14176	406	16.6	14.0	9.0	75.3
	leaf	0.7	0.9	3.7	11.7	3781	121	3.9	8.6	1.5	34.9
Hibiscus trionum	root	0.8	0	0	10.2	462	26.2	1.3	-	0.7	52.4
	leaf	0.5	0	0.6	10.3	852	95.2	1.9	0	0.7	52.5
Cirsium arvense	root	0.2	0	0	3.8	482	25.6	1.3	0	0.6	53.3
	leaf	0.4	0	0	6.1	1178	70.8	1.4	3.5	0	38.7
Matricaria maritima	root	0.8	1.1	1.8	11.5	1418	65.4	3.7	0	2.0	43.5
	leaf	0.3	0	0	29.2	462	149	3.6	9.0	0	73.9

Table 5

Heavy metal content of Hordeum distichon (μg/g dry matter)
Nagytétény

Elements	Root		Stalk		Leaf		Grain	
	average	extreme values	average	extreme values	average	extreme values	average	extreme values
As	0	0	0.01	0–0.05	1.7	0–3.9	0.2	0–0.9
Cd	1.6	0.8–4.0	0.5	0.3–1.3	0.5	0.2–1.5	0.09	0.04–0.13
Co	0.9	0.5–1.4	0.1	0–0.1	0.3	0–0.5	0.08	0–0.1
Cr	0.5	0.5–1.04	0	0	0	0	0	0
Cu	27.8	17.1–44.8	5.8	4.9–7.2	12.5	8.4–15.1	9.2	7.6–11.2
Fe	1632	695–2437	149.9	58.9–260	507	238–1016	115.6	67.3–170
Mn	56.7	36.3–75.3	23.5	11.1–41.2	25.4	2.8–88.1	23.7	21.51–26.3
Mo	0.3	0–1.0	1.2	0–2.5	1.5	0.2–2.8	1.8	0.8–2.5
Ni	14	6.3–40.3	9.4	7.1–9.5	10.7	3.3–19.4	5.8	4.5–7.4
Pb	17.1	6.7–32.9	2.0	1.1–4.6	5.7	2.2–14.0	1.3	0.5–2.4
V	4.4	4.4–4.9	0.8	0.64–0.9	1.9	1.4–2.4	0.5	0.5–0.6
Zn	238.6	102–393	169	88.9–283	149	49.6–232	92.8	76.3–124

Table 6

Element content in the organs of Zea mays (μg/g dry matter)
Ajka

Elements	Root	Stalk	Leaf	Grain
As	0	0	0	0
Cd	0.2	0	0	0
Co	0.7	0	0.3	0
Cr	2.5	0	0.8	0
Cu	5.1	2.4	11.9	0
Fe	2084	48.9	220	74.1
Mn	73.1	19.9	102.7	35.2
Ni	1.9	0	0	0
Pb	0	0	0	0
Se	0	0	0	0
V	0	0	0	0
Zn	15.2	9.1	35.1	31.3

In the roots of maize Cu, Fe, Pb and Zn can be found in larger amounts. In the Nagytétény samples heavy metals accumulated in the root indicate at the same time the extent of heavy metal load in the soil. The leaves also contain larger quantities of heavy metals. The Cr, Cu, Pb and Zn content of the roots as well as the Pb content of the leaves exceed the critical values for the plants (Table 7).

In the area of Nagytétény, Zn is also a loading element.

According to Klein et al. (1978, 1979) parallel with the Zn load of the soil, the Zn content also increases in the root, stalk and leaves. A Zn content of more than 440 μg/g in the root and stalk is toxic to the plant. The number of heavy metals translocated to the maize caryopsides is fewer than in the case of wheat or barley. In the maize caryopsides from Ajka, only three heavy metals could be detected.

In the maize caryopsides from Nagytétény, the quantities of Cd and Pb did not exceed the limit values for food consumption.

Owing to their heavy metal content, the leaves cannot be recommended for animal feeding. According to the transfer-factor calculations (Table 8), the heavy metals are blocked to a greater extent in the roots of maize, compared to wheat and barley; a translocation to the stalk and leaves is of lesser extent.

In the caryopsides, only 6 of the heavy metals could be detected.

The heavy metal accumulation capacity in the roots of maize was – except for Mn – higher than in the more frequent weed plants of the stand examined (Table 9).

Besides translocation, the heavy metal deposition originating from emissions also has an influence on the accumulation capacity and the heavy metal content in the leaves of the weed plants.

The roots of nitrophilous weed plants *(Cirsium arvense, Amaranthus retroflexus, Atriplex tatarica)* contain smaller amounts of heavy metals.

Table 7

Element content in Zea mays (μg/g dry matter)
(Nagytétény)

Elements	Root		Stalk		Leaf		Grain	
	average	extreme values	average	extreme values	average	extreme values	average	extreme values
Cd	0.6	0.38–0.9	0.06	0–0.2	0.2	0.19–0.23	0	0
Co	1.3	0.72–2.1	0	0	0.1	0–0.3	0	0
Cr	2.6	1.27–5.0	0	0	0.3	0–0.8	0	0
Cu	49.7	28.43–77.9	4.3	4.04–4.6	11.8	8.7–14.1	5.7	2.38–12.0
Fe	2552	1273–4460	133	99.05–161	542	445–606	42.0	31.59–56.6
Mn	54.9	26.3–94.5	8.6	7.31–10.6	29.9	23.08–36.4	6.2	4.95–7.8
Mo	0	0	0.2	0–0.5	0	0	0	0
Ni	4.0	2.22–6.6	1.2	0–2.7	1.7	1.12–2.5	4.0	0.5–10.5
Pb	65.9	32.2–112	3.5	3–3.8	12.8	10.01–16.3	1.8	0–5.5
V	2.6	1.43–4.5	0	0	0.5	0–0.8	0	0
Zn	143	115–160	78.6	53.68–107	91.7	60.99–110.0	47.1	30.4–80.1

Table 8

Transfer factor for maize organs (Nagytétény)

Elements	Root	Stalk	Leaf	Grain
Cd	0.10	0.01	0.32	–
Co	0.29	–	0.02	–
Cr	0.20	–	0.02	–
Cu	0.18	0.01	0.04	0.02
Fe	0.34	0.01	0.07	0.005
Mn	0.23	0.04	0.12	0.03
Ni	0.24	0.07	0.10	0.24
Pb	0.14	0.007	0.03	0.004
V	0.10	–	0.02	–
Zn	0.18	0.55	0.06	0.06

Conclusions

In the organs of wheat, barley and maize heavy metals are found in various quantities.

In industrial regions, accumulation of heavy metals can be expected. In the vicinity of the thermal plants and iron works examined, the heavy metal content in the soils has not yet exceeded the so-called tolerable value.

In the soils of Nagytétény, Cd, Cu, Pb and Zn can be detected in quantities above the tolerable value.

The heavy metals are accumulated in the intensive root-system of the cereal species. The element content of these plants is generally in proportion with the heavy metal quantity detectable in the soil.

In the Nagytétény area in the roots of maize Cu, Fe, Pb and Zn were found in larger amounts.

According to the decreasing quantity of heavy metals, the order of the plant organs is: root, leaf, stalk, grain.

The transfer factor calculated for the various plant organs indicates the extent of heavy metal translocation to the individual organs.

The quantity of the heavy metals detected in the grains did not exceed the limit values for food consumption.

Owing to the increasing load of heavy metals, a regular determination of the element content in crops grown in industrial regions is necessary.

The roots of monocotyledonous plants contain larger amounts of heavy metals than do those of the dicotyledonous weed plants.

Table 9

Potential heavy metal accumulation capacity of maize and its weed plants (μg/g dry matter)
Nagytétény

(On the basis of the maximum values obtained)

Sample		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Zea mays	root	0.98	2.15	5.05	77.9	4460	44.5	6.6	112	4.5	1160
	leaf	0.23	0.34	0.81	14.1	606	36.5	2.5	16.3	0.8	110
Convolvulus arvensis	root	0.5	0.65	0.91	36.8	1046	77.0	3.8	34.6	1.4	117
	leaf	0.3	0	0	58.8	699	104	2.7	14.5	0.7	79.5
Ambrosia elatior	root	1.0	0.9	0	268	824	75.0	3.8	28.2	1.6	117
	leaf	1.4	0.4	0	28.1	581	115	1.9	15.4	0.6	276
Cirsium arvense	root	0.56	0	0	37.7	437	19.9	2.2	25.8	–	66.6
	leaf	1.5	0	0	48.5	1094	51.9	3.6	39.5	–	251
Amaranthus retroflexus	root	0.66	0.5	0.6	21.1	759	23.5	6.7	29.6	0.8	103
	leaf	1.06	0.5	0	18.0	701	84.0	3.9	12.5	1.1	162
Atriplex tatarica	root	0.3	0.4	0.5	22.1	523	20.1	2.1	20.9	0.5	68.1
	leaf	0.2	0.5	0	15.0	762	68.0	2.1	10.9	0.9	127

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TRACE ELEMENTS LEVEL IN LEMON–SOIL INTERACTION

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Atomic absorption spectroscopic analysis was utilized for the determination of Ag, Au, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr and Zn in the different parts of lemon including fruit, mesocarp, pericarp, seeds and leaves, as well as in the soil samples taken from the immediate vicinity of lemon trees at 10, 30 and 60 cm depths from surface. Chloride was determined using chloride ion-selective electrode, and Ca by EDTA titration. The results show that most trace elements are concentrated in the pericarp of lemon and in the soil samples of 60 cm depth (the vertical distance from the lemon trees at which the soil sample was taken). This indicates that trace elements were absorbed by the plant from the zone of 10-30 cm depth. Leaves of lemon trees exhibit a high concentration of most trace elements, as a result of their accumulation there. Statistical analysis data show significant correlation coefficient values between trace elements present in lemon and in the soil samples (potassium shows a high positive correlation coefficient value at 60 cm depth, r=0.61), C. V. varies between 0.00 – 4.6 and 9.5 – 65, while S. D.: 0.00–78 and 0.004–70 for lemon and soil samples, respectively.

Keywords: trace element, atomic absorption, lemon, soil, leaves, ion selective electrode

Introduction

The present study is a part of a comprehensive programme designed to monitor the trace element levels absorbed by plants, and their distribution through the different parts of the plant, and to follow up deficiency of trace elements, and toxicological or nontoxicological effects on plants and soil after the construction of the High Dam Lake. After the construction of the High Dam, most mud sediments and silt precipitate in the Sudan part, up to Adendan in the most southern part of Egypt. Therefore artificial fertilizers are necessary to compensate for deficiency of trace elements required by plants.

Trace elements were determined by atomic absorption spectroscopic analysis in various types of wheats and wheat products (Zook et al., 1970), barley seeds (Bhana and Duffus, 1983), marine organisms (Medina et al., 1986), Egyptian crops (Sherif et al., 1979,1980; Awadallah et al.,1986). Egyptian cane sugar (Awadallah et al. 1985,1986), millet (Awadallah et al., 1986), purslane, beet, barley, fenugreek and lupin (Rashed, 1989) planted in the experimental farms of the High Dam Lake Authority.

Study area

The study area lies at the south-east part of the High Dam Lake region, about one km north the Abu Simbel airport, located between latitudes 22° 32' 30" - 22° 26' 06" E and longitudes 31° 31' 38" – 31° 39' 40" N. It was established on 60 feddans (4200 m²) in 1985 for experimental purposes. The geological area generally consists of Nubia sandstone formation of the upper cretaceous age. The soil formation ranges from shallow to loamy sand (clay minerals soil contains 5 to 23% gravel) and sand clay loam (coarse textured materials composed of 9 to 16% gravel). The surface is almost flat to somewhat undulating, covered with a pavement of pulverized gravel. The soil is characterized by shallow profiles of skeletal nature, underlain by ferrugenous Nubia sandstone and classified as: Entisols, Psamments, Quartizipsamments and Lithic Quartzipsamments (Regwa,1978).

Materials and methods

Five lemon samples set (leaves, fruit, mesocarp, pericarp and seeds) and five soil samples set (at 10, 30 and 60 cm depths taken from the immediate vicinity of lemon tree in February, 1985 before, and in November, 1990 after planting) were collected from the Abu Simbel experimental farm. The plant samples were washed separately and thoroughly with tap and bidistilled water, followed by deionized water, and allowed to drain on filter papers. When they were completely dry, the samples were blended by a stainless steel blender and surgical gloves were washed from outside with deionized water and utilized to prevent contamination. The subdivided lemon parts and soil samples were dried in an electrical furnace at 105 °C for 48 hours, then they were ground and powdered in an agate mortar, and kept in small polyethylene bottles.

Reagents and standard solution

1 - For Atomic Absorption Spectroscopy:

Bottles of 500 ml of 1000 ppm (A.R., 99.9%) atomic absorption spectroscopic standard solution for Ag, Au, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn (1 ml = 1 mg) were purchased from BDH, England, for the preparation of dilute standard solutions.

2 - For Ion-Selective Electrode:

Standard Cl (1000 ppm) was prepared by dissolving 1.65 g NaCl (A. R., 99.9%, BDH) in deionized water, transferred to 1 l volumetric flask and made up to the mark using deionized water

- Ionic strength adjustor (ISA) - 5M $NaNO_3$.
- Orion filling solution (Cat. No. 900002) in the inner chamber and (Cat. No. 900003) in the outer chamber of the double-junction reference electrode.
- 1M $NaNO_3$ was prepared from AnalaR $NaNO_3$ free from chloride.

Working standard solutions for both atomic absorption spectroscopy and ion selective electrode were prepared from the stock 1000 ppm standards.

Instruments

- SP 1900 Pye Unicam Flame Atomic Absorption Spectrophotometer digital and direct readout concentration with air-acetylene burner and cylinder.
- Hollow Cathode Lamps of Ag, Au, Co, Cu, Cr, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn.
- Orion EA 940 Ion Analyzer, ISE, Microprocessor.

– Orion (Cat. No. 94-17) Solid State Chloride Electrode.
– Orion (Cat. No. 90-02) Double-Junction Reference Electrode.

The experiment

Preparation of Samples for AAS and Measurement:

Two grams portion of plant (leaves, fruit, mesocarp, pericarp and seeds) samples were wet ashed in a teflon beaker with cover, using 20 ml of 1:1 HNO_3–$HClO_4$ acid mixture, followed by the addition of 3 drops of HF acid and allowed to dry. The cooled residue was dissolved in 5 ml conc. HCl and then the liquid was made up to 50 ml, using bidistilled water. One gram of soil samples was mixed with 20 ml (1:1) HCl – HNO_3 acids. The mixture was placed in an electrical furnace and heated until dry. The residue was extracted, using 2N HCl, and brought into 50 ml with bidistilled water.

Preparation of Samples for ISE and Measurement:

One gram portion of the finely dried subdivided samples was extracted by shaking in 1M $NaNO_3$. A ten-gram portion of soil samples was shaken with 100 ml free chloride bidistilled water for 30 minutes. The soil–water mixture was filtered off. One ml of 5M $NaNO_3$ (ISA) adjustor was added to all standard and sample solutions to provide a constant background ionic strength. Both the chloride and double junction reference electrodes were immersed in the standard solutions to break the range of the sample concentration, and calibrate the electrode for direct readout concentration. Then the electrodes were immersed in the sample solution and the concentration was given directly. Both standards and samples were brought to the same temperature and stirred constantly during measurement to prevent any changes in the slope of the electrode and the concentration. The single known addition technique was applied for plant which was given directly (Orion Research Incorp., 1974, 1982; La Croix et al., 1974; Samar et al., 1974).

Results and discussion

The data (mean values of triplicate determination of elements in each part) obtained on the analysis of leaves, fruit, mesocarp, pericarp and seeds of lemon taken from five trees, as well as those (triplicate determination) of the soil samples (from five sites at 10, 30 and 60 cm depths) before and after planting are reported in Tables 1 and 2, and represented graphically in Figure 1. Table 1 shows that Ag, Au, Ca, Cl, Cr, Co, Cu, Mg, Mn, Ni, Sr and Zn are concentrated in leaves more than in the other parts of lemon, indicating the vital necessity of these elements for plant and their selective absorption by plants, where they play a significant and important role in photosynthesis, production of colour, taste and smell, carbohydrate metabolism, chlorophyll (Malik and Srivastara, 1982; Bowling, 1982), activation of several enzymes and synthesis of amino acids (Weaver, 1972). Potassium and sodium accumulate in fruit which reduce Ca and Mg (this appears from the results of fruit analyses). Fe, K and Na in mesocarp, Cu, Fe, K and Na in pericarp and Fe, Mg and Na accumulate in the seeds of lemon. A higher concentration of these elements in leaves may be due to the fact that the content of these elements and their exchangeable capacity in the soil solution are high, or that the surface cells of leaves are large, or that the nitrogen content is high which enhances the import of K, Ca, Mg, Cu, Fe and Mn. This may be due to the fact that the plasmalemma (cytoplasmic membranes) prevents any loss of substances and allows water and inorganic nutrients, including these metals, to be translocated inside. The presence of Na and K in high concentrations in lemon fruit may be ascribed as being present as constituents of lemon fluid:

Table 1

Trace element concentrations in lemon samples

Element		Mean					L.S.D.					C.V.				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Ag	(ppm)	0.008	0.005	0.005	0.005	0.002	0.004	0.002	0.0	0.0	0.002	55	91	0.00	0.00	136
Au	(ppm)	0.146	0.056	0.019	0.068	0.057	0.013	0.008	0.006	0.017	0.006	9.5	15.9	34	26	11.7
Cl	(ppm)	46.4	12.2	24.4	10.6	40.8	7.6	2.9	4.9	0.89	6.9	16.3	24	20	8.4	16.9
Ca	%	4.99	1.38	1.01	2.08	0.89	1.5	0.16	0.16	0.69	0.46	31	12	16	33	5.2
Co	(ppm)	0.122	0.05	0.026	0.059	0.034	0.016	0.009	0.004	0.013	0.008	13.4	18.8	16	23	25
Cr	(ppm)	0.013	0.0	0.0	0.0	0.0	0.002	0.0	0.0	0.0	0.0	21	0.0	0.0	0.0	0.0
Cu	(ppm)	0.090	0.08	0.094	0.088	0.078	0.01	0.005	0.018	0.026	0.010	13.6	6.3	19.3	30.4	14
Fe	(ppm)	8.6	5.8	7.1	10.1	11.5	0.6	0.2	0.4	1.7	0.3	7.5	4.7	5.8	16	3
K	(ppm)	298	438	290	251	332	77	19.8	33	78	27	26	4.5	11.5	31	8.1
Mg	(ppm)	59.9	25.5	30.6	32.2	17.8	10.9	1.5	6.1	5.4	1.09	18.3	5.9	20	16	6.1
Mn	(ppm)	0.53	0.12	0.09	0.156	0.158	0.03	0.01	0.02	0.02	0.008	6	13.9	28	18	5.2
Na	(ppm)	3.81	3.38	4.06	5.16	3.16	1.14	0.64	0.74	1.06	0.11	30	19	18	20	3.7
Ni	(ppm)	0.15	0.05	0.04	0.074	0.06	0.016	0.010	0.008	0.015	0.010	10.8	18.8	19.9	20	16.6
Pb	(ppm)	0.06	0.09	0.011	0.017	0.017	0.003	0.007	0.006	0.005	0.004	4.6	82	59	33	26.0
Sr	(ppm)	2.92	0.62	0.30	1.04	0.91	0.29	0.15	0.02	0.34	0.03	10.1	25	7.9	33	3.4
Zn	(ppm)	0.25	0.058	0.039	0.138	0.07	0.07	0.013	0.019	0.06	0.015	29	24	50	44	22.0

a = leaf, b = fruit, c = mesocarp, d = pericarp, e = seeds

Table 2

Trace element concentrations at various depth (cm) in soil samples of Abu Simbel area before and after planting lemon trees

Elements ppm	Before planting												After planting											
	Mean			L.S.D.			R.E.			C.V.			Mean			L.S.D.			C.V.			R.E.		
	10	30 cm	60	10	30 cm	60	10	30 cm	60	10	30 cm	60	10	30 cm	60	10	30 cm	60	10	30 cm	60	10	30 cm	60
Ag	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.008	0.007	0.008	0.004	0.004	0.008	55	55	104	0.02	0.02	0.04
Au	0.13	0.13	0.14	0.03	0.03	0.03	0.07	0.07	0.07	22	21.7	20	0.11	0.12	0.13	0.04	0.04	0.03	37	35	29	0.08	0.08	0.07
Ca%	1.83	2.05	2.6	0.64	0.49	1.3	0.35	0.31	0.50	35	24	50	1.75	1.91	2.52	0.66	0.56	1.28	37	29	51	0.36	0.33	0.50
Cl	1.75	1.71	1.66	0.49	0.15	0.16	0.30	0.17	0.17	27	8.7	9.6	1.51	1.43	1.32	0.33	0.15	0.18	22	11	14	0.25	0.17	0.18
Co	8.4	5.8	4.2	2.1	1.4	1.3	0.64	0.52	0.50	25	24	28	0.24	0.24	0.28	0.06	0.09	0.09	26	37	35	0.10	0.13	0.13
Cr	1.2	1.17	0.74	0.61	0.58	0.20	0.34	0.34	0.20	51	24	31	0.38	0.47	0.48	0.13	0.14	0.17	34	30	35	0.16	0.16	0.18
Cu	3.51	3.17	2.37	0.75	0.88	0.56	0.38	0.41	0.33	21	49	27	0.20	0.27	0.22	0.04	0.15	0.13	21	56	59	0.08	0.17	0.16
Fe	347	381	360	67	78	78	3.6	3.9	3.9	19	20	21	312	334	327	37	56	70	12	16	21	2.7	3.2	3.7
K	28	35	37	6.8	7.5	9.2	1.1	1.2	1.3	24	21	25	20.2	25.3	28	6.7	5.1	8	33	20	28	1.1	1.0	1.2
Mg	32	38	4	3.3	7.3	8	0.81	1.2	1.2	10	19	17	26.6	31.2	37.4	7.2	7.8	10.9	27	24	29	1.2	1.3	1.4
Mn	6.5	6.4	5.7	1.3	1.6	1.2	0.50	0.56	0.48	20	25	21	5.3	5.73	5.38	0.52	0.82	0.79	10	14	15	0.4	0.39	0.39
Na	3.9	4.2	4.9	0.85	1.2	1.1	0.41	0.48	0.46	22	28	23	2.11	2.32	2.24	0.61	0.59	0.62	28	25	27	0.34	0.34	0.35
Ni	1.6	1.5	1.4	0.48	0.47	0.40	0.30	0.31	0.28	30	31	28	0.49	0.54	0.90	0.11	0.13	0.09	22	25	24	0.14	0.16	0.13
Pb	0.52	0.51	0.58	0.19	0.24	0.24	0.19	0.21	0.22	36	47	41	0.08	0.09	0.12	0.03	0.02	0.02	38	30	21	0.07	0.06	0.06
Sr	0.66	0.62	0.57	0.15	0.17	0.15	0.17	0.16	0.17	22	27	40	0.32	0.31	0.42	0.21	0.15	0.15	56	50	37	0.20	0.17	0.17
Zn	0.72	0.90	0.97	0.26	0.33	0.30	0.22	0.25	0.24	36	37	31	0.55	0.55	0.52	0.18	0.24	0.17	32	43	32	0.18	0.21	0.18

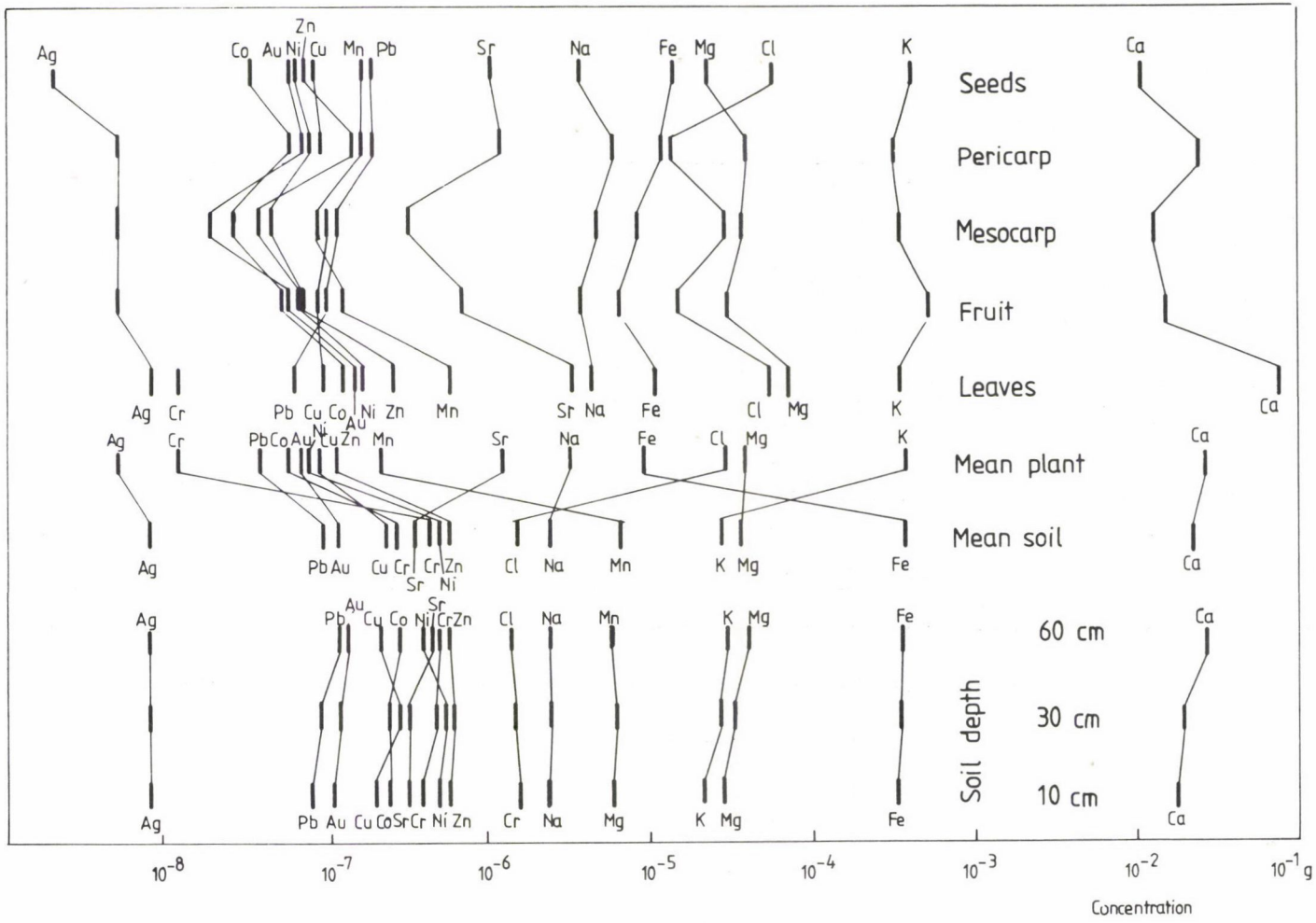

Fig. 1. Relationship between trace elements concentration in lemon and in soil samples (Abu Simbel area)

i.e. NaCl and KCl, and to compensate for the deficiency of Ca and Mg to stop generation and help growth. The presence of a high concentration of copper in leaves and pericarp is neutralized by Ca and Fe, as evidenced from the data (Yogadion, 1984). The high concentration of Zn in the leaves (Table 1) may be due to the fact that Zn exists in a relatively high concentration in the shallow to loamy sand soil of the experimental farms in the Abu Simbel area (Table 2) where it is more mobile and available, and it absorbs easily. (The normal range of zinc in Egyptian soil is between 15 to 35 ppm.) Also, the presence of the other elements such as Cu, Fe, Mg,...etc. in the mesocarp and seeds in low concentrations, may be due to the fact that the phosphorus content is higher and reduces these elements.The presence of cobalt in a high concentration in leaves may be explained on the basis that it is associated with ausin metabolism and promotes elongation of the cell envelop. Trace elements present in the different parts of lemon may exist as metal-high molecular weight complexes, mixed metal-mixed biological or enzymatic polycoordination complexes. The results (Table 2) show that the soil samples taken after planting at 60 cm depth contain the highest concentration of Ca, Mg, K, Zn, Cr, Sr, Co, Au and Pb, while those collected from 10 cm depth exhibit highest values of Mn, Cl and Ni, whereas soil at 30 cm depth possesses high values of Fe, Mn, Na, Zn, Ni, Cr and Cu. Thus, lemon trees absorb their essential nutrient trace elements from all depths, but with variable uptake parts, i. e. lemon trees absorb their needs from trace elements except Cl, Mn and Ni at 10 cm depth, absorb Ag, Au, Ca, Cl, Co, K, Mg, Pb and Sr at 30 cm depth but Ag, Cl, Cu, Fe, K, Mn, Na and Ni from soil solutions at 60 cm depth.

Soil dynamics

On the analysis of the soil samples taken from 0–10, 10–30 and 30–60 cm depths, the data obtained before and after planting of lemon trees (Table 2) show that Au, Cl, Cu, Fe, K, Mn, Ni, Sr and Zn decrease with depth, while Ca, Co, Mg, Na and Pb increase with depth. However, concentrations of the elements in the soil samples decreases after planting due to absorption of these elements by lemon roots from the surrounding soil solution, and accumulate in the different parts of lemon trees, so it is necessary to add fertilizers containing the essential trace elements to the soil (deficient trace elements soil) before next planting. This new beach land surrounding the High Dam Lake was reformed after covering it by the suspended mud existing in the water flood as a result of regression.

Statistical analysis of data

Correlation coefficient values, coefficient of variance, standard deviation and relative error values between element concentrations existing in lemon and soil samples at 10, 30 and 60 cm depths (Tables 3–8) are studied and discussed.

Results of the statistical analysis of the database of lemon show good, positive and interesting correlation values between elements ($r=0.67 - 0.84$). This indicates that these elements exist as essential trace elements and they are responsible for

Table 3

Trace element concentration ratio between plant and soil at 10, 30 and 60 cm depths

Elements	P/S10	P/S30	P/S60	P/S
Ag	0.63	0.63	0.63	0.63
Au	0.62	0.57	0.53	0.57
Co	1.4	1.2	0.9	1.2
Ca	17.0	18.0	20.0	19.0
Cl	0.24	0.23	0.21	0.22
Cr	0.034	0.028	0.027	0.029
Cu	0.44	0.32	0.40	0.38
Fe	0.028	0.025	0.026	0.027
K	16.0	13.0	11.0	13.5
Mg	1.3	1.1	0.9	1.0
Mn	0.038	0.036	0.039	0.037
Na	1.85	1.68	1.74	1.76
Ni	0.15	0.14	0.19	0.16
Pb	0.48	0.43	0.32	0.40
Sr	3.5	3.7	2.7	3.2
Zn	0.201	0.198	0.210	0.205

Table 4

Correlation coefficient values of trace elements present in lemon planted in Abu Simbel area

	Ag	Au	Co	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr
Ag	1.00														
Au	0.28	1.00													
Co	-0.08	0.76	1.00												
Ca	0.62	-0.39	0.76	1.00											
Cl	-0.35	-0.40	0.10	-0.07	1.00										
Cr	0.10	0.77	0.31	-0.10	-0.48	1.00									
Cu	-0.21	-0.72	-0.96	0.75	-0.23	-0.40	1.00								
Fe	-0.23	-0.26	-0.68	-0.36	0.35	-0.28	0.56	1.00							
K	-0.25	-0.55	-0.43	0.42	0.72	-0.18	0.22	0.25	1.00						
Mg	0.84	0.28	0.19	0.22	-0.30	-0.18	0.02	-0.59	-0.56	1.00					
Mn	0.75	-0.21	-0.87	0.67	-0.51	-0.28	0.71	0.06	-0.06	0.70	1.00				
Na	-0.21	0.83	0.80	-0.62	-0.02	0.75	-0.89	-0.16	-0.19	0.22	-0.71	1.00			
Ni	-0.78	-0.72	-0.34	-0.08	0.67	-0.43	0.18	0.27	0.73	-0.79	-0.50	-0.22	1.00		
Pb	-0.07	-0.24	0.35	-0.35	0.55	-0.77	-0.24	-0.84	-0.09	0.36	-0.03	-0.21	0.14	1.00	
Sr	0.71	0.11	-0.49	0.68	-0.77	-0.31	0.58	0.45	-0.28	0.43	0.80	-0.33	-0.61	-0.60	1.00
Zn	0.05	-0.45	-0.07	-0.08	0.12	-0.87	0.26	-0.50	-0.30	0.48	0.43	-0.63	0.03	0.80	-0.16

Table 5

Correlation coefficient values between trace elements present in the soil of Abu Simbel area before planting lemon trees

	Au	Ca	Cl	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	Pb	Sr	Ti	Zn
Au	1.00																
Ca	0.906	1.00															
Cl	-0.708	-0.843	1.00														
Co	0.183	0.010	0.206	1.00													
Cr	0.223	0.089	0.407	0.754	1.00												
Cu	-0.472	-0.571	0.427	0.687	0.208	1.00											
Fe	-0.006	-0.375	0.654	0.316	0.547	0.103	1.00										
K	0.017	0.029	-0.081	0.813	0.322	0.793	-0.242	1.00									
Mg	0.207	0.222	0.043	0.891	0.755	0.517	-0.107	0.827	1.00								
Mn	0.466	0.339	0.213	0.410	0.875	-0.279	0.567	-0.102	0.445	1.00							
Mo	0.036	-0.172	0.088	0.842	0.300	0.855	0.177	0.849	0.608	-0.093	1.00						
Na	0.195	0.588	-0.599	-0.441	-0.271	-0.534	-0.823	-0.106	-0.003	-0.096	-0.582	1.00					
Ni	-0.022	0.100	-0.603	-0.181	-0.756	0.235	-0.714	0.350	-0.172	-0.869	0.278	0.234	1.00				
Pb	-0.057	-0.089	-0.382	0.129	-0.554	0.530	-0.406	0.529	-0.022	-0.783	0.614	-0.161	0.910	1.00			
Sr	-0.521	-0.615	0.430	0.623	0.129	0.996	0.081	0.756	0.444	-0.353	0.830	-0.536	0.281	0.566	1.00		
Ti	-0.706	-0.453	-0.088	-0.070	-0.436	0.553	-0.625	0.435	-0.065	-0.755	0.181	0.235	0.596	0.520	0.594	1.00	
Zn	0.618	0.375	0.008	-0.004	0.436	-0.593	0.659	-0.529	-0.126	0.773	-0.282	-0.224	-0.679	-0.620	-0.631	-0.989	1.00

Table 6

Correlation coefficient values between trace elements present in soil of lemon at 10 cm depth (Abu Simbel farm)

	Ag	Au	Co	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr
Ag	1.00														
Au	-0.18	1.00													
Co	-0.031	0.92	1.00												
Ca	-0.70	0.75	0.93	1.00											
Cl	-0.49	-0.58	-0.48	-0.20	1.00										
Cr	-0.40	0.76	0.88	0.93	-0.05	1.00									
Cu	-0.18	0.95	0.95	0.88	-0.35	0.91	1.00								
Fe	-0.26	-0.73	-0.86	-0.79	0.70	-0.61	-0.72	1.00							
K	-0.12	-0.39	-0.47	-0.41	0.69	-0.08	-0.28	-0.78	1.00						
Mg	-0.62	-0.41	-0.46	-0.42	0.63	-0.09	-0.31	0.72	0.98	1.00					
Mn	-0.36	0.55	0.74	0.90	0.02	0.76	0.71	-0.61	-0.46	-0.52	1.00				
Na	-0.87	0.70	0.85	0.88	-0.06	0.97	0.84	-0.60	-0.02	-0.05	0.66	1.00			
Ni	-0.29	0.04	0.15	0.38	0.77	0.57	0.31	0.25	0.54	0.47	0.47	0.52	1.00		
Pb	-0.35	0.80	0.86	0.82	-0.18	0.95	0.89	-0.58	0.01	0.03	0.54	0.96	0.43	1.00	
Sr	-0.46	0.58	0.83	0.95	-0.03	0.92	0.75	-0.70	-0.24	0.21	0.81	0.93	0.48	0.82	1.00
Zn	0.07	0.84	0.97	0.92	-0.50	0.86	0.87	-0.91	-0.48	-0.43	0.68	0.87	0.09	0.86	0.87

Table 7

Correlation coefficient values between trace elements present in soil of lemon at 30 cm depth

	Ag	Au	Co	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr
Ag	1.00														
Au	0.23	1.00													
Co	0.32	0.96	1.00												
Ca	-0.09	0.60	0.52	1.00											
Cl	-0.69	-0.69	-0.83	-0.11	1.00										
Cr	0.46	0.46	0.86	0.51	-0.67	1.00									
Cu	0.64	0.64	0.79	0.30	-0.75	0.96	1.00								
Fe	-0.56	0.64	0.49	0.55	0.03	0.47	0.26	1.00							
K	-0.07	0.39	0.35	-0.38	-0.31	0.34	0.40	0.43	1.00						
Mg	-0.51	0.50	0.42	0.02	-0.10	0.29	0.19	0.81	0.82	1.00					
Mn	0.63	0.89	0.92	0.47	-0.87	0.93	0.93	0.24	0.22	0.13	1.00				
Na	-0.07	0.59	0.36	0.32	-0.007	0.69	0.61	0.70	0.49	0.52	0.36	1.00			
Ni	-0.10	0.70	0.55	0.12	-0.26	0.68	0.63	0.75	0.82	0.81	0.43	0.87	1.00		
Pb	0.03	0.96	0.88	0.75	-0.50	0.87	0.71	0.77	0.27	0.51	0.78	0.64	0.67	1.00	
Sr	0.74	0.73	0.69	0.21	-0.72	0.91	0.98	0.11	0.34	0.06	0.87	0.56	0.55	0.59	1.00
Zn	0.40	0.95	0.87	0.51	-0.66	0.99	0.95	0.53	0.44	0.37	0.90	0.71	0.73	0.89	0.88

Table 8

Correlation coefficient values between trace elements present in soil of lemon at 60 cm depth

	Ag	Au	Co	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr
Ag	1.00														
Au	-0.06	1.00													
Co	0.39	0.80	1.00												
Ca	-0.22	0.96	0.63	1.00											
Cl	-0.72	0.19	-0.19	0.34	1.00										
Cr	0.28	0.87	0.98	0.72	-0.15	1.00									
Cu	-0.02	0.98	0.80	0.95	0.25	0.86	1.00								
Fe	0.28	-0.29	0.06	-0.37	0.24	-0.06	-0.16	1.00							
K	0.61	-0.20	-0.13	-0.30	0.01	-0.04	-0.06	0.90	1.00						
Mg	0.64	-0.36	-0.03	0.41	-0.12	-0.17	-0.23	0.72	0.92	1.00					
Mn	-0.22	0.81	0.69	0.78	0.55	0.71	0.87	0.25	0.22	-0.04	1.00				
Na	0.42	0.83	0.99	0.68	-0.19	0.98	0.84	0.09	0.17	-0.01	0.70	1.00			
Ni	0.50	-0.20	-0.31	-0.11	0.85	-0.33	-0.10	0.65	0.36	0.18	0.35	-0.35	1.00		
Pb	0.36	0.80	0.99	0.63	-0.10	0.97	0.82	0.15	0.25	0.01	0.76	0.98	-0.20	1.00	
Sr	-0.52	0.82	0.37	0.91	0.68	0.47	0.83	-0.24	-0.28	-0.43	0.81	0.41	0.25	0.41	1.00
Zn	0.36	0.78	0.95	0.64	0.01	0.92	0.83	0.27	0.37	0.13	0.83	0.95	-0.07	0.98	0.46

growth, chlorophyll (Ca, Mg, K, Cl,...), amino acids, protein (Fe, Co, Cu,...) and ascorbic acid (Ca, Fe...), synthesis, and necessary for taste and smell (Mn), pigmentation and staining (Ag, Au, Cu, Cr, Ni, Mn, Fe), tissue and membrane formation (Zn, Cu, Pb), and cellular fluid creation (KCl, NaCl). The statistical analysis of the database of trace elements of Abu Simbel soil samples before planting lemon trees show positive, significant and interesting correlation coefficient values (r=0.659–0.996).Also, the analytical data of soil samples at 10 cm depth after planting of lemon trees exhibit high concentrations of Cl, Mn and Ni, indicating that these elements are present as essential detrital silicate minerals. Good positive correlations between the elements are obtained (r=0.25–0.77) indicating that Mn, Ni, Cl, Cr, K, Fe and Na occur as silicates, or metal-refractory organic carbon complexes and the other elements which are deficient in soil samples at 10 cm depth are rich in lemon and present as metal soluble exchangeable humates (Ag, Au, Ca, Mg,... – humates). Samples taken from 30 cm depth are more concentrated in Cr, Cu, Fe, Mn, Na, Ni and Zn than in the other depths. The significant positive correlation values (r=0.30-0.79) are observed between the elements. This suggests that these metals are associated with amorphous Fe/Mn/Al minerals or sulphides, carbonates or oxides, i.e. $CuFeS_2$, CuS_2, Fe_2O_3,$(FeCoNi)_3S_4$, $(NiMg)SiO_3$, H_2O, Al_2O_3, $ZnCO_3$, $CaMg(CO_3)_2$, $SrMg(CO_3)_2$, $CaCO_3$, $MgCO_3$, chromite ($FeCr_2O_4$), pyroxene, olivine, clay, silt, kaolinite, montmorillonite, muscovite, biotite, feldspar, illite and fine-grained clay minerals of the soil have large surface area and some of these metals exist as metal-humates, while the existence of the others are suggested to a small extent, as some refractory organic materials, and the adsorbed exchangeable, replaceable, and occluded forms of these metals are removed by aqua regia extract. Also, the results of the soil samples taken at 60 cm exhibit higher concentrations of Ca, Co, Cr, Mn, Pb and Sr, indicating that the concentration of these elements increases with depth. However, Ag, Au, Cu, Fe, K, Na, Mg and Zn show irregular distribution, while Ca, Cl, Mn, Ni and Zn decrease with depth. High positive correlation coefficient values between elements suggest that their existence is associated in mineral or complex forms, while low values suggest them to be distributed in low concentrations.

Conclusions

The data obtained by atomic absorption spectrophotometer (Ag, Au, Ca, Co, Cr, Cu, Mg, Mn, Fe, K, Ni, Pb, Sr and Zn), flame photometry (Na, K, Ca and Mg), spectrophotometry (Fe and Cu) and ion selective electrode (Cl) techniques are consistent for elements Ag, Au, Ca, Cl, Co, Cr, Cu, Mg, Mn, Na, Fe, K, Ni, Pb, Sr and Zn concentrations in lemon and in the soil samples, and within the desired permissible safety baseline levels. Leaves of lemon trees exhibit high concentrations of the most available essential and necessary nutrient trace elements. Also, soil samples taken from 30 to 60 cm depth exhibit a high concentration of the most trace elements needed by plants. Trace elements in plant/soil ratio and statistical analytical data have good correlations between trace elements present in the different parts

of the lemon trees and those in the soil samples (10, 30 and 60 cm depth). The Abu Simbel area (soil and atmosphere) is a clean environment and not polluted with toxic elements.

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OCCURRENCE OF CYCLIC HYDROXAMIC ACIDS IN THE TISSUES OF BARNYARD GRASS (ECHINOCHLOA CRUS-GALLI/L./P.B.), AND THEIR POSSIBLE ROLE IN ALLELOPATHY

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The tissues of barnyard grass *(Echinochloa crus-galli /L./ P.B.)* synthesize cyclic hydroxamic acids, which are secreted by its roots in free or glycosidic forms. Hydroxamic acids inhibit rice root growth. The concentration of hydroxamic acids accumulating in the rhizosphere exceeds the level that is necessary for a 50% root growth retardation of rice. Based on this, we assume that in the interrelationship between rice and barnyard grass, the cyclic hydroxamic acids secreted by barnyard grass roots have an allelopathic role by inhibiting the root growth of rice.

Keywords: Allelopathy, BOA, cyclic hydroxamic acids, DIBOA, DIMBOA, *Echinochloa crus-galli,* rice

Introduction

Weeds can cause considerable damage to our crop stands. The nature of the relationship between the two plants is still not fully understood. The relationship is usually of a negative nature for both plants, that is, they compete with each other for space, nutrients and water. A special relationship is found between a culture crop and a weed when the materials secreted by the latter retard growth of the former. This relationship between species is called allelopathy.

One of the most dangerous grass weeds is barnyard grass *(Echinochloa crus-galli).* This weed can be found in a number of crops, primarily on wet, heavy soils. It causes the most considerable damage in rice stands. It is a question that remains to be answered whether this plant of extremely intensive growth is only a "space parasite" to the culture crop or there is also some sort of special relationship between the two of them.

Apart from a few exceptions (seeds of *Acanthus mollis,* roots of *Scoparia*

Abbreviations:
BOA: benzoxazolinone
MBOA: 6-methoxy-2-benzoxazolinone
DIBOA: 2,4-dihydroxy-2H-1,4-benzoxazin-3[4H]one
DIMBOA:2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3[4H]one
HPLC: High performance liquid chromatography

dulcis) cyclic hydroxamic acids (1,4-benzoxazinone) have been identified in roots and shoots of grass species. In his comprehensive work Niemeyer (1988) reported 11 genera of grass species which produce hydroxamic acids. Their roles in biological systems are highly complex and cause much debate. Even now, it is primarily their roles in passive chemical resistance that are emphasized, but they may have a role in Fe(III)-uptake by grass as well (Tipton and Buell, 1970; Pethő, 1992b). Their specific role is also emphasized by Barnes et al. (1987). In aqueous extracts of rye straw they identified 2,4-dihydroxy-1,4(2H)- benzoxazinone-3-one (DIBOA) and its product of decomposition 2(3H)benzoxazolinone (BOA). It was also proven that these compounds inhibited root and shoot growth in *Lepidum sativum* and *Echinochloa crus-galli.* It was also assumed that they may be associated with allelopathic compounds having diffused from remains of rye shoots which inhibit weed growth on the area. Similar allelopathic effect, by rye residues, have also been reported earlier (Barnes and Putnam, 1986).

Initially, the activity of these compounds against bacteria, fungi and even insects was emphasized, but over the past few years scientists have paid more and more attention to other potential uses of cyclic hydroxamic acids. However, the limited occurrence of these compounds does not support a general physiological role which can be attributed to them. For this reason, testing their occurrence in cultivated and wild species of grass is more and more frequent (Zuniga et al., 1983). Argandona et al. (1980) reported that the cyclic hydroxamic acids tend to appear a few days after germination and their concentration increases gradually in the seedlings. Thus, we have analysed them in tissues of seedlings of some weed grasses *(Echinochloa crus-galli, Setaria italica, S. verticillata, S. viridis, Sorgum halepense).* We came to the conclusion that, out of the species tested, it was only the seedlings of barnyard grass that contained cyclic hydroxamic acids in detectable quantities, and this study is meant to give an account of this work.

Materials and methods

The seeds of barnyard grass were grown in the botanical garden of Debrecen Agricultural University and rice seeds (*Oryza sativa* L. cv. "Karmina") provided by the Irrigation Research Institute (Szarvas). The experimental plants were grown for different periods ranging from 1 to 5 weeks. They were placed on plastic nets in 100 and 200 ml dark walled glass pots. The week-old seedlings were transferred from water to the nutrient solution reported by Römheld and Marschner (1985). The seedlings were grown in a climatic chamber at 25/20 °C with light provided for 14 hours daily. About 10 g of the seedlings were homogenised with 30 ml water and the squeezed juice was kept at room temperature for one hour, so as to ensure enzymatic decomposition of glycosides. After acidification it was centrifuged and then the supernatant was extracted with ethyl acetate (3 x vol). The ethyl acetate phase was then dried in vacuum, redissolved in 1 ml 70% ethanol and chromatographed on Whatman 3 MM chromatography paper, using the solvent system ethyl acetate – formic acid – water (60 : 5 : 35). The chromatograms were dried with a cold airstream and analysed under a UV lamp at 254 nm. Cyclic hydroxamic acids absorb at short wave length UV (254 nm), and react with $FeCl_3$ to give a blue colour. The hydroxamic acids (Rf = 0.9) which gave a positive reaction with $FeCl_3$ were eluted with methanol, concentrated to a small volume and analysed by HPLC (Labor MIM Liquochrom) on a Chromsil C_{18} reverse phase column. The mobile phase was with 50% methanol (in: 0.02 mol acetate buffer, pH = 5.6) or a linear gradient of methanol (20 to 50%) generated by a solvent programmer and two pumps.

Cyclic hydroxamic acids, especially when ammonium ions are present (Pethő, 1992a), will quickly

change into corresponding benzoxazolinones with the formation of formic acid. Thus DIBOA will change into BOA, which has an absorption maximum of 270 nm (Bredenberg et al., 1962) while DIBOA absorbs at 254 nm with a shoulder at 282 nm. So, if the absorption of a compound at an identical retention time shows a decrease after heat treatment and parallel to it absorption at the retention time corresponding to that of BOA is rising at a 270 nm wavelength, we can be sure that DIBOA is present. Also DIMBOA was identified in a similar manner. The retention times and UV absorptions of the two benzoxazolinones are different, and hence the identification of the two compounds, as described above, is reliable.

Furthermore glycosides of cyclic hydroxamic acids were isolated from heat-treated tissues by aqueous extraction and from the exudates by 1-butanol separation. The preliminary purification was done with paper chromatography using the solvent system ethyl acetate – formic acid – water (60 : 5 : 35). The glycosides of cyclic hydroxamic acids move more slowly in this solvent system and their Rf value is around 0.1. Cyclic hydroxamic acids and their benzoxazolinones (Rf = 0.9), and the glycosides of hydroxamic acids (Rf = 0.12) were eluted with methanol and water, respectively (free and glycosidic fractions).

The cyclic hydroxamic acids and their glycosides secreted by the roots from the 2- and 4-week-old barnyard grass plants, in the 2-3 leave stage, grown on a nutrient solution, were transferred into an aerated distilled water for a period of 4 hours each morning. The water was then concentrated to 20 mL in vacuum and extracted three times with 1-butanol. The butanol phase was then concentrated and chromatographed on Whatman 3 MM paper using ethylacetate – formic acid – water (60 : 35 : 5) solvent system.

The hydroxamic acids were extracted from the root zone of 4- or 5-week-old barnyard grass plants, grown on perlite wetted with the nutrient solution, with ion free water. The extraction of cyclic hydroxamic acids and their glycosides from the aqueous solution was done in the manner mentioned above.

The data represent averages from four experiments.

Results and discussion

Through UV-detection and $FeCl_3$ reaction of the chromatograms of the analysed species we were able to detect hydroxamic acids in the seedlings of barnyard grass only. For this reason, in what follows, we are going to deal with this plant alone. The detected hydroxamic acids were eluted and analysed by HPLC. In the course of this analysis we found that seedlings of barnyard grass contained 2,4-dihydroxy-1,4(2H)-benzoxazinone-3-one (DIBOA).

The quantitative analyses of the cyclic hydroxamic acids were carried out in plants grown on perlite wetted with the nutrient solution over a period of five weeks. It was concluded (Table 1) that at this age considerable quantities of DIBOA and DIMBOA glycosides were present in both the shoots and the roots, and that DIMBOA was found in greater concentrations, especially in the roots.

In our earlier studies it was concluded that maize, wheat and rye (Pethő, 1992b) roots, placed in distilled water, will secrete considerable amounts of hydroxamic acids over a period of 4 hours. Proven that barnyard grass also contains hydroxamic acids, it is reasonable to analyse hydroxamic acid secretion by its roots. As Figure 1 indicates, both free cyclic hydroxamic acids and ones in a glycosidic bond can be identified from root extractions of barnyard grass seedlings. On the basis of the data in this Figure we can also state that it is DIMBOA, and DIMBOA glycoside and not DIBOA can be found in larger amounts in secretions of two-week-old barnyard grass plants.

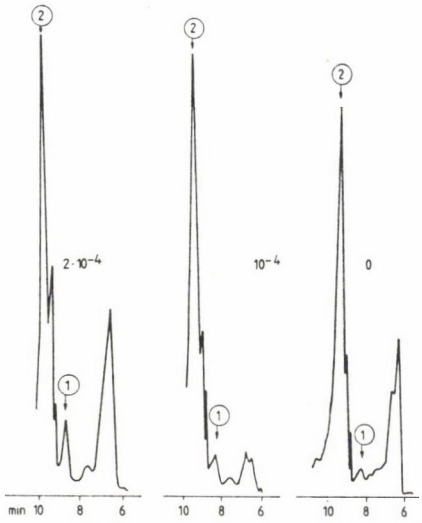

Fig. 1. Identification by HPLC of free and glycosidic hydroxamic acids secreted by roots of 2- week-old barnyard grass plants in 4 hours. A: glycosidic fraction; B: free hydroxamic acids detected at 290 nm; C: the same detected at 270 nm

Table 1

Amounts of hydroxamic acids and their glycosides in µg/g fresh weight as isolated from shoots and roots of five-week-old barnyard grass plants, and from the rhizosphere of four-week-old barnyard grass plants, and those secreted into distilled water in four hours

Hydroxamic acids	Shoot	Root	Isolated from rhizosphere	Secreted in 4 hours
DIBOA	–	–	4.75	1.54
DIMBOA	–	–	1.02	0.76
DIBOA-glycoside	7.5	4.05	10.18	5.54
DIMBOA-glycoside	8.7	18.85	6.89	4.19

On the basis of the data presented, it can be stated that like other plants containing hydroxamic acids, barnyard grass secretes cyclic hydroxamic acids through its roots. Since Barnes et al. (1987) have proven the allelopathic effect of DIBOA, we tested the effects of barnyard grass growing medium on root growth of rice. Germinating grains of rice were placed among ten-day-old barnyard grass seedlings. After 72 hours the lengths of rice roots were measured and compared to the root growth of barnyard grass-free incubated rice. The length of rice roots incubated on water was 84.9 mm while that of the ones on the solution of one-week-old barnyard grass was only 54.1 mm.

The effects of DIBOA on the growth of rice plants were analysed by purified crystalline compounds separated from rye. For the purposes of this analysis, germinating grains of rice were placed on the plastic nets of the pots containing DIBOA solution and after an incubation period of 72 hours the lengths of shoots and

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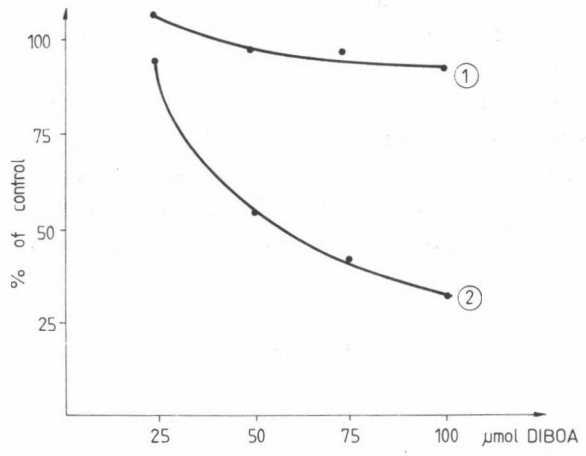

Fig. 2. Response of rice plants (% of control) to incubation on different concentrations of DIBOA solution over a period of 72 hours. Growth curves of shoots (1) and roots (2)

roots were measured. The data shown in Figure 2 indicate that DIBOA, even in fairly small concentrations, will considerably decrease rice root growth. Shoot growth decrease is moderate since it was only the DIBOA taken up that could have retarded it, as shoots had no direct contact with the DIBOA solution.

Barnes et al. (1987) found that the DIBOA concentration sufficient to bring about a 50% retardation (I50) in *Lepidum sativum* and *Echinochloa crus-galli* root growth was 0.36 mM. In our analysis, 50µM/L DIBOA concentration was sufficient to bring about a 50% retardation, which value was considerably smaller than that found by Barnes et al. (1987), and it shows that rice roots are extremely sensitive to DIBOA.

The amounts of the free and glycoside bounded cyclic hydroxamic acids accumulated in the rhizosphere of barnyard grass grown on perlite wetted with nutrient solution were determined after four weeks. The analyses show (Table 1) that there was a considerable amount of free and glycoside bonded cyclic hydroxamic acid accumulated in the rhizosphere of barnyard grass.

133 microgram DIMBOA glycoside/1kg wet perlite was isolated from the root zones of barnyard grass plants grown on perlite wetted with the nutrient solution. Therefore we determined the quantities (mM) of DIBOA, DIBOA-glycoside and DIMBOA-glycoside necessary for a 50% inhibition of root elongation of rice (I50) and found them to be 0.05, 0.26 and 0.14, respectively. It was concluded that free hydroxamic acids are more toxic than their glycosidic forms.

On the basis of the results it can be concluded that barnyard grass synthesizes cyclic hydroxamic acids and (DIBOA and DIMBOA) secretes them into the

rhizosphere. Rice roots are extremely sensitive to this compound. At present, there are no factual data available concerning the mechanism of inhibition.

Since cyclic hydroxamic acids tend to form complexes with Fe(III) it is possible that in this manner, i.e., disturbing the Fe-supply of the rice, they inhibit the growth of roots.

Nair et al. (1990) have recently proven that soil microorganisms transform BOA into 2,2'-oxo-1,1'- azobenzone, which is biologically more active than either DIBOA or BOA. Chase et al. (1991) have found that culture crops are more sensitive to these compounds than weeds. With these findings into consideration, the secretion of cyclic hydroxamic acids by weeds deserves special interest.

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POSSIBLE ROLE OF CYCLIC HYDROXAMIC ACIDS IN THE IRON UPTAKE BY GRASSES

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The iron complex of the cyclic hydroxamic acids is a source of iron available for plants. The complex taken up through the root is translocated to the shoot, where the iron increases the chlorophyll content and moderates the chlorosis. The roots of maize take up the iron complex of the DIBOA-glycoside and even transform it enzymatically. The intensity of uptake and the rate of transformation are determined by of the iron status: iron deficient crops take up more complex, their metabolism is increased compared to those grown in culture solution containing iron.

Uptake of the iron complex of hydroxamic acids can also be noted in rice and oat, which do not contain these compounds. The mechanism of uptake is not thus restricted to plants containing hydroxamic acid. The results render it probable that the cyclic hydroxamic acids represent a new form of the phytosiderophores.

Keywords: chlorosis, cyclic hydroxamic acids, DIBOA glycoside, DIBMOA glycoside, iron uptake, maize, oat, phytosiderophores, rice

Introduction

A definite quantity of iron has a vital importance for every living organism – from microorganisms to humans, since it is a component of many enzymes. At the same time, excessive quantities of it – especially in its reduced form – are toxic, because in that case free radicals may be generated through electron discharge (e.g. Fenton reaction). Under optimum conditions in the soils a considerable proportion of the iron is in an oxidized state, and this form is not readily available for the plants. The plants possess a special adaptation mechanism for the uptake of not readily available iron (Römheld, 1987). One of the components of this mechanism is the proton extrution by the roots, which increases the mobility of iron. This explains the "lime chlorosis" of soils with high lime content, where the iron uptake by the plants is hindered because the high pH value neutralizes the discharged protons. The grasses represent an exception, since these plants possess a special iron uptake mechanism. They transform the nicotianamine, a common compound in plants, into mugineic acid type compounds, which they exudate through their roots into the rhizosphere, where they form complexes with the oxidized iron. The grasses take up the mobilized chelates and the reduction takes place within the plant (Römheld and Marschner, 1986).

Abbreviations:
DIBOA: 2,4-dihydroxy-2H-1,4-benzoxazin-3[4H]one
DIMBOA:2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3[4H]one

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The exudation of compounds called phytosiderophores, of basic importance in the iron uptake by grasses, is determined by the iron supply. So the Fe-mobilizing effect (phytosiderophore activity) of the root exudate of barley in an iron deficient culture liquid is twentyfold, compared to that of plants raised in a culture solution containing iron (Marschner et al., 1989). Also, the iron deficient (chlorotic) plants took up five times as much iron complexed with phytosiderophore as plants well supplied with iron.

There are great differences in phytosiderophore activity between the plants. So maize and sorghum exude phytosiderophore in a quantity of 1/10 and 1/100, respectively, compared to barley (Kawai et al., 1988). In the case of these plants characterized by high organic matter production, besides the phytosiderophores of the mugineic family, an alternative mechanism is supposed to exist, which ensures an optimum iron uptake by these plants. Since both plants synthesize cyclic hydroxamic acids (Niemeyer, 1988), and in the iron uptake by microorganisms hydroxamate type siderophores play a role (Powell et al., 1982), it seemed to be expedient to examine whether the cyclic hydroxamic acids represented an alternative mechanism, besides the mugienic type phytosiderophores.

The cyclic hydroxamic acids form complexes with the Fe(III)-ions, and already Tipton and Buell (1970) supposed them to play a role in the iron uptake. Grasses of 12 genera have so far been proved to contain cyclic hydroxamic acids (Niemeyer, 1988; Pethő, 1993). Their occurrence can thus be considered as rather common in grasses, or at least is not restricted to a few species. Taking this for granted, we examined their concentration as a function of iron supply. We found that the DIMBOA-glycoside content of the maize roots, the amount of hydroxamates exudated by the roots, increased on $FeCl_3$ supply. In the presence of available iron (Fe-EDTA) this phenomenon was not observed (Pethő, 1992a). The roots of wheat and rye also exudate hydroxamates, and the amount of the exudate is a function of Fe-feeding (Pethő, 1992b). We supposed that, with a part of the grasses, the hydroxamic acids have a part in the iron uptake, possibly possessed an alternative phytosiderophore function; that is, they not only selected these compounds but also took up their complexes formed with Fe(III)-ions.

The question can be approached from various sides. If the plants take up the iron-hydroxamate complex, chlorosis appearing in iron deficiency ceases. An increase in the hydroxamate content of roots, or occasionally of shoots in the course of incubation in a solution of iron-hydroxamate chelates, may be a further evidence of the uptake of these complexes, the phytosiderophore-like function of the cyclic hydroxamates.

In this case the synthetic chelates (e.g. Fe-EDTA) can be replaced by these complexes.

The present paper gives an account of such experiments.

Materials and methods

For the experiments Pioneer 3950 MSc two-line maize, rice (*Oryza sativa* L. cv. "Karmina") placed at our disposal by the Research Institute of Irrigation, Szarvas, and oat (*Avena sativa* L. cv. "GK 3") were used. The plants were raised in phytotron, in an aerated hydroponic culture for 10-14 days from moistening. The conditions of the experiment were described in detail in earlier publications (Pethő, 1992a, b). In short: the pregerminated grains after the radicle had appeared were transferred to plastic nets in dark glass vessels containing ion-free water; then after 3 days to a culture liquid described by Römheld and Marschner (1986). The culture solution was renewed every 3 days. It was prepared with twice distilled water and its initial pH was 5.6.

The chlorophyll content in the blades of the youngest leaves was determined by the method of Arnon (1949). The glycosides of the cyclic hydroxamic acids were determined from 2-4 g plant material inactivized by 4 minutes of boiling. This procedure is necessary to inactivize the glycosidases, because the aglucons readily decompose. With the view of a more exact determination, it is reasonable to examine the glycosides. The heat treated material was rubbed in mortar with 20 ml boiling water in the presence of quartz sand; then, after the addition of 20 ml ethanol, extracted in horizontal shaker for 1 hour at room temperature. After centrifuging, the solution was evaporated in vacuum to a low volume (about = 0.5 ml), applied to 15 cm wide Whatman 3 chromatographic paper, then chromatographied with the upper phase of a mixture of ethyl acetate, formic acid and water (60:5:35) solvent over 25 cm. The place of the hydroxamate glycosides was determined on the basis of blue colouring obtained by alcoholic $FeCl_3$ solution treatment, or by detecting at 254 mn. From the horizontal stripes the hydroxamic acid glycosides were extracted with 35% ethanol. The alcoholic extract was evaporated until dry in vacuum, the residue taken up in 35% ethanol, filtered through microfilter, and analysed by means of a Labor MIM type Liquochrom 2010 liquid chromatograph. From the solution of 2g fresh weight/ml concentration 20 µl was applied to a 4.6x25 cm Chromsil C_{18} reverse phase column. Elution took place with a mixture of methanol and water. The initial 20% methanol concentration was increased by 5% per minute for 10 minutes, then elution was continued at that concentration for further 4 minutes. The rate of through-flow was 1.0 ml/minute. Detection took place at 254 nm with DIBOA-glycoside and at 266 nm with DIMBOA-glycoside. The quantitative analyses were carried out on the basis of the peaks of calibration curves, drawn with chromatographically purified samples.

For the examinations chromatographically purified hydroxamate glycosides were used, which were complexed with $FeCl_3$ and, after the appearance of the blue colouring, diluted to the required concentration. The inhubation time was 1 to 6 hours given in detail for each experiment.

With the reliability of the experiments kept in view, we carried out eight experiments with maize, six with rice and two with oat. The data are the averages of a minimum three replications per sample.

Results

Iron complexed with DIMBOA-glycoside as iron source

The youngest leaves of two-week-old maize plants raised on an iron-free culture solution already show expressed chlorosis. If the maize plant is able to take up iron in the form of its complex with hydroxamic acid, the iron thus supplied has to moderate the chlorotic symptoms. With iron complexes of synthetic compounds (e.g. Fe-EDTA) the most frequently used concentration is 10^{-4} mol/l. We used the same concentration. We added 10^{-4} mol/l DIMBOA-glycoside titrated with $FeCl_3$ to the culture solution, and on the third and sixth day following the iron supply compared the chlorophyll content of the fourth, youngest leaf with that of plants kept on an iron-free medium (Table 1).

The data of the table show that, in the case of plants grown on iron-free culture solution, the degree of chlorosis increased. The iron complex of the cyclic hydroxamic acid proved a good source of iron. Even three days after its application the chlorosis

was considerably subdued, and six days following the treatment it practically ceased. The chlorophyll content of the fourth, youngest leaves increased nearly threefold compared to the control leaves.

According to the results of the experiment, iron in a complex formed with DIMBOA-glycosid probably can also be taken up by the roots of maize and translocated to the leaves.

Table 1

Effect of the iron(III) complex of DIMBOA-glycoside on the chlorophyll content of maize leaves

Treatment	3 days after the addition of the complex		6 days after the addition of the complex	
	µg/g	%	µg/g	%
Fe-free control	782±74	100.0	590±72	100.0
10^{-4} mol/l Fe(III)-complex	1195±106	153.3	1614±113	273.6

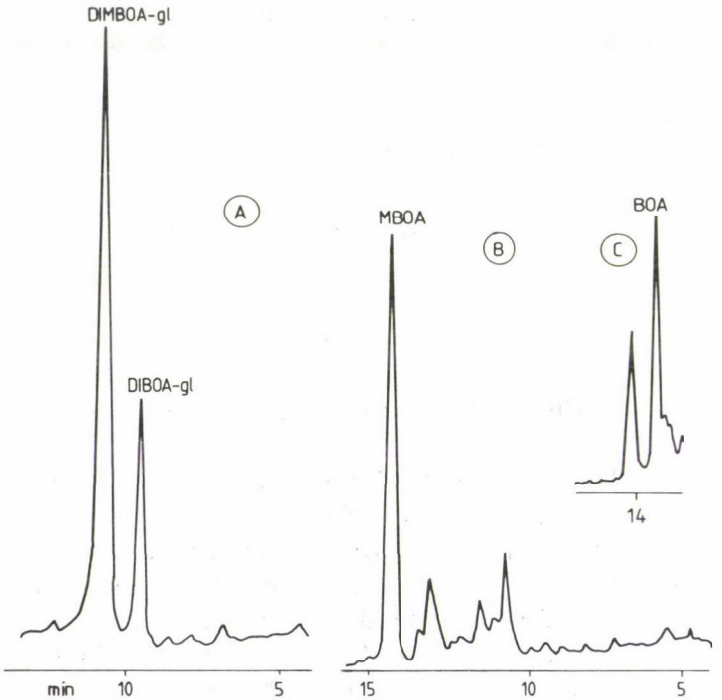

Fig. 1. Hydroxamic acid glycosides in roots of maize plants incubated in distilled water (0), $FeCl_3$-titrated 10^{-4}mol/l and $2 \cdot 10^{-4}$ mol/l DIBOA-glycoside solution. 1: DIBOA-glycoside; 2: DIMBOA-glycoside. From 2 g fresh root/ml concentration solution 20 µl was applied to 4.6×25 cm Chromsil C_{18} column. Elution with linear gradient of methanol-water. Initial methanol concentration 20%, increased by 5% every minute. Rate of through-flow 1 ml/minute. Detection at 255 nm, absorbance about 9 minutes changed from 0.5 to 2.0 (the line in the figure breaks)

Uptake and transformation of the iron complex of DIBOA-glycoside

In the tissues of maize DIMBOA-glycoside is dominant while the amount of DIBOA-glycoside is considerable lower. With other plants it is just the other way round. We started from the assumption that if the root of maize took up the iron complex of DIBOA-glycoside, its DIBOA-glycoside content would increase. In the course of these experiments roots of maize plants raised on an iron-free mediumwere incubated on iron complex of DIBOA-glycoside of 10^{-4} mol/l, and $2 \cdot 10^{-4}$mol/l concentration for five hours with constant aeration. The surface adhered and apoplasmically fixed complex was removed by washing for 10 minutes in distilled water, and the hydroxamic acid-glycoside content of the roots was then liquid chromatographically analysed.

As seen in Fig. 1 showing the characteristic phases of the liquid chromatograms, the hydroxamic acid content of the roots increased at both concentrations of DIBOA glycoside, compared to the roots incubated in distilled water. It was surprising that not only the amount of DIBOA-glycoside supplied, but also the amount of DIMBOA-glycoside increased too, which suggests that the maize not only took up but also transformed the hydroxamic acid it had taken up. The quantitative relations of the two hydroxamic acids are shown by the fact that in the course of measuring the absorbancy of the instrument had to be changed. During the detailed examinations, the test solution was therefore diluted after the determination of the DIBOA-glycoside, and the quantity of DIMBOA-glycoside was determined from the diluted solution at 266 nm.

Hydroxamate uptake and metabolism of roots of maize plants of various age

Roots of 10- and 13-day maize plants were incubated on DIBOA-glycoside solution of different concentration with constant aeration for six hours. After 10 minutes of soaking, the hydroxamic acid-glycoside content of the roots was determined (Table 2).

Table 2

Hydroxamic acid contents in maize roots incubated with the iron complex of DIBOA-glycoside

Concentration of the complex, mol/l	DIBOA-glycoside		DIMBOA-glycoside	
	µg/g	%	µg/g	%
	Experiment with 10-day-old plants			
0	2.53±0.76	100.0	729.3±36.8	100.0
10^{-4}	6.67±0.52	263.6	849.3±52.9	116.4
$2 \cdot 10^{-4}$	9.13±1.83	360.8	853.3±37.7	117.0
	Experiment with 13-day-old plants			
0	2.02 ±0.29	100.0	412.0±37.5	100.0
$2 \cdot 10^{-4}$	19.53±2.23	966.8	507.0±32.2	123.1

On the basis of the data given in the table the following statements can be made:

1. The hydroxamic acid content of the maize roots decreases in time, which is in accordance with the literary data (see later).
2. In the roots fed with DIBOA-glycoside not only the amount of the DIBOA- but also that of the DIMBOA-glycoside increased; but while with the former the increase was relative, with the latter it was of absolute significant.
3. In the older plants iron deficiency appeared more expressedly, the youngest leaves showed symptoms of chlorosis. This accompanied the increased hydroxamic acid uptake. In the 13-day plants the DIBOA-glycoside content grew nearly tenfold.
4. The roots of maize probably not only take up but also transform the iron complex of the hydroxamic acid: the benzene ring is supposedly first oxygenated, then methylated. These enzymatic processes seem to be intensive, since during the six hours of incubation the amount of the DIMBOA-glycoside remarkably increased. The question has not yet been settled.
5. On the basis of the function of enzymes responsible for the development of the metoxy group, the complex is likely to be transported in symplasmic way. That is, the membrane transport of the absorbed hydroxamic acid is highly probable.

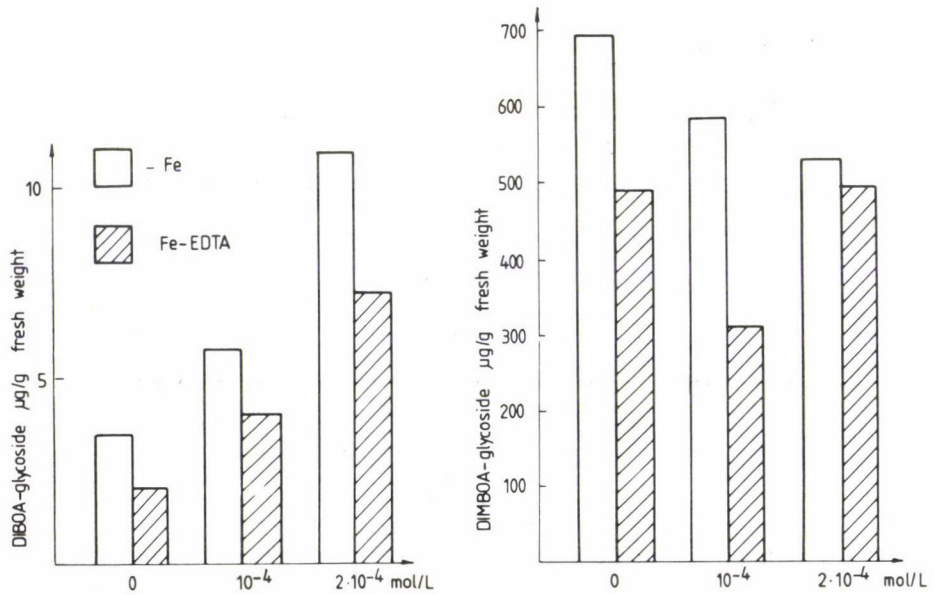

Fig. 2. Iron-hydroxamate uptake and DIBOA and DIMBOA-glycoside content in plants raised in an iron-free culture solution and in one containing iron in the form of 10^{-4} mol/l Fe-EDTA. On the horizontal axis the concentration of the DIBOA-glycoside-iron complex of the incubation medium is indicated

Effect of iron supply on the hydroxamic acid content of maize roots and uptake of its iron complex

If we take the role of hydroxamic acids in iron uptake for granted, it is probable that iron deficient plants take up larger quantities of iron in the form of hydroxamic acid complex. In order to settle the questions, we raised maize plants on culture solutions free of iron and containing 10^{-4} mol/l Fe-EDTA, respectively. The plants were placed in the culture solution (and supplied with iron) a week after the beginning of the experiment. After three days the culture solution was replaced, and the plants were then incubated in distilled water, on 10^{-4} mol/L iron complex of DIBOA-glycoside at room temperature, with constant aeration over 5 hours. After that the enzymes of the roots were inactivated by boiling and the hydroxamic acid-glycosides extracted. The extracts were chromatographically purified, then the hydroxamic acid-glycosides determined by liquid chromatography. The results are contained in Fig. 2.

The youngest leaf of the two-week-old (4-leaved) plant was chlorotic on the iron-free medium, the quantity of chlorophyll a + b was 930 µg/g compared to 1.490 µg/g in leaves of plants grown on iron-containing medium, which means a 63% higher chlorophyll content. The absence of iron increased both the DIBOA- and the DIMBOA-glycoside content. The iron deficient plants took up more of the iron complex of the DIBOA-glycoside than did the plants raised on a medium containing iron.

The data obtained show that the iron given in the form of hydroxamic acid complex is available for the maize plant, and the uptake is a function of the iron status. Iron deficient plants take up more hydroxamic acid complex than plants previously raised on a medium containing iron.

The maize roots contain DIMBOA-glycoside about two orders more than the form of glycoside not containing metoxy group (DIBOA). This is probably due to the fact that the oxygenation then methylation of the benzene ring is more intensive in this plant than in other plants, where the DIBOA-glycoside is dominant. It is also likely that the iron status influences these enzymatic processes too, and not only the hydroxamic acid synthetized in the root, but the absorbed one is also a substrate of these enzymes.

According to the data, the iron supply moderates the transformation processes. The reaction is relatively quick, because not only in the roots of plants raised on an iron-containing medium was the amount of DIMBOA-glycoside smaller, but the iron taken up in the course of the five-hour incubation also moderated the activity of the enzymes responsible for the development of the metoxy group.

Iron (III)-complex of hydroxamate taken up by rice plants

In the course of earlier experiments (Pethő, 1993) we found that the root and shoot growth of rice plants was inhibited by the cyclic hydroxamic acids. This is only possible if these compounds are taken up by the rice. Taking this fact as a basis, we attempted to find out whether rice plants grown on an iron-free medium took up iron

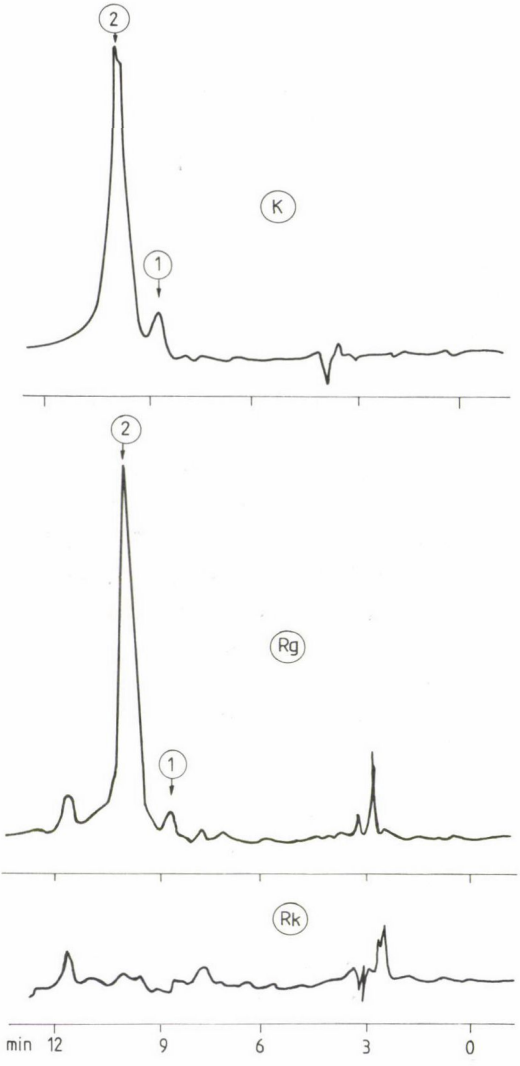

Fig. 3. Hydroxamic acid-glycoside uptake by rice roots. K: hydroxamic acid-glycosides isolated from maize shoots.
1: DIBOA-glycoside;
2: DIMBOA-glycoside.
Rg: extract of rice roots incubated in 0.63 mmol/l solution of the previous preparation for 18 hours.
Rk: extract of control rice roots incubated in distilled water.
Detection at 266 nm, absorbance 1.0

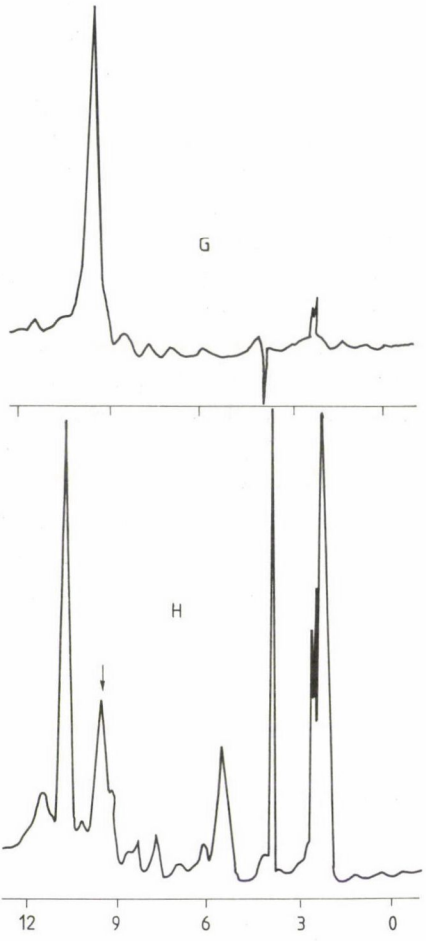

Fig. 4. Liquid chromatographic analysis of roots (G) and shoots (H) of two-week rice plants incubated in $FeCl_3$-titrated hydroxamic acid-glycoside solution for 16 hours.
The root extract corresponds to 2 g, the shoot extract to 10 g fresh weight per ml.
From this solution 20 µl was applied to C_{18} column.
Detection at 266 nm, absorbance 0.5

in the form of hydroxamate complex. That is, the rice plant does not contain hydroxamic acids, so a successful incubation on DIMBOA solution unambiguously proves the uptake of the complex.

From etiolated maize seedlings we made the hydroxamic acid-glycoside preparation and produced its Fe complex, then adjusted the concentration of the complex to 0.63 mmol/L, and placed ten-day-old rice plants onto the complex. After 18 hours of incubation we placed the roots in distilled water for 1 hour in order to remove the iron adhering to the surface and fixed in the apoplasma, respectively. After that, the homogenate of the heat treated roots we extracted with n-butanol, took the residue of the vacuum-dried butanol phase in 35% etanol and purified it chromatographically. Then we analysed the hydroxamic acid content of the extracts by liquid chromatography. The extract of rice roots incubated in water and the solution of intake were analysed in parallel.

The retention curves shown in Fig. 3 prove that the rice roots took up both hydroxamic acid-glycosides. Accordingly, the uptake mechanism is not restricted to plants containing hydroxamic acid. Then we attempted to detect the absorbed hydroxamate in the shoot too, and performed the experiment with oat plants as well.

Hydroxamate content in roots and shoots of rice- and oat plants incubated on the iron complex of hydroxamic acid glycoside

As seen in Fig. 4, with a retention time of 9.4 minutes, the DIMBOA-glycoside can be detected both in the root and in the shoot; it was taken up by the root and translocated to the shoot of rice. While in the root 81 μg/g, in the shoot 8.0 μg/g DIMBOA-glycoside was measured after 16 hours of absorbtion time. Then the plants were placed back into the culture solution. After 24 hours 26.5 μg/g DIMBOA-glycoside was measured in the shoot, and after 48 hours the earlier chlorotic leaves turned green, while their chlorophyll content increased. This shows that the complex gradually translocated from the root to the shoot, considerating that the effect of iron and the presence of the DIMBOA-glycoside was indicated.

After that, the concentration of hydroxamic acid was decreased to $2 \cdot 10^{-4}$ mol/l and the time of uptake to 5 hours, and the plants were placed back into the culture solution for 24 hours. The experiments were carried out with rice and oat plants. The amount of DIMBOA-glycoside in the roots and shoots of the plants are given in Table 3.

According to the data, the roots of oat also take up the iron complex of the DIMBOA-glycoside, moreover, the latter even translocates to the shoot. The mass of the oat shoot was larger, so the hydroxamic acid taken up by the root was diluted to a greater extent.

Table 3

DIMBOA-glycoside content in rice- and oat plants incubated with DIMBOA-glycoside complex of iron for 5 hours, 24 hours after the time of uptake

Plant	DIMBOA-glycoside, µg/g	
	in root	in shoot
Rice	13.7±1.32	6.5±0.57
Oat	15.4±1.81	1.7±0.25

Discussion

With iron complex of DIMBOA-glycoside added to the culture solution of maize at a concentration of 10^{-4} mol/l, the chlorotic symptoms moderated. This concentration corresponds to that used with other, synthetic chelates. Thus, the iron(III)-hydroxamate complex proved to be an adequate source of iron.

On the root of maize, the hydroxamic acid concentration decreases with age. The same was found by Argandona and Corcuera (1985). When the roots of maize were incubated on Fe(III)-complex of DIBOA-glycoside, the amount of DIBOA-glycoside increased in the roots; in the case of chlorotic plants, it became nearly tenfold, compared to the DIBOA-glycoside content of the control plants.

Furthermore, the uptake of the iron(III)-hydroxamate complex by iron deficient plants was found to be a function of the iron status: iron deficient plants took up more DIBOA-glycoside than the plants raised in a medium containing iron. This result agrees with the data published by Marschner et al. (1989): the Fe(III)-mobilizing effect of barley root exudates is remarkably higher in the case of plants raised in an iron-free culture solution. Sugiura and Nomoto (1984) also found that the lack of iron increased the mugineic acid production.

The concentration of the main hydroxamic acid of maize (DIMBOA-glycoside) lessens even during a five-hour incubation under the influence of iron-hydroxamate complex. This agrees with the result published by Takagi et al. (1984), namely, that following the iron supply the mugineic acid production of iron deficient plants rapidly decreased. A comparison with these literary data shows that the cyclic hydromamic acids are in many respects similar to the phytosiderophores, members of the mugienic acid family.

The iron-hydroxamate complex is also taken up by the roots of the rice and oat plants, and is even translocated to the shoot. When, after the time of uptake, the plants were placed back into the complex-free culture solution, the hydroxamate content of the shoot continued to increase, indicating that the complex taken up by the root gradually translocated into the shoot, and the chlorophyll content of the leaves increased. It is likely that the iron-hydroxamate uptake system is not restricted

to plants containing hydroxamate. As for the place of transport, only Argandona and Corcuera (1985) can be referred to, who measured a higher hydroxamate concentration in the transporting tissues than in the ground tissues. At the same time, their presence in the xylem sap in any detectable quantity was not proved. This question requires further investigations.

In agreement with the literary data, we may assume that the cyclic hydroxamic acids take part in the uptake of iron (and possibly other microelements), and even in its transport. This group of compounds appears to possess a phytosiderophore function.

On the basis of the data published by Tipton et al. (1973), Niemeyer (1988) thinks it probable that in the biosynthetic chain of the benzoxazines the glycosides of the lactames are oxygenated, then methylated, and ultimately through the oxygenation of the lactames, the hydroxamic acids are formed. On the other hand, our results show that in the maize root the oxygenation, then methylation of the benzene ring of the hydroxamic acids is not excluded either. In plants containing hydroxamic acids, the metoxy group-free DIBOA is more frequent, but in the tissues of wheat and maize the DIMBOA can be found in larger quantities (Zuniga et al., 1983). In these plants the enzymatic processes of oxygenation and methylation are probably more intensive, and the processes are not restricted to the lactames, but also take place with the hydroxamic acids. Further investigations are necessary to settle the question.

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MICROPHENOLOGY OF FLOWERING IN TWO APPLE VARIETIES

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In the apple variety collection of the Debrecen State Farm, Pallag the microphenological phenomena of flowering were followed in the varieties Watson Jonathan and Golden Delicious between 1988 and 1990.

We found significant differences in the duration and course of anther dehiscence between both the varieties and the dates of observation. The dehiscence of anther was more responsive to changes in temperature than the ageing of stigma. In warm and sunny weather some dehisced anthers can be found in the flower even at the beginning of flower opening, while when the weather is cool and cloudy the dehiscence of anthers begins 3–4 hours later. The stigma reaches a receptive stage before the flower opens and the anthers dehisce, the apple flower can be regarded as a male antecessor.

In warm and sunny weather the stigmae lose viability, in 1.5–2 days, while in cool, cloudy weather, they begin to turn brown after 4–5 days. The maximum stigmal secretion that helps the adhesion of pollen is between 11 a. m. and 3 p. m. In warm, humid and cloudy weather the stigmal secretion is produced in abundance. In the warm motionless air of the parchment isolator secretion, ageing of stigma and dehiscence of anthers begin and end earlier than in the open air.

Keywords: apple varieties, flowering, microphenology, functioning ability of sexual organs, anther, anther dehiscence, viability of stigma, secretion, pollen germination, isolation

Introduction

A large proportion of the cultivated fruit varieties are self-sterile. To achieve a suitable measure of fruit setting all apple varieties need pollen donors. For choosing the pollen donor varieties a thorough knowledge of the flowering times is necessery; it can be acquired through the examination of the microphenological phenomena of flowering (Davary-Nejad and Nyéki, 1990; Davary-Nejad, 1992).

The flowering index elaborated for the herbaceous horticultural crops (*Adonis vernalis* L., *Capsicum annuum, Matricaria chamomilla* L.) (Máthé, 1977; Máthé and Bahadli 1989; Máthé et al., 1985) has recently been found to be suitable for the numerical expression of the apple flowering, too (Máthé et al., 1993). This index may enable a more exact determination of the extent of simultaneous flowering.

Microphenology belongs to the observation methods of flowering phenology; it is concerned with the functioning ability of sexual organs in relation to the meteorological factors.The method makes it possible to establish the time of the secretory activity of stigma, the dehiscence of anthers and the pollen shed (Nyéki, 1975; Ifju and Nyéki, 1977). The stigmas were viable for 2–6 days and the dehiscence of anthers took 1–5 days. The latter depends on the duration of flowering;

the shorter it is, the shorter will be the time the sexual organs are able to function. The viability of stigmas and the pollen shed are influenced by air temperature, precipitation and other meteorological factors. In case of too high temperatures, the anthers may even begin to dehisce before the flowers open. The daily maximum of pollen shed in apple is generally between noon and 4 p. m. (Percival, 1955; Soltész, 1982).

In apple and pear varieties the stigmas become mature 1–4 days earlier than the anthers, therefore those varieties are primarily suitable to be pollen donors, in which the time of pollen shed coincides with the functioning period of the stigma of the variety to be pollinated (Gyuró et al., 1976). In the apple flower, the anther fully opens in 2 days.

Williams (1966) introduced the concept of "effective pollination period" (EPP), the difference between the life of the embryo sac and the length of time required by the cells of the pollen tube to reach the oosphere; it varies with the variety of apple. The effective pollination period is generally shorter than the duration of the viability of stigma (Williams, 1966; Williams and Smith, 1966; Williams and Wilson, 1970).

According to Marro et al. (1976) the EPP is a function of the development level of the embryo sac: the embryo sac degenerates at a certain rate, so that the late pollination of flowers is unsuccessful. When pollination took place later, varieties with embryo sacs of longer life showed better fertilization.

According to the classification of Heslop and Harrison (1976) the stigma of taxa in the subfamily Pomoidae belongs to the wet type. Such stigmas are covered by drops of secretion in their receptive phase.

According to Mowaffak (1985) the daily amount of secretion drops on the stigma varied with the temperature.

The length of the pollen shed period is 1–5 days in apple (Soltész, 1982). The dehiscence of anthers takes 2–3 days, so even in full blossom only a part of the flowers open and give pollen (Lalatta, 1982).

Materials and methods

Our observations were made in the apple variety collection of the Debrecen State Farm at Pallag on 'Watson Jonathan' and 'Golden Delicious' varieties. The trees were planted in 1978 with MM 106 as rootstock at a spacing of 6 × 4 m, and trained into an open spindle crown form. The plantation was regularly tended, and fallow cultivation was used, but irrigation was not carried out.

Changes in the colour of stigma (and phases of its viability, respectively) and the amount of stigmal secretion were followed every hour between 7 a. m. and 6 p. m. in free standing, pollinated and parchment covered flowers. Ten flowers per tree and treatment beginning to open were marked out in the outer part of the crown at eye level. The force of wind, the clouds and the temperature were also recorded.

The amounts of secretion on the stigma were given the following points:

1 – the stigma is green and produces much secretion;
2 – the stigma is green and produces a medium amount of secretion
3 – the stigma is green and little secretion can be found on it;
4 – the stigma is green and there is no secretion;
5 – the stigma is dull;
6 – the stigma is brown.

The dehiscence of anthers in the flowers was followed every hour simultaneously with observations of changes in the stage of stigma. The proportion of the hourly dehiscing anthers was given as a per cent of the total number of anthers.

The microphenological phenomena were studied in free-standing flowers of both varieties. In addition, in the case of the variety 'Watson Jonathan' the phenomena of artificially pollinated and isolated flowers were also examined.

Results

Time of anther dehiscence

The pollen transferring, pollinating activity of the pollen-collecting bees is very efficient. Their presence can, however, be reckoned with only after the anther dehiscence. The first anthers dehisce 3–7 hours after the opening of the flower, on the average (Table 1). In a higher proportion (30%) the anthers dehisce 6–24 hours following the flower opening, depending on the weather.

According to the period of anther dehiscence there are significant differences between apple varieties and years. In 1988 (when under the influence of high temperatures the anthers rapidly dehisced) the two varieties examined did not differ from one another, while in 1989 and 1990 the anthers of Golden Delicious dehisced more rapidly than those of the variety Watson Jonathan. *Changes in the temperature were found to have a very great effect on the speed of anther dehiscence.* The dehiscence of the anthers began latest in 1990 (5.9 and 7.0 hours, respectively), and the longest time until the dehiscence of the last anther elapsed in the same year (62.1 and 82.9 hours, respectively). During the period of observation the temperature was low compared to the previous two years, the daily temperature maximum did not reach 20 °C.

The relationship between the length of the period of anther dehiscence and the two elements of weather is shown in Table 1. In 1988 under the influence of the clear sky and high temperature (above 17 °C almost throughout the period of anther dehiscence) the anthers dehisced at a very fast rate. In 1990, on the other hand, the temperature hardly rose above 17 °C during our observations, and there was a 47% cloudiness; as a consequence, the anther dehiscence was long drawn-out.

In the flowers under the isolator the anthers dehisced at a faster rate in all three years of examination, and the stigmas were viable for a shorter time than in flowers standing free.

The course of anther dehiscence

The course of anther dehiscence – just like its duration – is decisively determined by the weather. In warm, sunny weather some dehisced anthers can be found in the flower even at the beginning of flower opening, while in the case of a cool, cloudy weather the dehiscence of anthers begins 3–4 hours later.

The dynamic of anther dehiscence varies both with the time of examination and the variety. In 1988 nearly 60% of the free standing flowers dehisced in the very

Table 1

Duration of stigma viability and anther dehiscence in apple varieties (Debrecen, 1988–1990)

Year	Treatment	Duration of anther dehiscence	Duration of stigma viability	Time from flower opening to anther dehiscence throughout		Temperature above 17 °C	Temperature below 17°C	Clouds above 40%
		(hour)	(hour)	(hour)	(hour)	(hour)	(hour)	(hour)
1988	Watson Jonathan	34.4	35.7	2.7	37.1	34	2	0
	Watson Jonathan (in isolator)	25.0	29.9	3.1	28.1	29	0	0
	Watson Jonathan (pollinated)	33.3	36.1	2.1	35.4	30	6	0
	Golden Delicious	37.4	28.3	2.8	40.2	25	3	0
	$L.S.D._{5\%}$	8.29	7.31	1.24	8.81	–	–	–
1989	Watson Jonathan	43.6	60.7	3.8	47.4	31	29	28
	Watson Jonathan (in isolator)	31.0	49.2	2.5	33.5	27	22	16
	Watson Jonathan (pollinated)	48.8	63.3	2.4	51.2	30	33	16
	Golden Delicious	38.5	57.6	3.3	41.8	34	23	28
	$L.S.D._{5\%}$	6.45	3.0	0.5	6.3	–	–	–
1990	Watson Jonathan	89.2	121.9	7.0	97.2	14	107	47
	Watson Jonathan (in isolator)	66.7	103.3	6.0	72.0	21	82	38
	Watson Jonathan (pollinated)	82.7	112.9	6.9	89.0	15	98	42
	Golden Delicious	62.1	105.3	5.9	68.0	17	88	40
	$L.S.D._{5\%}$	3.9	8.6	1.4	3.1	–	–	–

first day, the proportion of dehisced anthers was about 40% on the second day, and only a few percentages on the third day.

In 1989, when the weather was cooler at the time of flowering, fewer anthers dehisced on the day of flower opening, and the dehiscence of anthers was of a slower rate. The anther dehiscence reached 40–45% on the first, 45–50% on the second day, while the rest 12–13% dehisced on the third day.

In 1990 the weather was cooler than in the previous two years. As a consequence, the dehiscence of anthers took 5 days with 'Watson Jonathan' and 4 days in the case of 'Golden Delicious'. The anthers of both varieties dehisced in the largest proportion on the day following flower opening.

In Figs 1–4 changes in the functioning ability of the sexual organs are shown. With all varieties and treatments similar differences can be observed between the years. *In 1988* the rapid anther dehiscence was characterized by very high peaks. *In 1990*, under the influence of the cool weather the dehiscence of anthers was much more even.

The dynamic of anther dehiscence was in close correlation with changes in the daily temperature. *Most anthers dehisced between 10 a. m. and 3 p. m.*

The great difference in temperature between the years is indicated by the maximum proportion of anthers dehiscing in an hour; it was 11.5% in 1988, 9.8% in 1989 and 6.5% in 1990.

Stigma viability

The end of the viability of stigma was shown by the change of its colour. When the light green or yellow stigmas have turned mat brown, they are ineffectively reached by the pollen and fertilization thus occurs in very low proportions. The duration of the viability of stigma changed in the same way as the duration of anther dehiscence. It was shortest in 1988 (28.3 hours with 'Golden Delicious' and 35.7 hours in the case of 'Watson Jonathan') and longest in 1990 (105.3 and 121.9 hours, respectively). The weather caused a nearly 4-fold difference in stigma viability between these two years.

The relationship between the duration of the viability of stigma and the conditions of weather is shown in Table 1. In warm and sunny weather (1988) the stigmas loses viability in 1.5–2 days, while in cooler, cloudy weather they begin turning brown after 4–5 days. The flowers of the two varieties examined did not essentially differ in the duration of stigma viability. The stigmas of 'Watson Jonathan' lost viability later in all three years, but the difference was only significant in 1990.

We examined 10 flowers per variety to determine the duration of the viability of stigma. The scatter among the flowers and the averages characterizing the varieties are contained in Table 1. The decrease in the proportion of viable stigmas is shown in Figs 1–4.

The stigma becomes recipient even before the opening of the flower, while the anthers begin to dehisce either at the time of flower opening or several hours later.

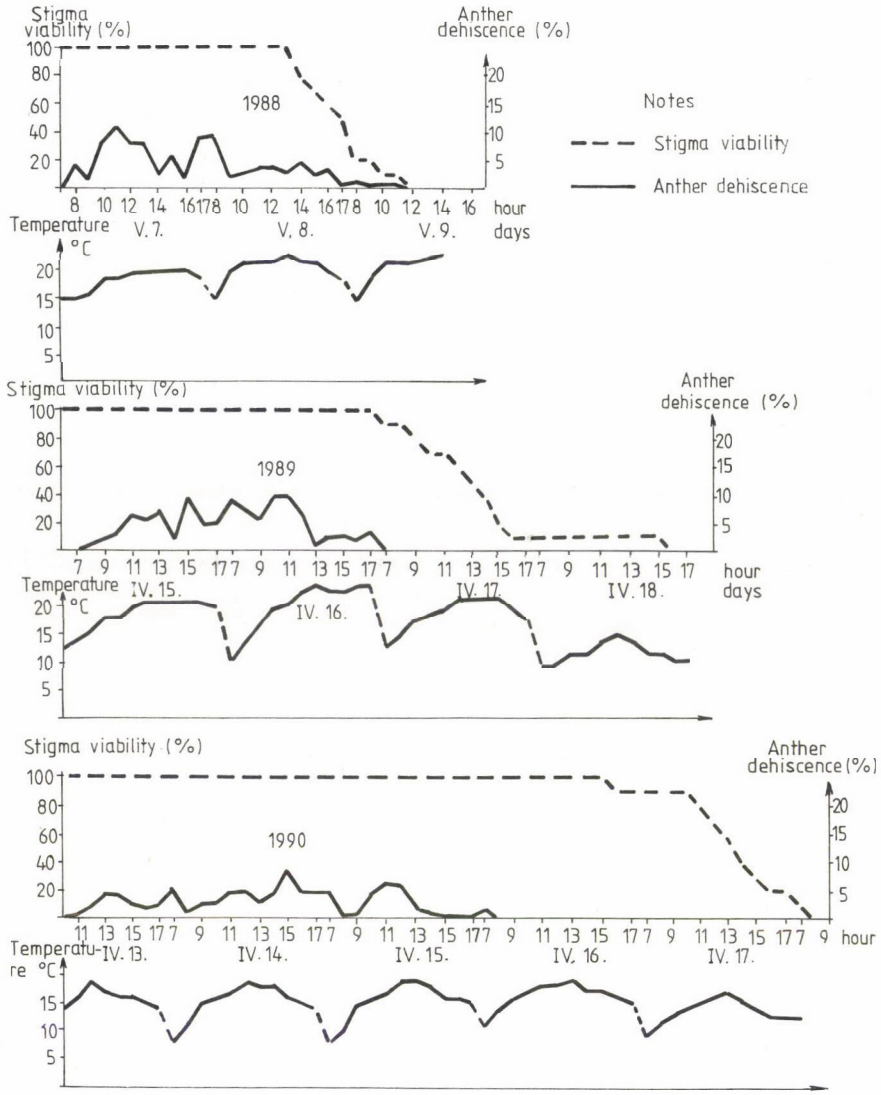

Fig. 1. Functioning ability of reproductive organs in isolated, non-pollinated flowers of the apple variety 'Watson Jonathan'

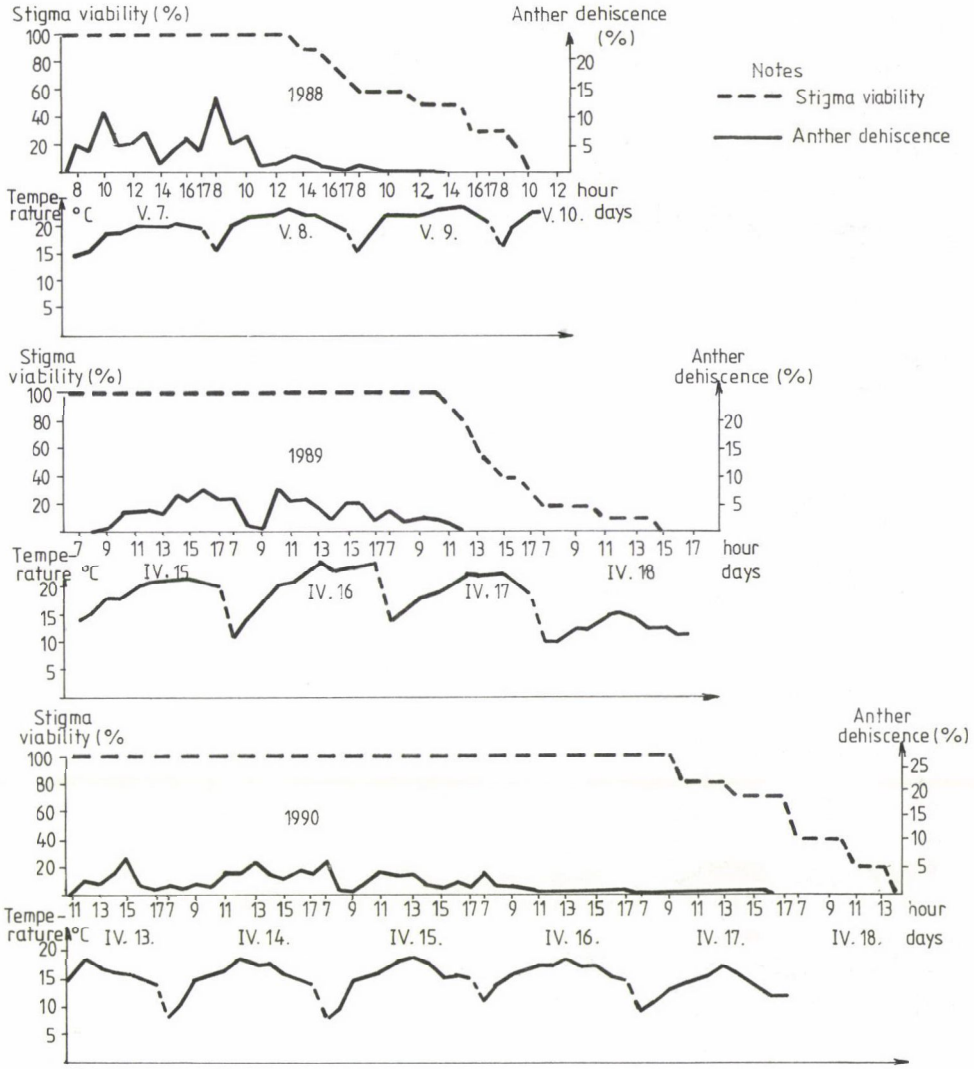

Fig. 2. Functioning ability of reproductive organs in free flowers of the apple variety 'Watson Jonathan'

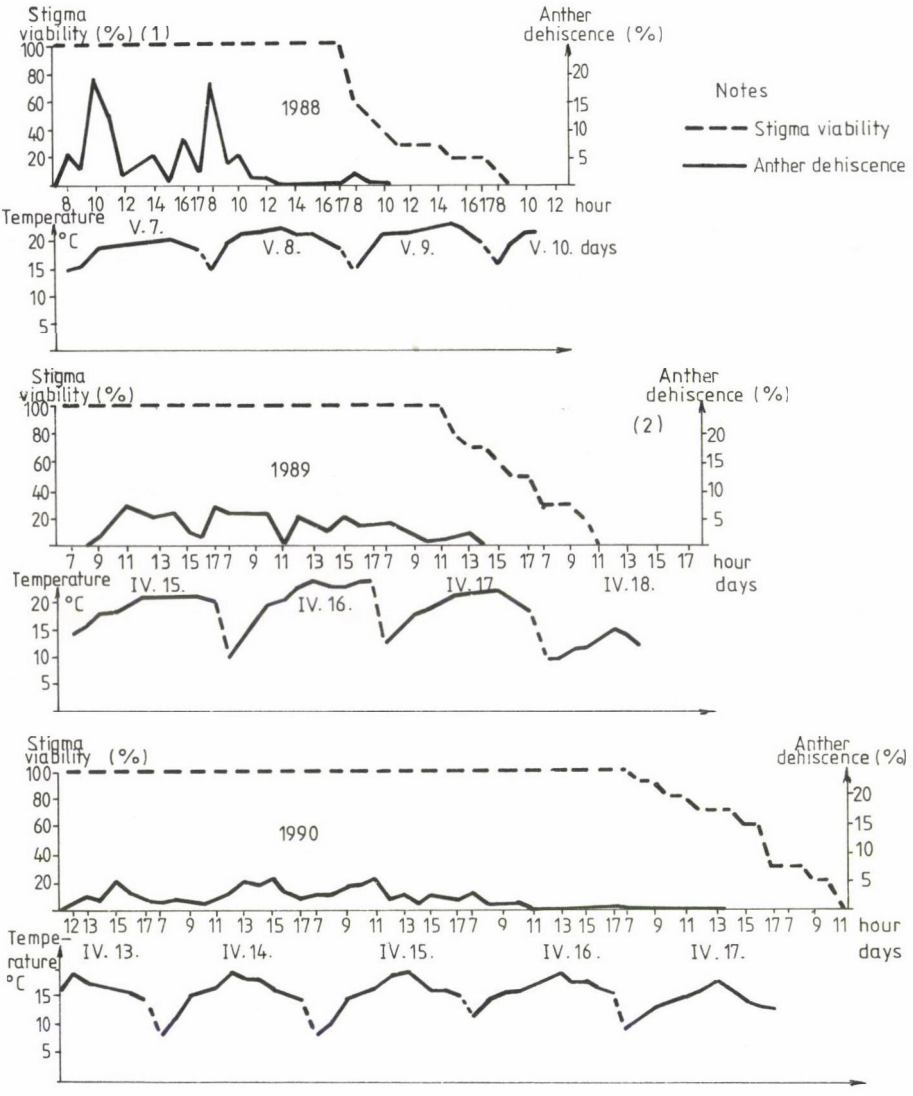

Fig. 3. Functioning ability of reproductive organs in free-standing, artificially pollinated flowers of the apple variety 'Watson Jonathan'

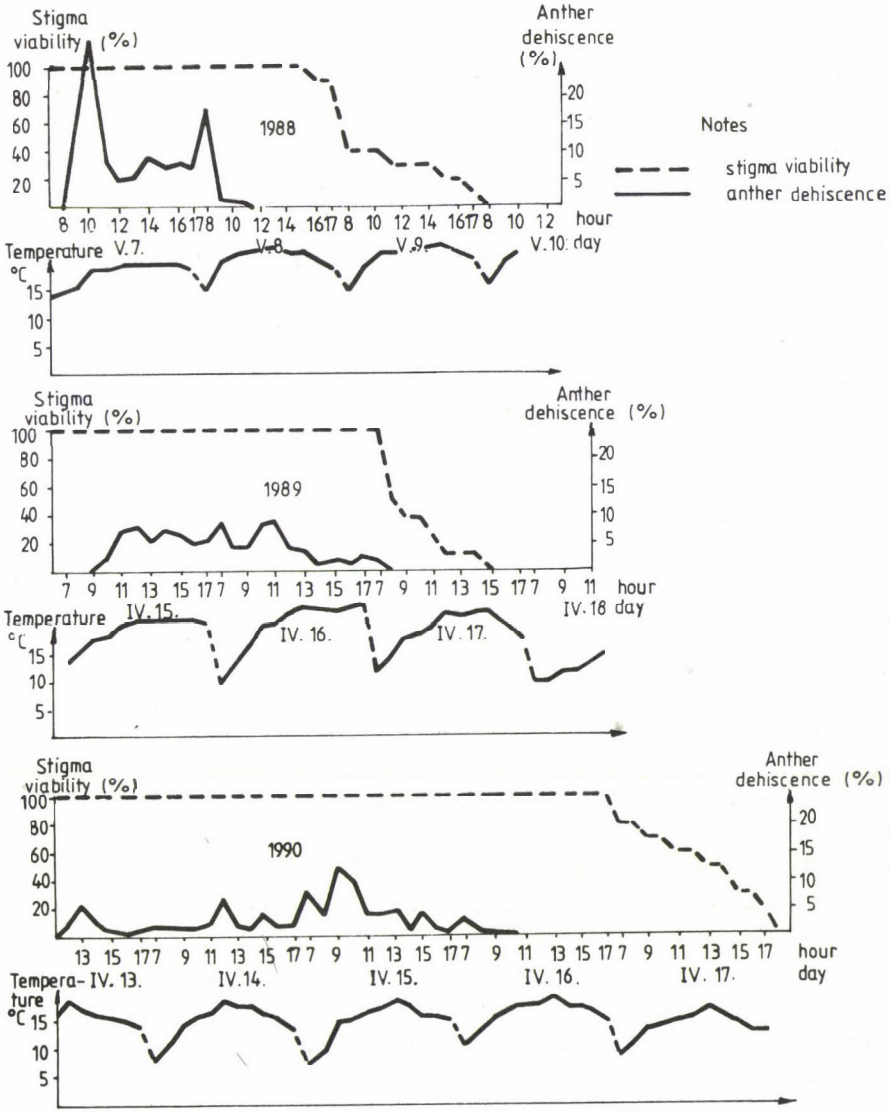

Fig. 4. Functioning ability of reproductive organs of the apple variety 'Golden Delicious'

According to Soltész (1982) in warm weather the dehiscence of anthers in balloon stage occurs with the apple too. Accordingly, the apple can be regarded as a *male antecessor.* In most flowers the stigmas retained viability longer than the period of anther dehiscence.

On choosing the pollen donor variety it is of absolute importance that the pollen shed of the pollen donor overlap the viability of stigmas in the variety to be pollinated. Favourable pollen supply is ensured by the pollinator variety whose flowers open 2 days earlier than those of the main variety.

The adherence and germination of the pollen is promoted by the appearance of the stigma secretion. Our observations between 1988 and 1990 proved that in the case of either variety ('Golden Delicious' and 'Watson Jonathan') the stigma secretion did not appear at the beginning of flower opening, the secretory activity started 4–7 hours later.

The process of secretion is influenced by the weather: in warm weather it beginas earlier, in cool weather later. *Maximum secretion can be observed between 11 a. m. and 3 p. m. (above 19 °C air temperature).* The amount of stigma secretion is very large in warm, humid, cloudy weather. When the colour of the stigma changes, its surface becomes mat, the secretory activity decreases in intensity or stops.

On examining the secretory activity we found differences between the varieties. 'Watson Jonathan' produced stigma secretion over a longer period than the variety 'Golden Delicious'.

Effect of pollination and bagging on the viability of the reproductive organs of flower

Ten pollinated and ten non-pollinated flowers of the variety 'Watson Jonathan' kept under paper bag were examined for the viability of the reproductive organs in comparison with the free standing flowers. The aim was to study the effect of pollination and of the microclimate under the parchment isolator. Nyéki (1974) found that in sunny, warm weather the air in the paper bag was warmer (30–40 °C) than the surrounding air. Experiences have shown that at night the bagged air-space cools down in a greater measure owing to the loss of emission and the absence of air motion. This microclimate influences the course of flowering, the viability of stigma, the pollen shed and the development of pollentube. Higher temperatures accelerate the development of the reproductive organs.

The effects of treatments and years appeared in combination. In comparison to the reproductive organs of free-standing flowers, significant difference was observed only in 1990. In the previous two years the development processes passed off quickly under the influence of the higher temperature.

The effect of the parchment bag was much more remarkable compared to the pollination and appeared in all three years. In 1988 the stigma viability was 5.8 hours and the period of anther dehiscence 9.5 hours shorter in the bagged flowers than in the free ones. In 1989 the duration of both phenomena was shorter by 12.4 hours, while in 1990 22.5 and 19.6 hours shorter periods were recorded, respectively.

Under the bag, in the warmer and motionless air secretion on the stigmas of flowers begins earlier, its process is more intensive and passes off at a faster rate than in the free flowers. In 1990 secretion on the day of flower opening was observed only in the isolated flowers.

According to the results of our examinations *the stigmas of pollinated flowers lost viability earlier.* As shown by the data of Table 1 the secretory activity in the pollinated flowers stopped earlier than in the free flowers in all three years. All this is supposed to be the effect of pollination, and, in the case of the adhered pollen or the pollen that develops, the secretory activity loses its function.

Discussion

Studying the microphenology of flowering we have arrived at the same conclusions as Gyuró et al. (1976), namely that, on choosing the pollen donor varieties, the microprocesses of flower opening must also be taken into consideration. The pollen discharge of the pollen donor variety has to reach maximum when most flowers of the variety to be pollinated are in a receptive phase.

It is necessary to study the functions of the reproductive organs of flowers in order to be able to determine their viability, and through the activity of bees and pollination to understand the process of fertilization.

In agreement of Gyuró et al. (1976) and Lalatta et al. (1978) our observations proved the phenomenon of *male antecedence.* We have confirmed the statement of Nyéki (1974) that the secretory activity of stigmas, the dehiscence of anthers and the shed of pollen depend primarily on meteorological factors. We have to add, however, that *the dehiscence of anthers is much more responsive than the lost of stigma viability to changes in temperature.*

According to our observations, in agreement with those by Lalatta (1982), the duration of anther dehiscence was 2–3 days. In cool weather, however, it may be protracted to 5 days (Soltész, 1982).

The dynamic of anther dehiscence was found to be in very close correlation with changes in the daily temperature. Most anthers dehisced between 10 a. m. and 3 p. m., which is in agreement with what Percival (1955) described in his publication.

According to our observations, the viability of stigmas in the flowers lasts longer in most cases than the period of anther dehiscence. The viability of stigmas ranged from 1 to 5 days depending on the variety and – above all – on the weather. According to Brózik and Nyéki (1975) the stigmas of apple are viable for 2–4 days following the opening of flower.

Studying the formation of secretion drops we found that the amount of secretion on the surface of the stigma changed as a function of temperature, which confirms the observation of Mowaffak (1985). The secretory activity was most intensive between 11 a. m. and 3 p. m.

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SOME MORPHOGENETIC CHARACTERS OF SERBIAN AND ROMANIAN PLUM VARIETIES

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Between 1987 and 1992 seven Serbian, 11 Romanian and one French plum varieties were examined in Cegléd. The trees, 10–16 years old, had been grafted to myrobalan seedling rootstock, and the dimensions and correlations of the flowers and leaves of the varieties led to some important conclusions of pomological selection and botanical value.

The petal median of the Čačanska varieties of female character and of the male sterile Romanian plum varieties differed in the first place from that in the other varieties, and the Vinet de românesc also was an exception. In the latter Romanian variety and in Č. sečer and Č. II/II 80/59 the pistil is very long; the high stamen number in Agen 707, in the Čačanska series and in Busuioace de Georgiu deserves mention. The relative stamen number positively confirmed the above. In the varieties examined except the Tuleu gras group the pollen germination is generally satisfactory. The flower parts can be considered stable, with the exception of the results obtained in 1990.

Most of the varieties differentiated more than 24 stamina, and the most frequent pistil length was 11–12 mm; examples of polyand hypoandria were equally found among the varieties examined. The length of petiole is also characteristic of the varieties, and at least so are the length and width of leaves and their pointedness. The leaf-shape index was hardly variety specific.

The author eventually found the close correlations of the various vegetative and reproductive morphogenetic characters to be of use in variety research and acclimatization, in selection work and even in investigations of virus susceptibility. It may be rightly supposed that similar conclusions can be arrived at in a wider scope of variety and species.

Keywords: plum varieties, morphogenetic character, leaf, flower morphology

Introduction

The beginning of the 20th century was a very interesting period for the research of plum varieties, when a great many varieties became widespread, and numerous new varieties were produced by crossing. Hedrick (1911), a follower of earlier great botanists, collected many plum varieties and described them in his pomology, but the activity of some prominent Hungarian botanists was also important [cf. Surányi (1985): the names of Péter Meliusz Juhász (1578), János Apáczai Csere (1653), János Lippai (1667), Mátyás Bél (1742), János Nagyváthy (1826), and later Ferenc Entz (1854–1859), Máté Bereczki (1877–1887), then Dezső Angyal (1925–1926), László Herszényi (1934), Mátyás Mohácsy (1940) must by all means be mentioned].

After the Peace Treaty of Trianon a new situation was created in Hungary; a long process began which ultimately decided whether, after the apple, the plum would keep its distinguished place in the Hungarian fruit growing. As it turned out, the situation did not fundamentally change in the period of large-scale fruit production. Moreover, the international successes of plum breeders gave an impetus

to plum cultivation in Hungary. The autocracy of the Besztercei plum seems to have ceased, its sharka susceptibility, fractional fruit production and small fruits cause problems; despite this, it takes a high position in the variety structure of Hungary (40.6%) (Hungarian Central Statistical Office, 1992).

Among others, great attention has been paid by the Hungarian growers to the Serbian and Romanian varieties and prospective varieties appearing on the border areas of the Carpathian Basin. The latter are of particular importance because the local varieties have been rapidly 'built in' the updated variety structure. Tuleu gras and Vinet de românesc can be regarded as basic varieties (Bordeianu et al., 1965; 1969), and the former is one of the parent varieties of a new range of variety (Tomcsányi, 1979; Tóth and Surányi, 1980).

The pomological evaluation of theČačaki hybrids was first given by Paunović et al. (1975, 1978) who referred to their fertility problems too (Ogasanović, 1985). These varieties have wider spread so far than the Romanian plums, although according to our experiences their production and composition values do not confirm their superiority to the latter. Out of the parent varieties of the Čačaki series the favourable characteristics of the Wangenheim, Pozegača or Ageni variety range are dominant in the hybrids: Č. rodna (high yielding), Č. lepotica (fine), Č. rana (early), Č. sečer (sugar), Č. najbolja (best).

A sign of the reasonable interest in plum is that an increasing number of publications deal with the questions of variety use, including the analysis of changes (Hunyady, 1985; Hungarian Central Statistical Office, 1992). Both the theoretical botanical and the practical cultivation aspects are covered by the Hungarian publications and the work of the institutes. Virus susceptibility, fruit size, fertility and fractionality are the main problems, though recently the question of root-stock has also aroused interest (Tóth and Erdős, 1987; Tóth and Surányi, 1980).

The vegetative and reproductive organs of the varieties and their morphogenetic characters still keep results in store, which may be of use both in the breeding (genetic) work and in the production-centric pomology. Many regularities of practical importance have so far been disclosed (Tukey, 1936; Weinberger and Thompson, 1962; Surányi, 1973; Cifranik, 1978; Brózik Jr. et al., 1985), to which attention was called already by Tydeman (1957) (cf. Tóth and Surányi, 1980).

In the case of the varieties and prospective varieties included in the study self-fertility, a genotypic character is of basic importance (Tóth, 1969), even if it has many phenotypic components (Surányi, 1978, 1986), because the age, growth characteristics, nutrient status and health condition of the trees and the actual environmental effects (including the root-stock) greatly influence the annual fertility conditions and eventually the fruit set (Surányi, 1973).

The fertility conditions of the varieties included in the study are not uniformly described in the literature; first of all the old varieties (cf. Tóth and Surányi, 1980) but even the Bluefre, the Stanley, the Čačanska najbolja and the Santa Rosa are good examples of the inconsistent data (Nyéki et al., 1985). As a further difficulty, even the extent of frost damage with the fruit (Bluefre) and the pollen germination and fertilizing ability (pollen donor) are inadequate (Besztercei Bt. 2) (Nyéki et al., 1985).

The endogenous hormone balance, the quantitative and qualitative changes of materials playing a decisive role in metabolism, the tissue structure, the ecological tolerance and the phenological characters are all components of frost resistance in the varieties. The state of dormancy is otherwise highly important in the plum varieties too; it explains the sensitivity of Bluefre in winter, the fairly good frost resistance of Ageni, Č. najbolja, Č. rana, Č. rodna and Tuleu timpuriu as well as the excellent frost resistance of Pescarus, Silvia, Stanley, Tuleu gras, Besztercei (=Pozegača) and Č. II/II 80/59 (Szabó, 1987; Szabó and Nyéki, 1991).

As we have mentioned, the question of self-fertility cannot always be unambiguously settled, because its degree may depend on the growing site and the method of examination alike. For this very reason works by Nyéki et al. (1985), Nyéki (1989) and Surányi (1986, 1991) deserve attention (cf. Tóth and Erdős, 1987). Therefore a group of variety (Szabó et al., 1990; Szabó, 1988; Erdős and Tóth, 1988–1992, unpublished and our own investigations: Surányi, from 1989 on), or even the plum varieties in general (Szabó and Gyuró, 1990) call for clearing up the flowering and fertility conditions in the course of acclimatization (Nyéki et al., 1985; Szabó et al., 1989; Surányi, 1991).

Since the end of the fifties, an ever more serious problem has been caused to the plum growers by the sharka virus which attacks other species as well. This question is not actually dealt with in the present paper, though it cannot be separated from the variety question (Surányi and Erdős, 1992), and there is a really important observation: differences certainly occur in fertility and tolerance between the plum varieties (cf. V. Németh, 1986; Tőkés, 1988; Szabó et al., 1991). Those are the perspectivic plum varieties which exceed the others in respect to fertility, ecological tolerance, virus resistance and composition value of fruit alike. The relationship between fertility and sharka virus resistance is perhaps the most difficult problem which might be solved in the future by gene technological methods; at the same time the consequences of bee pollination with respect to virus infection must also be considered.

Materials and methods

Between 1987 and 1992 seven Serbian and one French (control) varieties, furthermore 11 Romanian local varieties and hybrids were examined in Cegléd; the trees – with myrobalan seedling rootstock – had been planted in 1977–79. In the variety collection, a regular virus survey was carried out on two occasions (at the beginning of June and in mid-August), the leaves and fruits were marked with empirical numbers, showing the symptomatic condition of the trees and fruit from 1 to 5.

In full blossom the petals of flowers with 70–80% openness (30 in number) were measured one by one under stereomicroscope; the length and width of the petal, and the pistil length were given in mm. The number of formations morphologically passing for filament was recorded for each flower, then the relative stamen number in each flower was calculated (Surányi, 1989).

Pollen germination was examined at the time of anthesis, that is not from anthers collected from buds then dehiscing on watch-glass. About 300 pollen grains were kept in 15% saccharose solution suspension drop for 24 hours, and the extent of germination was given in percentage. The pollen whose tube length reached in 24 hours four-times the length of the pollen diameter was regarded as viable. Three samples per variety supplied the basic data for the statistical analysis.

Not only the varieties were compared for the data obtained, but – taking the varieties for replication – the years too; the frequency of the characteristic values of the reproductive organs was determined for both variety groups.

The plum varieties were compared for 5 aspects of the leaves; the differences between the varieties were determined on the basis of the length of petiole, the length and width of the leaf-blade, its shape index, and the percentage proportion of the apical part (pointedness). The leaf samples were collected in 1988, 1989 and 1992; 50 leaves per variety were removed from the 3–4th internode above the base of the year's growth; the years were evaluated together, because the number of replications could not be regarded as sufficient for establishing the year effects.

The examined characters of the leaves and flowers were analysed in correlations too, because many surprising correlations could be proved that had been reported e.g. by Dahl (1935), Röder (1940), Tóth (1957) or by Brózik Jr. et al. (1985). In the present paper a new range of the morphological characters (leaf) is determined, and we did not wish to include its correlation with the production of the varieties and trees (yield, fruit size and components) in this account, although according to Tomcsányi (1979) it would have been reasonable.

Results and discussion

The size of petal was rather varying among the Serbian and Romanian varieties alike, Čačanska II/II 80/59 excelled in petal median with its great pistil length compared to Č. Šečer. Pozegača (like Besztercei) and the hybrid mentioned before differentiated much fewer stamina than the other varieties on the average of 6 years, but the unfavourable change of the relative stamen number towards both the gynoecium (Č. II/II 80/59 and Č. Šečer) and the androecium (Č. lepotica) could be observed. All in all, it can be said that the pollen germination exceeds 40%, so in respect of viability even if not in fertilizing ability they are considered good varieties.

Figure 1 contains morphogenetic data obtained in the flowers of the Romanian varieties too. Vinet de românesc produces flowers with particularly large petals, and in this it does not differ from the plum varieties. Vinet de românesc has an extremely long pistil, while that of the Gras ameliorat is very short. Tuleu gras and the hybrids of which it was one of the parent species developed few stamina in the flower; Tuleu gras and Busuioace de Georgiu represented the two extremes. The latter and Silvia have high relative stamen number, a consequence of the less than 11 mm pistil and the comparatively high stamen number. Tuleu gras and its progeny (Centenar, Pescarus, Silvia, Tuleu timpuriu etc.) produce very little if any pollen, so they can be regarded as male sterile. They indeed demand a pollinator partner just like the self-sterile varieties of the Čačanska series. The influence of the rootstock too has recently been proved in the experiment at Cegléd (Surányi, 1991).

Figure 1 analyses the influence of the years too, on the basis of 5 data series; the petal median is generally larger in the Romanian (mostly male sterile) varieties than in the Serbian plums, but the role of the years can hardly be proved, least so on the basis of pistil length and stamen number. The relative stamen number cannot evidently give a different result, that is, the influence of the year is insignificant beside the effect of variety. The pollen germination, on the other hand, seems to be a phenotypic character, at least it is a character influenced by too many factors to be possibly taken into consideration.

In the flowers of the plum varieties examined, the 11–12 mm long pistil and

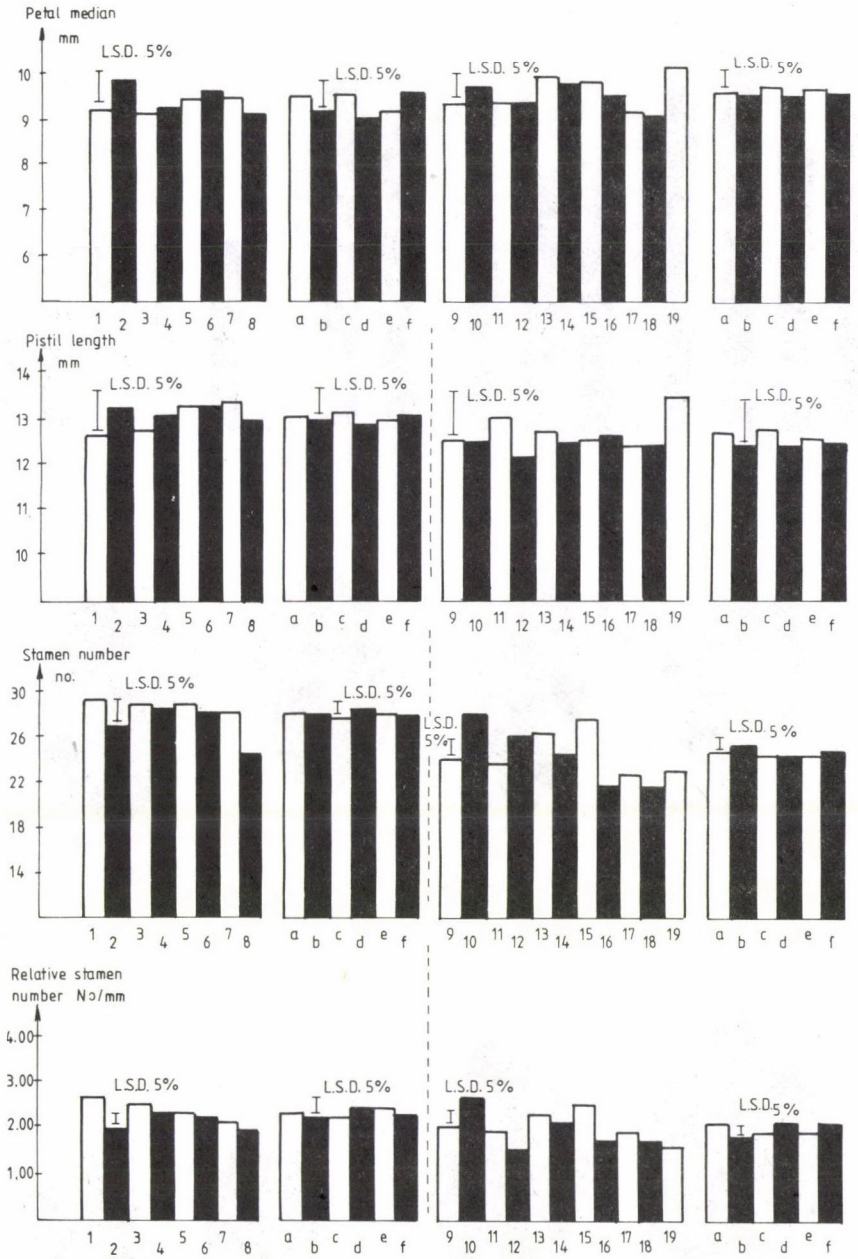
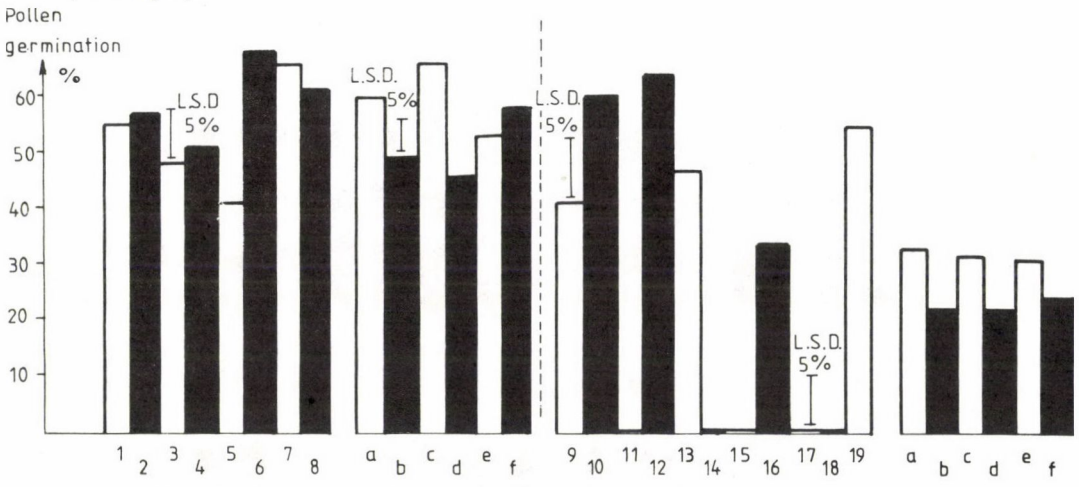


Fig. 1. Changing of the flower remarks according to cultivars (of Serbian and Romanian) and years
Note: 1 - Agen 707 2 - Čačanska II/II 80/59 3 - Čačanska lepotica 4 - Čačanska najbolja 5 - Čačanska rana 6 - Čačanska rodna 7 - Čačanska sečer 8 - Pozegača 9 - Albatros 10 - Busuioace de Georgiu 11 -Centenar 12 - Gras ameliorat 13 - Gras Dames 14 - Pescarus 15 - Silvia 16 - Tuleu dulce 17 - Tuleu timpuriu 18 - Tuleu gras 19 - Vinet de românesc
a- 1987, b-1988, c-1989, d-1930, e-1991, f-1992

(cont. p. 230.)

(cont. Fig. 1)



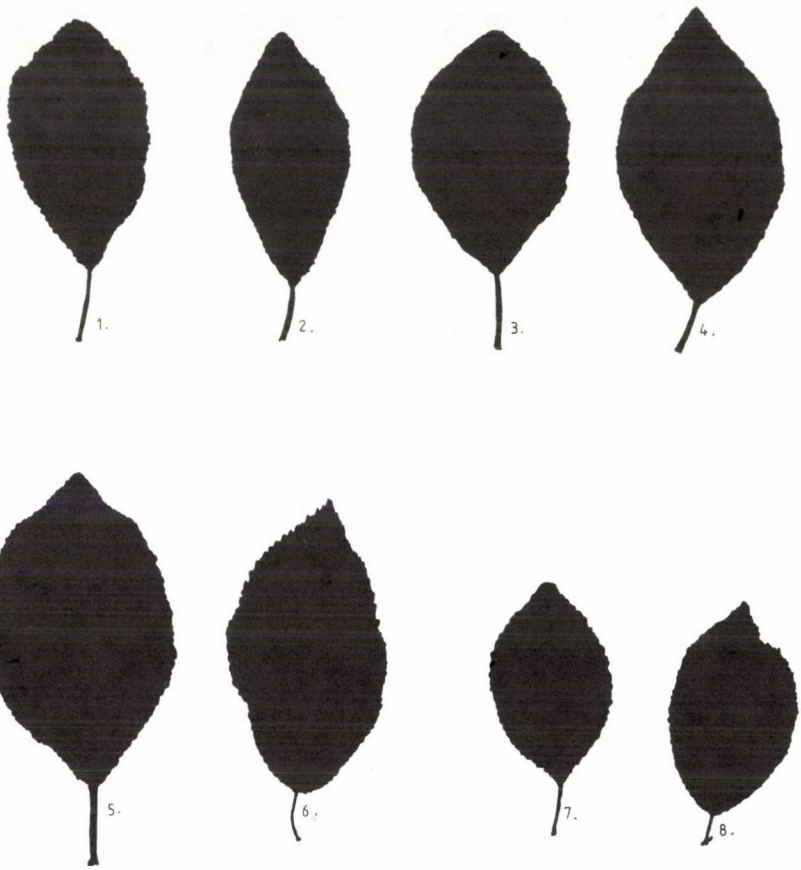


Fig. 2. The leaf forms of Serbian plum cultivars (size: × 0.71)
1 - Agen 707 2 - Čačanska II/II 80/59 3 - Čačanska lepotica 4 - Čačanska najbolja 5 - Čačanska rana 6 - Čačanska rodna 7 - Čačanska sečer 8 - Pozegača

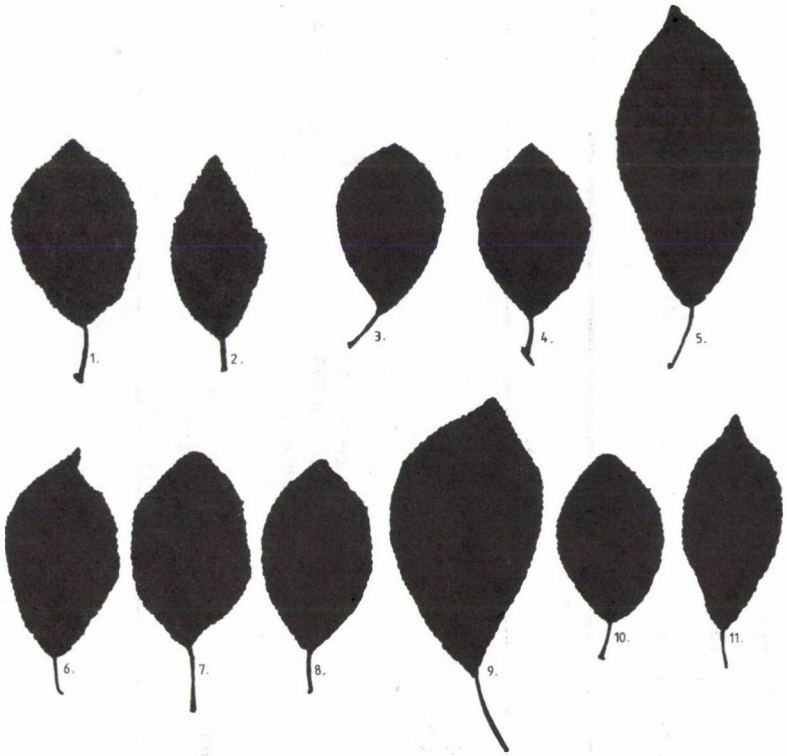


Fig. 3. The leaf forms of Romanian plum cultivars (size: × 0.71)
1 - Albatros 2 - Busuioace de Georgiu 3 - Centenar 4 - Gras ameliorat 5 - Gras Dames 6 - Pescarus 7 - Silvia 8 - Tuleu dulce 9 - Tuieu timpuriu 10 - Tuleu gras 11 - Vinet de românesc

the more than 24 stamina, but with Agen 707 the polyandria are not convincing; some Čačansca varieties (13–14 mm), on the other hand, develop remarkably large pistil (Č. sečer, Č. rodna, Č. II/II 80/59). A comparatively short pistil and few stamina a consequently small (greenish) flower characterize the Tuleu gras group and the Romanian varieties Pozegača and Vinet de românesc.

Special emphasis has recently been laid on the stamen number (Surányi et al., 1992), because the close correlation between the colour of nectary and the number of stamina can be proved in the plum varieties. The colour of the nectary showed the following variation in the different varieties:

– *green:* Pozegača, Albatros, Centenar, Pescarus, Tuleu dulce, T. timpuriu, T. gras and Vinet de românesc

– *greenish yellow – yellowish green:* Agen 707, Č. II/II 80/59, Č. lepotica, Č. najbolja, Č. rana, Č. rodna, Č. sečer, Busuioace de Georgiu, Gras ameliorat, Gras Dames and Silvia.

The green (n=8) and the transitional (n=11) colour of the nectary gave a highly significant result concerning the stamen number. On the average of the 8 varieties

Table 1

Main frequency of sexual organs in Serbian and Romanian plum cultivars

	Stamen number, pc.			
Pistil length, mm	16–19	20–23	24–27	28–31
9–10	T. dulce 28%	Albatros 61% Pescarus 36%		Agen 707 23%
11–12	T. gras 32% T. timpuriu 30%	Pozegača 38%	Silvia 53% Busuioace de g. 40% Gras ameliorat 39% Gras dames 33%	Č. Najbolja 47% Č. Rana 38% Č. Lepotica 34% Č. Rodna 27% Agen 707 23%
13–14		Vinet de rom. 32%	Č. II/II 80/59 41% Č. Šečer 33% Č. Rodna 27%	

Note: underlined letters = Serbian plums
the rest = Romanian plums

19.8 stamina/flower compared to 26.9 stamina/ flower in 11 varieties proved to be in close correlation with the colour of the nectary (p=0.1%).

The shape and size of the leaves can be seen in Figs 2 and 3 Č. najbolja and Č. rana have remarkably large leaves, like Gras Dames and Tuleu timpuriu, exceptional in the case of the Romanian varieties. The length of the petiole ranged between 11.8 and 27.5 mm, the length and width of the leaf-blade showed similarly characteristic differences. The shape index of the leaves, a highly important character (Filarszky, 1911) hardly varied with the variety. We tried to characterize the leaf shape with the percentage proportion of the apical part (pointedness) too; Agen 707, Č. rodna, Pozegača and Busuioace de Georgiu differed, accordingly, to a considerable extent from the other varieties (Figs 2 and 3).

Since in the two variety groups the plum varieties were closely related, we thought the relationship between certain morphological characters of the leaf and flower to be of special importance; so the correlation between the length of the petiole and the length of the leaf-blade turned out to be weak and uncertain, while the length of the petiole and the width of the leaf-blade proved to be in very close correlation. The leaf-shape index is unsuitable for making distinctions between the varieties, but a demonstrable correlation with the petal median could be found at 2% level, and the r-value of the leaf-shape index and the relative stamen number also deserves attention (Tables 1 and 2).

Table 2

Important relationships of leaf and flower morphological characteristics

Relationships		r-value	p %
Length of petiole	– Length of leaf-blade	+0.3610	~10
The same	– Width of leaf-blade	+0.8378	0.1
The same	– Leaf index	–0.1594	–
The same	– Median size of petal	–0.2447	–
Length of leaf-blade	– Width of leaf-blade	+0.8684	0.1
The same	– Median size of petal	–0.0717	–
The same	– Pistil length	+0.1129	–
Width of lead-blade	– Median size of petal	–0.3010	–
The same	– Relative stamen number	+0.1998	–
Leaf index	– Median size of petal	+0.5659	2
The same	– Pistil length	+0.2390	–
The same	– Stamen number	–0.1496	
The same	– Relative stamen number	–0.3151	–

After Tóth (1957), Tydeman (1957), Brózik (1960) we tried to analyse such morphogenetic characters which might help in describing the variety on the one hand, and make the breeders' selection work quicker and more efficient, on the other. That is certainly how the correlation between the leaf petiole and the average fruit weight, and the dependence of the fruit size on the width of the leaf-blade, may be of importance (Fig. 4). We think that the size, colour and phenological properties of

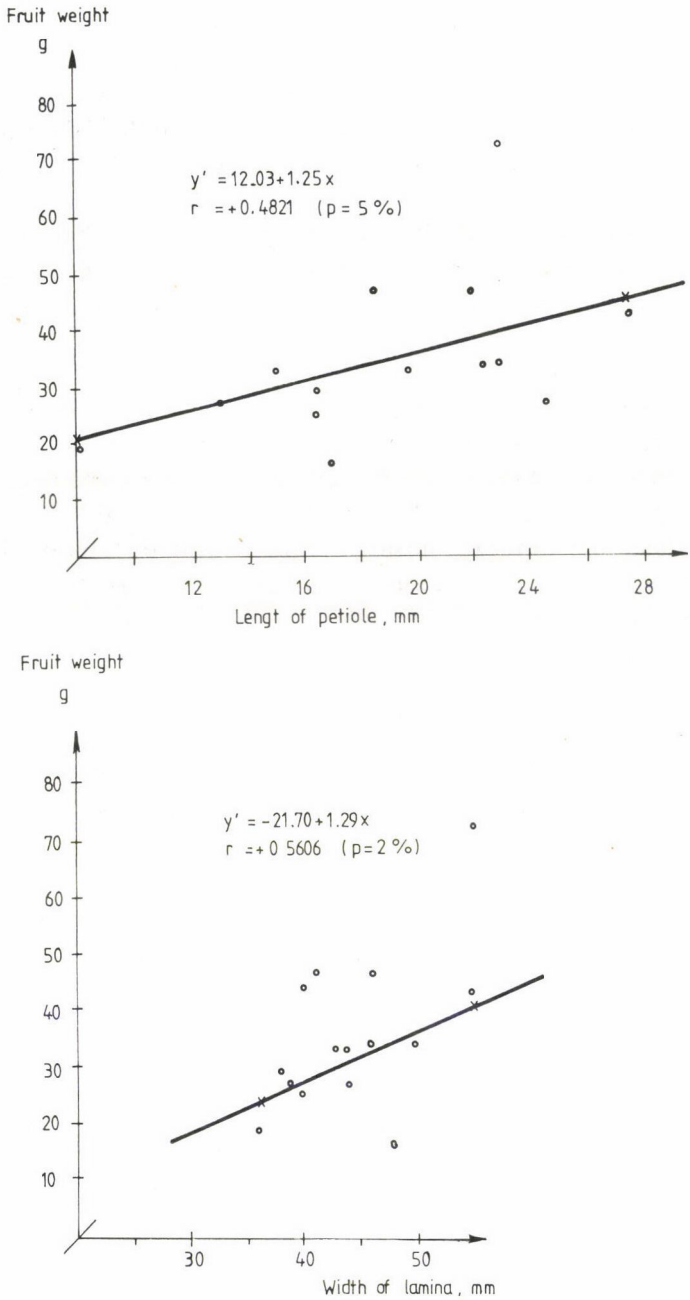


Fig. 4. Dependence of fruit weight on the parts of leaves

the leaves play a part not only in the juvenility research of the trees, but also in the production biology examinations, and even in the work of breeding and variety maintenance.

Similar results can surely be obtained from a botanical material wider than the present varieties, so the usefulness of the morphogenetic investigations will become more evident, and the breeders can also make use of the results. Thus, our observations were concentrated on new and popular varieties that have spread mostly from the Eastern Central European region. Agen 707, Pozegača and Tuleu gras were basic varieties in our study. According to our preliminary results the susceptibility to sharka virus and to some fungal diseases also showed elements possibly correlated with the morphological characters of leaves; this in itself may justify us in continuing our work.

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NECTARY STRUCTURES IN CHERRY CULTIVARS

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The intrafloral nectary of cherry lines the adaxial surface of the widening receptacle between the rise of stamina and pistil. The gland can be epimorphic, automorphic and transitional. The nectary can be separated into three tissue regions: epidermis, glandular tissue and nectary parenchyma. In the cherry cultivars the ornamentation of the cuticle covering the epidermis is uniformly striated. The patterns of the cuticle are characteristic of the cultivar. The rich striation prevents the secretion from running off or evaporate quickly for a long time, which attracts the insects over a considerable period. The thickness of the cuticle and the position of the nectary stomata in relation to the epidermis cells refer to the ecological type of the cultivar. There is a close correlation between thick cuticle and sunken stomata. The small, square cells of the glandular tissue with their large nucleus and abundant plasma form rows parallel to the surface. The number of cell rows in the glandular tissue is characteristic of the cultivar. On the basis of the size of the glandular tissue, the insect attraction of the cultivars can be foretold and their apicultural importance assessed.

Keywords: cherry, nectary, pollination

Introduction

The data of floral nectary structure concerning the Rosaceae family were summarized by Fahn (1953), who described that the nectary gland of the Rosaceae lines the concave adaxial side of the receptacle between the rise of the stamina and the ovary. The nectary histology description of several genera of the family is found in Kartashova (1965).

On the structure of the cuticle covering the surface of the gland in the cherry varieties examined, there are no data available. The diagnostic value of the cuticular peculiarities was emphasized by Stace (1965) and Sinclair and Sharma (1971). Still, the pattern of the cuticle has been occasionally used for identifying the taxa. Dunn et al. (1965) having examined 226 dicotyledonous species found the cuticle of the xerophilous species, always rugose, while in the mesophilous or hygrophilous taxa it was smooth-surfaced, Mueller (1966) believed the ornamentation of the cuticle to be genetically controlled.

The relatively few literary data concerning the ultrastructure of the nectary surface was summarized by Durkee (1983). Results of examinations of some taxa of the subfamily Prunoideae have been recently reported (Orosz-Kovács, 1988, 1989, 1991; Orosz-Kovács et al., 1989, Orosz-Kovács et al., 1990a, b).

The shape, size and place of the epidermis cells of the nectary and the position of the stomata relative to the epidermis cells are considered by Kartashova (1965) to be characteristic of the variety. The secretion of the nectar usually takes place through the stomata. According to Gulyás (1975) the nectary stomata are generally isolated or are placed in groups (twin-stomata), and very often keep their functioning ability; by opening and closing the nectary fissure, they regulate the duration and intensity of secretion (Frey-Wysslig and Häusermann, 1960).

The glandular tissue under the epidermis can be recognized by its small isodiametric or polyhedric, deeply staining cells, each with a large nucleus and rich in plasma, similar to the meristemic cells. But by its plastid apparatus it can be distinguished from the meristematic tissues (Kartashova, 1965; Gulyás 1968, 1975). The development and structure of the glandular tissue were described for Persica vulgaris, one of the species of Prunoideae by Kartashova (1965). The ontogenesis of the nucleus in the glandular cells from the beginning to the end of secretion was shown by Kartashova (1965), Kálmán and Gulyás (1974) and Gulyás (1975).

Durkee (1983) and Fahn (1988) also summarized the ultrastructure of the glandular cells.

In recent decades, the attention of the floral anatomists turned towards the correlation between structure and function. According to Schmid (1988), their conclusions were not convincing.

In the course of investigations in Hungary, Gulyás (1968) and Kincsek (1977) found connections between the size of the nectary and the nectar production of the species of Labiatae and Fabaceae.

More recent examinations indicated a rather close correlation between the size of the glandular tissues and the sugar value of the volume of nectar measured at the time of production maxima in Pándy sour-cherry clones (Orosz-Kovács and Gulyás, 1989) and in apple cultivars (Orosz-Kovács, 1989; Orosz-Kovács et al., 1990). It was estabilished that, based on the size of the glandular tissue, the production can be forecasted. From the structural characteristics of the nectary, some conclusions can be drawn on the sugar production of the flower, thus also on the attraction exercised on the insects visiting the flower. We have therefore made comparative structural examinations of the intrafloral nectary in cherry cultivars.

Materials and methods

Between 1986 and 1990 we examined the intrafloral nectary structures of 27 cherry cultivars at three growing sites (Table 1). For the comparative analyses, samples from the same year and same growing site were taken into consideration.

For the histological examination of the nectaries young open flowers with unripe anthers during two or three years originated from one or two growing sites were usually taken. The samples were collected from bearing spurs of the second branch storey on the south-eastern side of at least three trees. Ten sections of each of 5 flowers per year, or of 10 flowers of the same year supplied the histological data; that is, 100 measurments of the sections of 10 flowers per cultivar were recorded.

Parameters of the histological examinations:

– thickness of cuticle

– length and thickness of epidermis cells

– occurrence of sunken stomata and extent (number of cell rows) of sinking
– length and thickness of glandular tissue
– thickness of nectary parenchyma
– thickness of receptacle.

Since on the medial-longitudinal cutting of the flower the glandular tissue is more or less a regular parallelepiped, the size of the glandular tissue is given by the product of Length x Thickness.

Sections were made by Mrs. L. Zorn and processed by paraffin or paraplast inbedding and toluidine blue staining. For the scanning electron microscope (SEM) preparations, glutar aldehyde fixing, Na-cacodylate washing and ethyl alcohol dehydration were employed. After drying at the critical point, gold coating and gold shadowing took place in a Jeol vacuum steamer. Micrographe were made by means of an ASID-4 SEM adapted to Jeol 100 °C in the EM laboratory of the Medical University of Pécs. The SEM technique was performed by Mr. Ferenc Kaposvári.

Table 1

Material, place and time of investigations

Variety	Root-stock	Investigation	
		Place	Time
Annabella	Vadcseresznye	Érd-Elvira	1987
Bigarreau Burlat	"	Érd-Elvira	1987
Bigarreau Burlat	"	Ceglédbercel	1988,1989
Bigarreau Marmotte	"	Érd-Elvira	1987
Germersdorfi	"	Danic-puszta	1986
Germersdorfi/3	"	Érd-Elvira	1987
Germersdorfi/3	"	Ceglédbercel	1988,1989
Germersdorfi/57	"	Érd-Elvira	1987
H-165	"	Ceglédbercel	1988,1989
H-171	"	Érd-Elvira	1987
Hedelfingeni óriás	"	Érd-Elvira	1987
Hudson	"	Érd-Elvira	1987
Jaboulay	"	Érd-Elvira	1987
Jaboulay UF 155	"	Érd-Elvira	1987
Katalin (H.261)	"	Érd-Elvira	1987
Margit (H.66)	"	Ceglédbercel	1988,1989
Májusi hosszúszárú	"	Érd-Elvira	1987
Merton Bigarreau	"	Érd-Elvira	1987
Münchebergi korai	"	Érd-Elvira	1987
Münchebergi óriás	"	Érd-Elvira	1987
Pinchon Early	"	Érd-Elvira	1987
Solymári	"	Danic-puszta	1986
Solymári	"	Érd-Elvira	1987
Solymári-16	"	Érd-Elvira	1987
Stella	"	Érd-Elvira	1987
Szegedi óriás	"	Érd-Elvira	1987
Van	"	Érd-Elvira	1987
Van	"	Ceglédbercel	1988,1989
Van F. 635	"	Érd-Elvira	1987
Valerij Cskalov	"	Érd-Elvira	1987
Vadcseresznye		Mecsek	1989
Vadcseresznye Altenweddingeni		Cegléd	1989,1990
Vadcseresznye CT. 2493		Cegléd	1989,1990

Results and discussion

The intrafloral nectary of the cherry lines the adaxial surface of the receptacle between the rise of the stamina and pistil. The wide bag of this receptacle has a wavy surface, often somewhat ribbed. The apical part of the nectary may reach the protrusions of the rise of stamina, where it may form bulges. The colour of the gland is yellow, yellowish green or green, depending on the cultivar.

The glandular tissue band in the medial-longitudinal section of the flower clearly indicates the shape of the gland, which is either sunken in or emerges from the receptacle. The former are epimorphous, the latter automorphous type nectaries. There are, however, transitional forms too, which sink in the receptacle at the basal part and emerge in the apical part (Fig. 1).

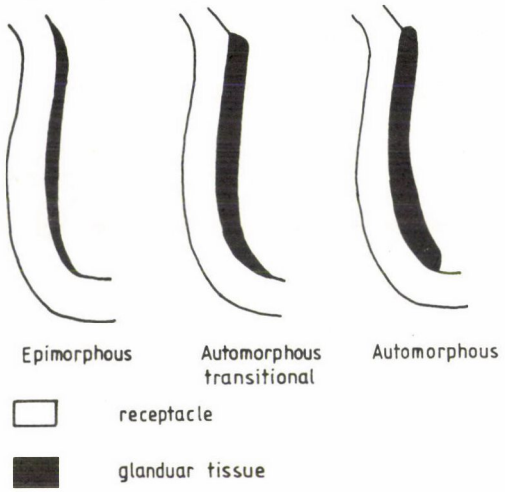

Fig. 1. Structural types of nectary in cherry cultivars

Out of the cherry cultivars "Annabella" "H-165", "Hudson", "Jaboulay", "Májusi hosszúszárú" (long-pedicelled), "Merton Bigarreau", "Müncheberg early", "Pinchon early", "Solymári" and "Valeri Chkalov" have an epimorphous nectary, while "H-171" and "Altenweddingen" wild cherry have an automorphous nectary; the other varieties show transitional automorphism.

The one cell-layer epidermis of the nectary is covered by cuticle. Nectary cuticles had a striate surface in all sweet cherry varieties examined.

Out of the wild cherries and cherry cultivars examined, four characteristic types of cuticle are shown here. In the Altenweddingen wild cherry the cuticle is thinly striated. The ribs are narrow, undulate, and often do not cover the entire epidermis cell, in the interstomatal regions they mostly are completely irregular, and around the stomata they show a radial pattern. On the primary striation, secondary towerings seldom occur. Above the stomata a wide circular cuticular slit permits the outflow of secretion. Around the slit the surface consists of smooth or slightly plicate sheets superposed in several layers surrounded by radially set, prominent ribs.

The stoma levelling the epidermis cells indicates a mesomorphous character, while the thin cuticular ornamentation refers to a slightly hygromorphous ecological type (Fig. 2A, B).

The wild cherry sample [originating from the Mecsek Mountain in southern Hungary] is characterized by parallel culticular ribs running in the longitudinal direction of the receptacle, over several cells in the interstomatal space. The ribs, thickly towering over the primary structure cover the surface. The structure arranged radially around the stomata hardly opens at the nectar slit. The thick striation, and the stomata placed planarly or slightly sunken between the epidermal cells, indicate a mild xeromorphism (Fig. 2C).

The gland surface of the wild cherry CT. 2493 selected at Cegléd is covered by thick cuticle ribs. The ribs are wide and gently rugose, here and there reminiscent of crumpled sheets. Around the stomata the cuticle ribs are characteristically towering. The nectar fissure is moderately opened. The stomata lie level with the epidermis cells. Accordingly, the wild cherry CT. 2493 can be classified into the mesomorphous type.

The "Germersdorf" sweet cherry variety has a very characteristic cuticle design. The stomata are densely set. The cuticle ribs are radially arranged around the guard cells. Since the stomata are more or less sunken, the radial structure of the cuticle ribs repeats scale-like in several storeys. The secretion flowing through the nectar fissure rises in the rib-formed furrows as microcapillaries, and spreads over the surface. The radial pattern forms concentric circles around the stomata in at least three cell rows. In the "pit" of the sunken stoma and in the rich, spongy cuticular striation the produced secretion remains for a long time, and keeps attracting the insects even when it is no longer produced. Owing to the densely set stomata, the radial pattern around them makes the nectary surface characteristic of the cultivar. In the interstomatal area there is hardly any epidermis cell row in which the cuticle ribs, unlike the radial design around the secretion fissure, run parallel to the longitudinal axis of the flower. The sunken stomata, and the highly undulate striation of the thick cuticle, indicate xeromorphism (Fig. 2E, F).

The nectary cuticle of the cherry cultivars can be 1.5 – 6 μm thick (Fig. 3). Cultivar like "Bigerreau Marmotte" or the long-pedicelled "May-cherry" with the thinnest – under 2 μm – cuticle are hygromorphous by their other characters (e.g. protruding stoma), too. "Pinchon Early", "Van F. 635", "Van F. 630", whose cuticle is the thickest (5–6 μm), are xeromorphous even based on sunken stomata.

The structure and thickness of the cuticle are also important for purposes of nectar retention. The nectary of the Germersdorf cultivar retains the nectar in the furrows of the cuticle for a long time, while from the smoother surface of the wild cherry's nectary the secretion more evaporates quickly.

The tightly closed cell rows of the nectar epidermis are interrupted only by the anomocytic stomata in the sweet cherry cultivars. The epidermis cells are extremely difficult to study because the small thin-walled cells closely adhere to the subepi-

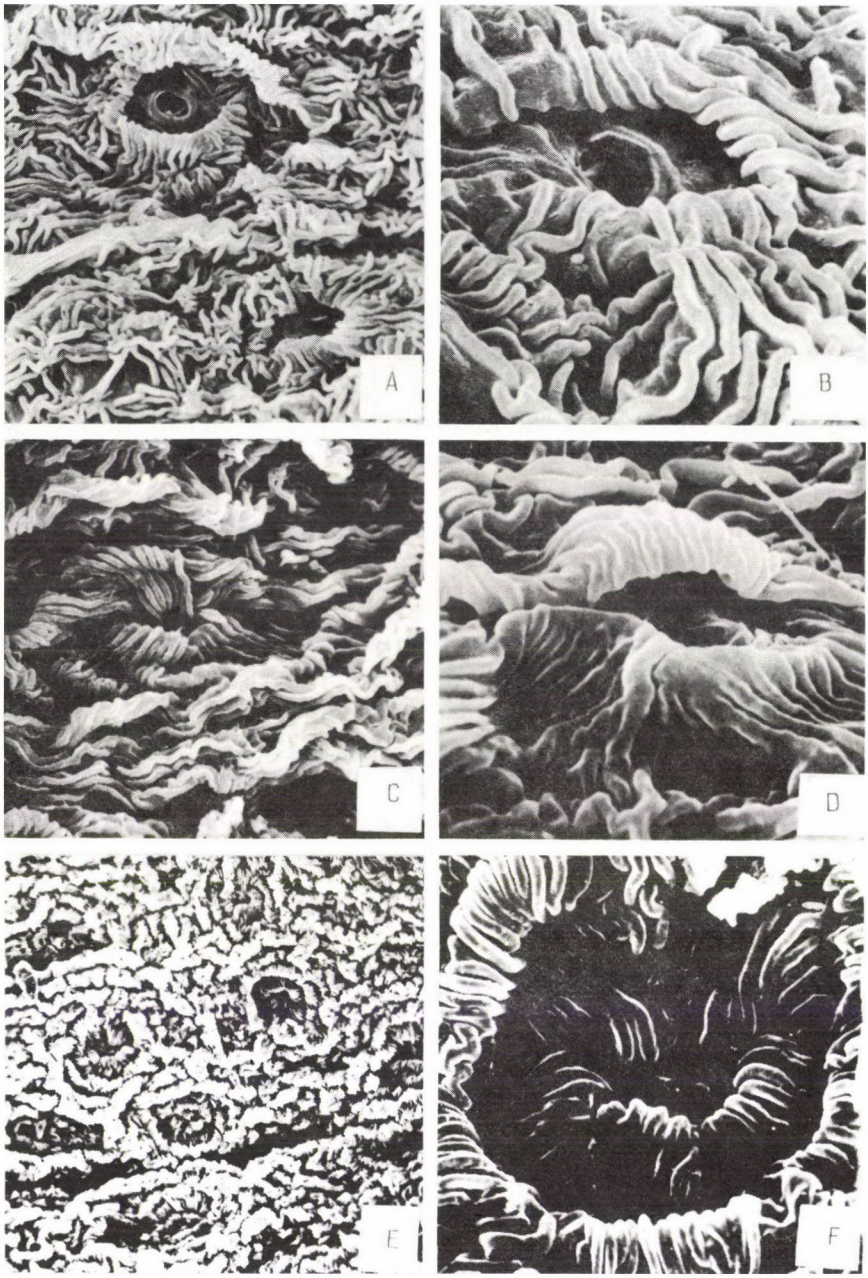

Fig. 2. Nectary surface in cherry cultivars A-B. Thin cuticular pattern of the wild cherry Altenweddingen. SEM × 1000 (A) and × 3000 (B) C. Nectary cuticle of wild cherry from the Mecsek Mountain (SEM × 1000). D. Gland surface of the CT. 2493 wild cherry (Cegléd) (SEM × 3000). E-F. Nectary surface of the cherry cultivar Germersdorf giant (SEM × 300 and × 3000)

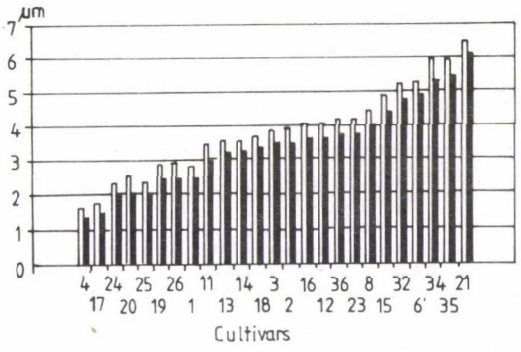

Fig. 3. Cuticular thickness of nectary epidermis in cherry cultivars (on wild cherry root-stoch). Érd, 1987

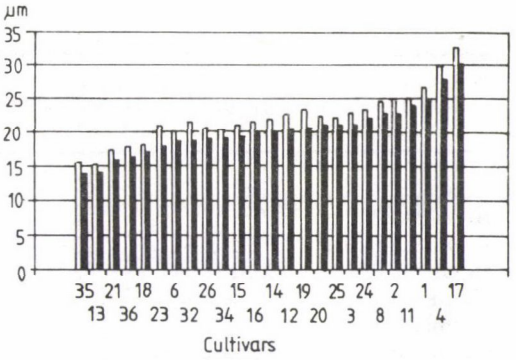

Fig. 4. Length of cells of nectary epidermis in cherry cultivars (on wild cherry root-stoch). Érd, 1987

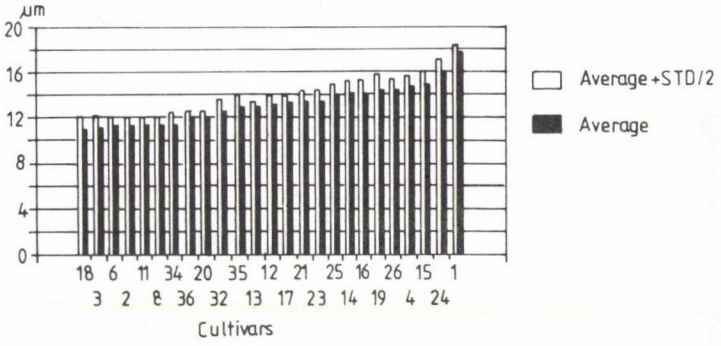

Fig. 5. Thickness of cells of nectary epidermis in cherry cultivars (on wild cherry root-stoch). Érd, 1982

dermal cell-row of the glandular tissue. Besides the impossibility of preparing excoriates, the SEM methods also fail to provide satisfactory information on the epidermis, because the dense cuticular striation covering the cells conceals the borders of cells. The examinations were therefore carried out in the medial-longitudinal section of the flower.

The shape of the epidermis cells in the longitudinal axis of the flower is a parallelepipedon, square, circle or oval, with its axial tangential wall often protruding and papilla-shaped. The epidermal cells are papillar, e.g. in the cherry cultivars H. 165. H. 66 and Altenweddingen. The epidermis cells can be 14–30 µm long. They are shortest in the cultivars Hedelfingen giant and Valeri Chkalov and longest (over 24 µm) in "May long-pedicelled" and "Bigarreau Marmotte" (Fig. 4, Table 2). The width of the epidermal cells of the nectary ranges between narrower limits of variation than does the length: 11–18 µm (Fig. 5, Table 2). Significant differences can only be found between the lowest and the highest values.

Table 2

Summarized measuring data of nectary epidermis in cherry cultivars

Cultivars, clone	Site of sampling	Time of examination	Epidermis cell length µm	Epidermis cell thickness µm	L/T	Cuticle thickness µm	Sunken stoma
Anabella	Érd	1987	24.83	17.79	1.40	2.43	0.40
Bigarreau Burlat	Érd	1987	22.91	11.26	2.03	3.46	0.1
Bigarreau Burlat	Ceglédbercel	1988	21.25	11.14	1.91	3.46	0.4
Bigarreau Marmotte	Érd	1987	27.78	14.84	1.87	1.41	0.0
Germersdorfi	Pécs	1986	16.00	12.54	1.28	2.82	0.9
Germersdorfi-3	Érd	1987	18.94	11.26	1.68	4.86	1.0
Germersdorfi-3	Ceglédbercel	1988	22.02	11.65	1.89	4.35	0.6
Germersdorfi-57	Érd	1987	22.66	11.39	1.99	3.97	0.2
H. 66	Ceglédbercel	1989	17.41	13.95	1.25	3.71	1.0
H. 265	Ceglédbercel	1988	16.77	16.26	1.03	2.43	0.0
H. 171	Érd	1987	23.94	11.26	2.13	2.94	0.4
H. 261	Érd	1987	20.61	13.06	1.58	3.58	0.3
Hedelfingeni Óriás	Érd	19.87	14.34	12.93	1.11	3.2	0.0
Hudson	Érd	1987	20.1	14.21	1.41	3.2	0.1
Jaboulay	Érd	1987	19.33	14.98	1.29	4.35	0.5
Jaboulay UF.155	Érd	1987	19.71	14.21	1.39	3.58	0.3
Májusi Hosszúszárú	Érd	1987	29.82	13.31	2.24	1.54	0.0
MertonBigarreau	Érd	1987	17.28	11.01.	1.57	3.33	0.6
MünchebergiKorai	Érd	1987	20.74	14.34	1.45	2.43	0.0
Münchebergi Óriás	Érd	1987	21.12	12.03	1.76	2.05	0.0
PinchonEarly	Érd	1987	16.00	13.44	1.19	6.02	2.4
Solymári	Pécs	1986	27.65	8.32	3.32	3.07	0.2
Solymári	Érd	1987	18.3	13.44	1.36	3.71	2.0
Solymári-16	Érd	1987	22.14	16.00	1.38	2.05	0.0
Stella	Érd	1987	21.25	14.08	1.51	2.05	0.0
Szegedi Óriás	Érd	1987	19.07	14.46	1.32	2.43	0.2
Vadcseresznye	Mecsek	1989	19.46	12.42	1.57	4.35	0.4
Vadcseresznye Altenweddingeni	Cegléd	1989	22.40	14.08	1.59	5.12	1.5
Vadcseresznye Altenweddingeni	Cegléd	1990	20.86	14.59	1.43	4.86	1.3
Vadcseresznye CT. 2493	Cegléd	1988	17.66	16.64	1.06	3.07	0.5
Vadcseresznye CT. 2493	Cegléd	1989	17.06	28.29	0.62	3.2	0.5
Van	Érd	1987	18.94	12.54	1.51	4.74	1.8
Van	Ceglédbercel	1988	16.51	12.8	1.29	4.99	1.4
Van F. 630	Érd	1987	19.2	11.39	1.69	5.25	1.1
Van F. 635	Érd	1987	14.21	12.80	1.11	5.38	1.3
Valerij Cskalov	Érd	1987	16.51	12.03	1.37	3.71	0.0

Abbreviations:
L/T: length/thickness
Sunken stoma: occurrence of sunken stoma, extent of sink
0: hygromorphous or mesomorphous type, no sunken stoma
0.1 – 1: stomata sunken at most to depth of 1 cell-row
1.1 – 2: stomata sunken at most to a depth of 2 cell-rows
above 2: stomata sunken deeper than 2 cell rows

The arrangement of the stomata among the cherry cultivars differs highly. According to Kartashova (1965) the stomata can be planar with the epidermis cells, or sunken under the adjacent cells, or even raised above the epidermis, depending on the position of the gland. She states that in tubular flowers with narrow or closed throats the stomata are always set on emergences, while in the open, choripetalous flowers they are sunken below the level of the epidermis cells. According to our receptacular sinus, the above are but partly true, since the hypanthium is similar, more or less closed in all sweet cherry cultivars. Even in these closed, humid situations there are sunken stomata, just as prominent stomata can be observed in the apical part of the gland in some cultivars. The position of the stomata indicates the ecological type of the cultivars, rather than the exposure of the nectary (Table 2). The cultivars concerned can be placed in the following types proposed by us (Fig. 6):

Type 1 (Fig. 6A): *hygromorphous* – The surface of the nectary is highly undulate. On the top of the waves, the stomata are situated on emergences. Examples of such cherry cultivars are, Szegedi giant, Hedelfingen giant, "Germersdorf-3" and "Solymár-16". In the latter the stoma on the top of the emergence is slightly sunken. It should be noted that the Germersdorf cultivars collected at Pécs (Danic puszta) were positively xeromorphous, while the Germersdorf-3 clone grown at Érd was unambiguously hygromorphous.

Type 2 (Fig. 6B): *hygro-meso-xeromorphous* – At the base of the nectary the stomata are on emergences, level with the epidermis cells but, in the apical part of the nectary near the surface, sunken in the glandular tissue. The sweet cherry variety Germersdorf-57 e.g. belongs to this type.

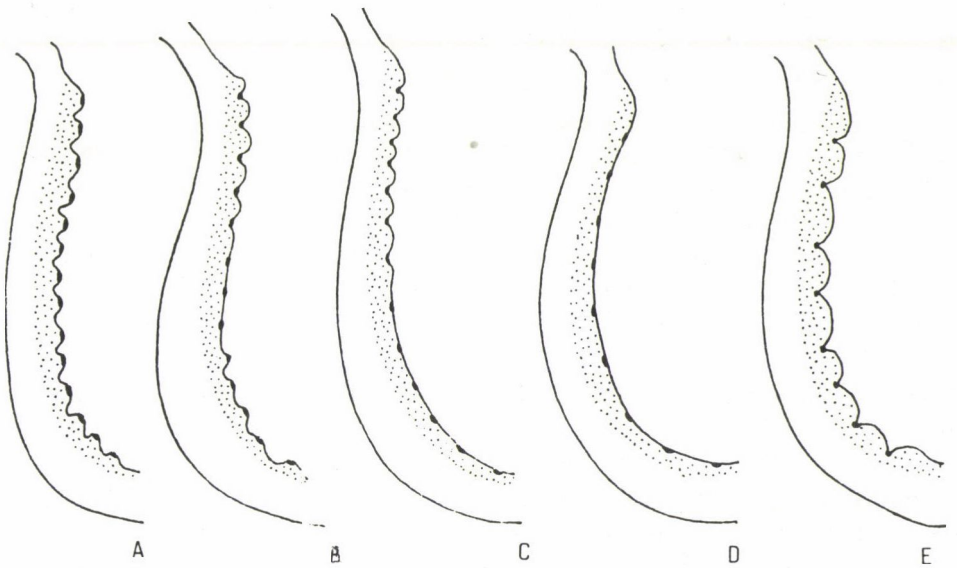

Fig. 6. Arrangement of nectary stomata and structure of glandular tissue in cherry cultivars A. hygromorphous type B. hygro – meso – xeromorphous type C. meso – xeromorphous type D. mesomorphous type E. xeromorphous type F. Stomata on emergences in the Solymar-16 cherry cultivar (× 160) G. Slightly sunken stoma and the glandular tissue in the CT. 2493 wild cherry

Type 3 (Fig. 6C): *meso – xeromorphous* – The stomata occur near the base, level with the epidermis cells, but apically more or less sunken. E.g. Annabella, H. 66, Morton Bigarreau, Jaboulay UF. 155.

Type 4 (Fig. 6D): *mesomorphous* – The nectary stomata are situated level with the epidermis cells, or sunken to a maximum half cell-row. E.g. Altenweddingen wild cherry.

Type 5 (Fig. 6E): *xeromorphous* – All stomata are sunken below the epidermis, at a depth of 1-3 cell-rows. This occurred with the samples of H-165 and of the Pécs (Danic puszta) samples of Germersdorf giant.

Under the stomata, especially in the hygromorphous types, a wide vessel of fluid can be found (Fig. 6F–G).

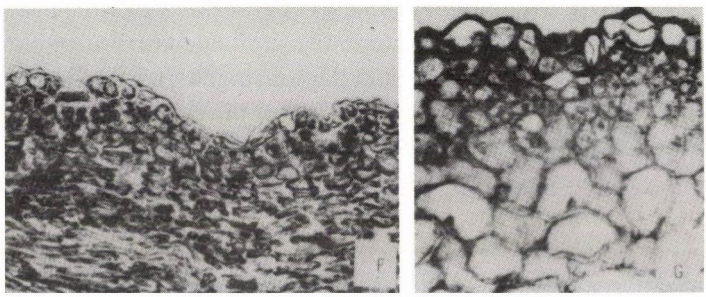

Fig. 6F–G

To know the ecological demand of the plant is equally important both for producers and breeders, because the cause of fruit drop may radicate in the xerophilous environment of a hygromorphous cultivars, not only in the deficient fruit set.

Between the thickness of the nectary cuticle and the sunken stomata there is a very close correlation r=0.8 (y=6.2–0.57x), which indicates the close relation of these two histological indices with that of xeromorphism.

The ratio of length to thickness of the epidermis cells (L/T) expresses the shape of the latter as appearing in the longitudinal section of the flower. The ratio may be below 1, in wich case the epidermis cells are more or less orbicular or oval, or it may be above 1, when the epidermis cells are parallelepipeds. In the case of flattened, elongated parallelepipeds, L/T exceeds 2, e.g. in Bigarreau Burlat and Germersdorf-57 (Table 2).

The cells of the glandular tissue are small, square-shaped and closely set in longitudinal section. They have abundant plasma and large nucleus, and form rows parallel to the surface. In old glands inclusions and crystal druses frequently separate out. Owing to their presence the nectary tissues are less damaged by animals. Ageing of the glandular tissue is indicated by the development and growth of vacuoles.

In the sweet cherry cultivars, the glandular tissue is usually uniformly thick. In some cases, however, the glandular tissue, which slightly widens under the stomata, narrows in the interstomatal area (Fig. 6G).

Table 3

Measuring data of nectaries in cherry cultivars

Cultivar, clone	Examination		Glandular tissue lengththickness		Number of glandular cell-rows	LxT*	Thick-ness of nectary parenchyma	Thick-ness of recept-acle
	place	time	µm	µm		µm²	µm	µm
Annabella	Érd	1987	3434.34	48	3.5	165 000.9	102.66	184.45
Bigarreau Burlat	Érd	1987	3055.92	31.1	2.4	95 064.13	65.28	112.38
Bigarreau Burlat	Ceglédbercel	1988	3020.22	32.77	2.5	99 337.62	80.51	128.51
Bigarreau Marmotte	Érd	1987	4028.39	41.22	3.3	166 348.1	91,01	160.51
Germersdorfi	Pécs	1986	3023.08	41.22	3.2	124 247.4	74.88	91.61
Germersdorfi-3	Érd	1987	3284.4	46.08	3.6	151 840.5	75.01	169.47
Germersdorfi-3	Ceglédbercel	1988	3075.91	40.96	3.1	125 609.2	137.34	211.33
Germersdorfi-57	Érd	1987	3345.8	34.94	2.4	117 245	82.68	121.6
H. 66	Ceglédbercel	1989	3648.54	30.08	2.3	109 867.8	72.32	130.43
H. 265	Ceglédbercel	1988	3255.85	50.30	3,9	162 674.1	91,78	164.1
H. 171	Érd	1987	3311.53	30.59	2.4	100 854.7	51.7	59.52
H. 261	Érd	1987	3565.72	34.05	2.7	120 869.6	83.58	95.36
Hedelfingeni óriás	Érd	1987	3060.2	18.3	1,4	55 405.49	76.8	98.69
Hudson	Érd	1987	2803.16	31,87	2.5	89 010.32	69.63	79.23
Jaboulay	Érd	1987	2200.55	43.52	3.7	95 791.61	136.06	138.37
Jaboulay UF.155	Érd	1987	2788.88	44.67	3.4	124 433.9	83.58	120.19
Májusi hosszúszárú	Érd	1987	3517.16	72.06	5.6	253 238.1	83.2	94,85
Merton Bigarreau	Érd	1987	3370,08	41.6	3.3	141 434.6	64.64	85.76
Münchebergi korai	Érd	1987	3901.3	37.12	2.9	145 112.2	80	137.34
Münchebergi óriás	Érd	1987	3342.95	42.62	3.3	142 825.6	98.69	136.03
Pinchon Early	Érd	1987	3440.05	43.01	3.8	147 477.4	86.78	196.86
Solymári	Pécs	1986	2844.58	35,07	2.9	99 381.49	117.89	99.2
Solymári	Érd	1987	3697.66	32.26	3.2	119 692.4	58.37	150.66
Solymári-16	Érd	1987	3522.88	46.21	3.3	164 851.1	78.59	101.38
Stella	Érd	1987	4308.20	69.25	5.2	298 824.4	34.18	122.37
Szegedi óriás	Érd	1987	3005.94	50.05	4.0	150 564.7	72.83	126.21
Vadcseresznye	Mecsek	1989	2463.30	31.74	2.3	78 84o.22	101.76	111.36
Vadcseresznye Altenweddingeni	Cegléd	1988	2663.22	15.10	1.1	40 291.08	85.25	201.98
Vadcseresznye Altenweddingeni	Cegléd	1990	2698.92	18.43	1.3	47 079.67	92.03	209.92
Vadcseresznye CT. 2493	Cegléd	1988	2574.68	27.14	2.2	69 702.85	111.87	89.86
Vadcseresznye CT. 2493	Cegléd	1989	2554.69	27.52	2.1	69 739.41	109.06	90.62
Van	Érd	1987	2931.68	22.27	2.4	64 769.51	33.15	83.46
Van	Ceglédbercel	1988	3103.04	30.46	2.2	94 389.66	74.88	184.7
Van F. 630	Érd	1987	3551.44	44.54	3.5	157 367.9	76.42	99.97
Van F. 635	Érd	1987	3878.85	50.56	4.0	145 815,9	107.14	177.66
Valerij Cskalov	Érd	1987	3314.39	39.94	3.2	132 546.7	48.51	82.94

*L × T: Length × thickness

As to the rise of the nectary, there are types that spring directly from the pistil, while others start at a distance from the ovary. In the cherry cultivar "H-165" e.g. the basal part of the nectary begins with a protuberance quite close to the rise of the ovary, indicative of automorphism. Between the rise of the hypanthium and the pistil a more or less horizontal widening of the receptacle can be observed in several varietes, which offers a space for nectar to gather at the base of the flower.

The number of cell-rows in the glandular tissue is characteristic of the variety and this is not influenced by the yearly fluctuations of the weather. In the cherry cultivars the gland consists of 1 to 6 cell-rows. The lowest and highest value seldom occur.

The length of the glandular tissue is 2000–4500 μm. The nectary of "Jaboulay", "Hudson", "Van", "Van F. 635", and "Szegedi giant" is short, that of "Solymári", "Solymári-16", "Van F. 630", "H. 261" is long, while the varieties Münchenberg early, "Bigarreau Marmotte" and "Stella" have a very long nectary (Fig. 7, Table 3).

Based on the thickness of the glandular tissue, sharp distinctions can be made between the cultivars (Fig. 8, Table 3). The nectary is thinnest – below 30 μm – in the varietes Hedelfingen giant. Altenweddingen wild cherry and "Van", in which its glandular tissue consist only of 1–2 cell-rows. The medium thick, 30–50 μm nectaries consists of 3–5 cell-rows. The Stella and Májusi long-pedicelled varieties have the thickest nectary, close to or even exceeding 70 μm, and the glandular tissue usually consists of 5–6 cell-rows.

The product of length and thickness characterizes well the size of the glandular tissue. This tissue is smallest in Hedelfingen giant and Van. The gland is also small in the cultivars Hudson, Bigarreau Burlat, Jaboulay, H.171 and Germersdorf-57, while largest in "May long-pedicelled", and "Stella" (Fig. 9, Table 3).

The correlation (r=0.91) between the size of glandular tissue and nectar production at the time of maximum nectar secretion was determined (Orosz-Kovács, Gulyás, 1989) for the Pándy sour cherry clones.

Schmid (1988) denies the existence of correlation between the size of nectaries and amount of nectar production. This conclusion may probably originate from the fact that the nectar produced by open pleomorphous flowers can be partly lost by evaporation in certain periods of the day.

As explained in our earlier papers (Orosz-Kovács, 1988,1989), the nectar is produced periodically in rhythmical quanta and this rhythmical activity can be detected by a continuous sampling, repeated every hour. Therefore Schmid's viewpoint is to be modified in the sense, that direct correlations between nectary size and nectar production can be found only in the periodically active flowers, based on the nectar quantity produced during the periods of maximal production. Gulyás and Kincsek (1977) also discovered correlations between structure and product in the species of Labiatae and Fabacease, although they did not consider the periodicity of nectar secretion. The positive result may be attributed to those flowers of tubular corolla (Labiatae), or those with bent petals (Fanbaceae), in which the secretion does not evaporate during the 24-hour isolation.

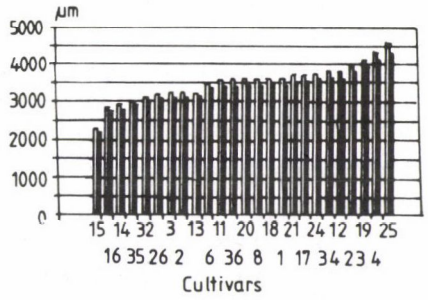

Fig. 7. Length of glandular tissue of nectary in cherry cultivars (on wild cherry root-stock). Érd, 1987

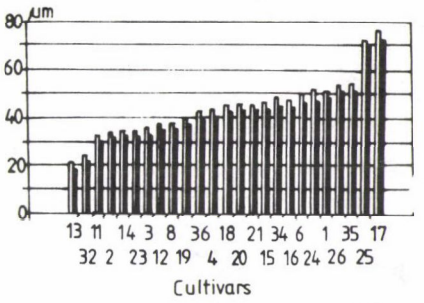

Fig. 8. Thickness of glandular tissue of nectary in cherry cultivars (on wild cherry root-stock). Érd, 1987

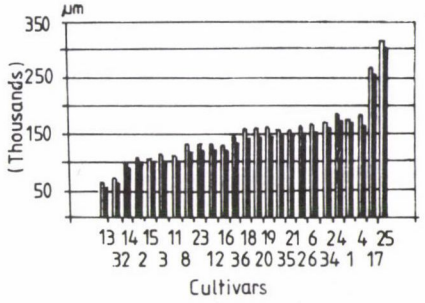

Fig. 9. Size (length × thickness) of glandular tissue of nectary in cherry cultivars (on wild cherry root-stock). Érd, 1987

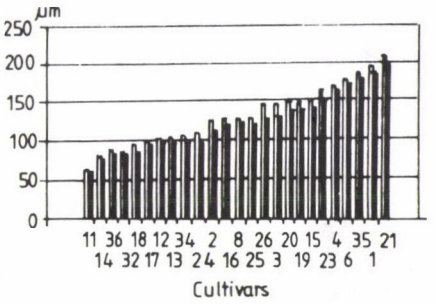

Fig. 10. Thickness of nectary receptacle in cherry cultivars (on wild cherry root-stock). Érd, 1987

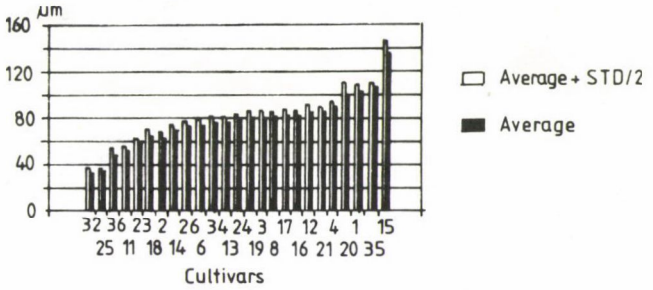

Fig. 11. Thickness of nectary parenchyma in cherry cultivars (on wild cherry root-stock). Érd, 1987

Unfortunately, we could not find the correlation between nectary structure and nectar production in cherry cultivars because, in arid periods, flowers with thin glandular tissue do not produce secretion at all, and during the years of studies between 1987 and 1990 there was very small amount of precipitation in the critical periods on the experimental areas. Nevertheless, the correlation of structure and production is highly probable in the cherry cultivars, similarly to the sour cherry varieties, where the size of the glandular tissue forecasts the prospective product and is thus indicative of the apicultural value of the cultivars. Neither did the thickness

of the *receptacle* and *nectary parenchyma* (Figs 10 and 11) show any kind of correlations with the size of the glandular tissue, nor with the surface of the nectary. The cells of the nectary parenchyma are larger than the glandular cells. Nectary parenchyma can be easily distinguished from the glandular tissue by the giant vacuoles developing relatively early in the cells. The vascular bundles in the receptacle run longitudinally. Never was observed the branching of the bundles and their entering the nectary parenchyma or glandular tissue.

It can be established that the surface of the nectary helps to retain the nectar of flowers and indicates the ecological demand of the cultivars. By the size of the glandular tissue, the cherry cultivars can be easily differentiated; and, on the basis of the nectary structure, the prospective nectar production and consequently the insect attraction of flowers can be forecasted.

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CALLUS CULTURES AND PLANT REGENERATION FROM MATURE EMBRYOS IN WINTER WHEAT

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A three-step method was developed for the regeneration of plants from callus cultures from mature embryos of different winter wheat cultivars. Embryos were removed from seeds after 24 h inhibition and then incubated on callus-forming media for 17 days, followed by 21 days in the light. Rates of regeneration among regeneration media was as high as 40 – 50% for the best cultivars which were similar to regeneration rates of calli from immature embryos.

Keywords: regeneration, somatic embryogenesis, *Triticum aestivum*

Introduction

Regeneration has been successfully achieved from immature embryos in numerous wheat cultivars through the use of appropriate nutrient media, hormone concentrations and composition, and culturing conditions (Zhang and Seilleur, 1987; McKinnon et al., 1987; Hayashi and Shimamoto, 1988; Chu et al., 1990; Wang and Nguyen, 1990). However, the production of callus cultures from immature embryos is both time-consuming and expensive. The present experiments were aimed at developing a method of embryogenic callus formation and plant regeneration from mature embryos of various Hungarian winter wheat cultivars.

Materials and methods

Eight winter wheat cultivars developed in the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, were used: Martonvásári 4 (Mv 4), Martonvásári 8 (Mv 8), Martonvásári 12 (Mv 12), Martonvásári 14 (Mv 14), Martonvásári 15 (Mv 15), Martonvásári 17 (Mv 17), Martonvásári 21-85 (Mv 21-85) and Martonvásári 18 (Mv 18). For surface sterilization, the seeds were soaked in 70% ethanol, 3% H_2O_2 or, in 0.1% $Hg Cl_2$ for 5 minutes. The seeds were left to swell for 24-h in sterile distilled water, during which time the seed coat split. The following media were employed: MS-A medium containing the mineral salts and organic additives of modified Murashige and Skoog (1962) medium, together with 30 g/l saccharose and 0.1 mg/l kinetin solidified with 8 g agar, and MS-B medium containing the mineral salts of modified Murashige-Skoog medium, together with 100 mg/l myo-inositol, 0.5 mg/l vitamin B1, 150 mg/l asparagine, 20 g/l saccharose and 50 mg/l adenine solidified with 8 g agar. The supplements of MS-A and MS-B media are summarized in Table 1.

For the purposes of callus formation, these were supplemented with 10 µl/l 2,4-D (MS-A 10 and MS-B 10). Calli were incubated in the dark for 17 days at 25 °C, then for 21 days in the light, with an 18-h illumination period (light intensity was 6000 lux) on the same nutrient medium. Regeneration took place for 21 days with an 18-h illumination period (10000 lux) on MS-B nutrient media containing no 2,4-D, (MS-B) 0.5 µl/l 2,4-D (MS-B 0.5), or 20 mg/l $AgNO_3$ (MS-B $AgNO_3$). The extent of callus formation and regeneration was evaluated at the end of each incubation period.

Table 1

Supplements of MS-A and MS-B media

Supplement mg/l	MS-A	MS-A	MS-B 0.5	MS-B 10	MS-B $AgNO_3$	MS-B
Kinetine	0.1	0.1	–	–	–	–
2,4-D	–	2.21	–	2.21	–	–
Myo-Inositol	100	100	100	100	100	100
Nicotinic acid	0.5	0.5	–	–	–	–
Piridoxine-HCl	0.5	0.5	–	–	–	–
Thiamine-HCl	0.5	0.5	0.5	0.5	0.5	0.5
Glycine	2	2	–	–	–	–
Asparagine	–	–	150	150	150	150
Adenine	–	–	50	50	50	50
Saccharose	30000	30000	20000	20000	20000	20000
$AgNO_3$	–	–	–	–	20	–

Results

Over 80% of the embryos formed a callus on both MS-A and MS-B media when the 2,4-D concentration was 10 µM/l. As the hormone concentration decreased, there was an increase in the proportion of embryos that germinated rather than formed a callus. The ratio of callus formation to germination at a concentration of 10 µM/l 2,4-D on MS-B medium is shown in Table 2 for each cultivar.

Table 2

Callus formation and embryo germination by cultivars of mature embryos during incubation in the dark on MS-B 10 medium

Cultivar	Total number cultured	Callus number	Callus %	Plantlet number	Plantlet %
Mv 4	450	403	89.6	35	7.7
Mv 8	460	412	89.5	46	10.0
Mv 12	450	388	86.2	62	13.8
Mv 14	500	389	77.8	82	16.4
Mv 15	450	409	90.8	39	8.6
Mv 17	450	378	84.0	63	14.0
Mv 21-85	450	408	90.6	27	6.0
Mv 18	460	414	90.0	21	4.6

The callus formation on MS-B medium was optimum after incubation in the dark for 17 days at a 2,4-D concentration of 10 µM/l, giving values of 77.8 to 90.8%, depending on the cultivar, with a 4.6 to 16.4% germination without any callus formation. At lower 2,4-D concentrations, the percentage of germination was

higher, reaching 80%. The MS-A nutrient medium also supported a callus formation, depending almost exclusively on the 2,4-D concentration (data not presented). After three weeks in the dark, the cultures were subcultured to MS-B 10 media, and incubated in the light. These were evaluated after 3 weeks. Macroscopically undifferentiated, but compact, yellowish-white calli and those that were turning green were regarded as embryogenic calli (Table 3).

Table 3

Callus differentiation and plantlet formation by cultivars on MS-B 10 medium. Cultures here incubated in the light

Cultivar	Total number cultured	Embryogenic yellowish-white %	Calli greenish %	Plantlet %
Mv 4	403	60.7	39.5	0.5
Mv 8	412	46.8	46.1	6.8
Mv 12	388	55.6	40.8	3.5
Mv 14	389	78.8	19.2	1.0
Mv 15	409	41.7	46.5	11.2
Mv 17	378	48.0	41.3	10.7
Mv 21-85	408	61.9	15.7	22.3
Mv 18	414	68.9	28.4	2.7

It can be seen from the table that, depending on the cultivar, the ratio of calli that turned green during the three weeks ranged from 15.7% to 46.5%, while the degree of germination was 0.5–22.3%. In cultivars that later proved to possess the best regeneration ability (Mv 4, Mv 12 and Mv 17), there was a relatively high ratio of calli that turned green during the 3-week period and a low percentage of spontaneous regeneration.

In preliminary experiments, regeneration was attempted using several types of nutrient media (MS-A and MS-B media containing no 2,4-D and 10, 20 or 50 mg/l silver nitrate) and various hormone concentrations (5, 0.5 and 0 μM/1 2,4-D). On the MS-A medium, there was a higher ratio of nonembryogenic calli, which did not turn green and later became fragile, and of calli that were only capable of root formation. The best results were achieved with MS-B, the MS-B $AgNO_3$ and MS-B 0.5 media, and these were used for regeneration in later experiments. If the concentration of 2,4-D in the nutrient medium was gradually reduced, from 5 μM/l to 0.5 μM/l, there was an increase in root formation without the capability of plantlet regeneration (data not shown).

Embryogenic calli underwent extensive plantlet regeneration (Table 4). On MS-B and MS-B $AgNO_3$ media, the plantlet formation was as high as 47.7 and 49.3 % in the best cultivars (Mv 12 and Mv 17 respectively). On MS-B 0.5 medium there was a higher proportion of undifferentiated calli and of calli producing only roots.

Table 4

Extent of regeneration from embriogenic calli on MS-B medium, on MS-B $AgNO_3$ medium and on MS-B 0.5 medium

Cultivar on MS-B medium	Total number cultured	Plantlet %	Root %	Callus %
Mv 4	134	44.2	45.1	10.7
Mv 8	108	15.5	84.5	0.0
Mv 12	125	47.7	52.3	0.0
Mv 14	127	18.4	74.5	7.1
Mv 15	120	24.5	74.5	0.0
Mv 17	112	49.3	45.5	5.2
Mv 21-85	106	25.9	13.7	60.4
Mv 18	134	25.5	1.4	73.1
Cultivar on MS-B $AgNO_3$ medium				
Mv 4	120	41.8	47.6	10
Mv 8	138	13.8	74.0	12.2
Mv 12	130	46.7	41.4	11
Mv 14	135	15.3	67.0	17.7
Mv 15	141	31.3	54.9	13
Mv 17	116	44.6	51.2	4.2
Mv 21-85	127	24.9	66.7	8.4
Mv 18	145	18.4	74.3	7.3
Cultivar on MS-B 0.5 medium				
Mv 4	132	24.3	70.4	5.3
Mv 8	130	9.6	30.6	60.0
Mv 12	131	13.9	73.0	13.1
Mv 14	120	12.6	64.4	23
Mv 15	135	15.9	61.5	14.5
Mv 17	118	36.8	56.9	6.3
Mv 21-85	138	18.4	55.3	16.3
Mv 18	117	16.5	65.7	17.8

Discussion

An efficient regeneration method was developed using the mature embryos of various winter wheat cultivars. Elena and Ginzo (1988) found that regeneration from mature wheat embryos could be achieved on nutrient medium with a reduced auxin content. If the embryos were cut up, or the scutellum was removed, the cultures obtained from the embryo axis and the plumula formed calli that were either

compact, white and capable of regeneration, or fragile. The former were capable of shoot regeneration, while the white, fragile calli formed from the radicle were incapable of shoot regeneration.

Zhang and Seilleur (1987) used both intact, mature embryos and embryos dissected in various ways for regeneration. Of these, calli originating from embryos deprived of their scutellum and hypocotyl gave the best regeneration, with a frequency of 13.3 to 61.5% depending on the nutrient medium and the cultivar.

McKinnon et al. (1987), who used whole, mature embryos, obtained 45–52 regenerated plantlets from 100 embryos, depending on the cultivar.

Since it was previously found that no calli were formed in a tissue culture from the scutellum of mature embryos, whole embryos were used in the present work. The MS-B nutrient medium containing 10 μM/1 2,4-D proved optimum for callus formation. When embryogenic calli were placed on various differentiating media, 9.6–49.3% regeneration was achieved, depending on the cultivar and the nutrient medium. A reduction in the 2,4-D concentration to 0 in a single step, or the addition of a 20 mg/l concentration of $AgNO_3$, was equally efficient in inducing regeneration, while a nutrient medium containing 2,4-D at a concentration of 0.5 μM/l tended to shift differentiation in the direction of root regeneration only. Less favourable results were obtained with $AgNO_3$ than were those reported by Purnhauser et al. (1987) in the wheat variety GK Kincső. This can probably be attributed partly to differences in the nutrient media used and partly to differences in the wheat cultivars.

The cultivars examined gave a good callus formation and, although their growth intensity was lower than that observed in the usual dicotyledonous test plants, e.g. tobacco, it was high enough for them to be used in biochemical and physiological analyses. The extent of plant regeneration achieved in the best cultivars is comparable with the results reported for a callus induction from immature embryos.

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USE OF EXCISED-LEAF WATER CONTENT IN BREEDING TEF (*Eragrostis tef* (Zucc.) Trotter.) FOR MOISTURE STRESS AREAS

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Excised-leaf water loss (ELWL) has shown promise in many cereals regarding its potential to be used as a physiological selection criteria in breeding for low moisture areas. In this study, the merit of this trait was assessed in tef using a random selection of twenty-one pure lines of tef from the more than 2000 land race collections. These were grown in contrasting environments (Optimum vs moisture stress) for two seasons in a randomized complete block design.

The results showed that there were significant differences among tef genotypes in ELWL during vegetative stage coupled with non-significant genotype-environment interaction in low moisture environments. The trait was positively related to grain yield under moisture stress where slow water loosers gave higher yields than fast water loosers. In addition, the trait is affected by crop phenology such that mean ELWL was higher during tillering stage followed by jointing stage and anthesis in that order. It is, therefore, concluded that ELWL can be used as a selection criteria in breeding tef for low moisture areas. However, further research needs to be done on its inheritance.

Keywords: tef, *Eragrostis tef* (Zucc.) Trotter., excised-leaf water loss, drought resistance

Introduction

Tef is considered to be the most widely adapted of all major cereal crops (maize, sorghum and wheat) in Ethiopia because of its drought tolerance and adaptation to a wide range of soil conditions (Seyfu, unpublished). As a result, a considerable amount of it is grown in moisture stress areas including the rift valley region. Breeding tef for drought-prone areas has been one of the major objectives of the tef improvement programme in Ethiopia. In such an effort, the use of physiological and morphological traits as a selection criteria was said to be useful (Clarke, 1987). However, the utility of a particular characteristics as a selection criteria in a breeding programme will depend upon the ease and cost of screening and its relation to an easily measured agronomic trait, such as yield. One of the less expensive and easily measured physiological traits, excised leaf water loss, was examined in detail in tef to see its potential as a selection criteria, as it proved to be so in some other cereals.

Sandhu and Laude (1958) showed that excised plants of drought-resistant winter wheat cultivars lost water more slowly than less resistant cultivars, and ELWL seems to be positively related to yield under drought. Similarly, Clarke and McCaig (1982), Dedio (1975) and Jaradat and Konzak (1983) found differences in ELWL among wheat genotypes. However, differences among cultivars seem to

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disappear after anthesis (Clarke, 1987). So, the trait is operative during vegetative growth stage and makes selection very fast, i.e. no need to wait until physiological maturity. Genotype-environment interaction have also been reported (Jaradat and Konzak, 1983). Nevertheless, this was more of ranking than significant difference. Hence, it is possible to be used under a variety of conditions. From this evidence, it is apparent that the trait in question is promising as a selection criteria in breeding for moisture stress environments. The objectives of the present study, therefore, are:

1. to see correlation among grain yield, drought susceptibility index (DSI) and ELWL measured at various growth stages.
2. to assess variation of tef genotypes for ELWL at various stages and its interaction with environment.
3. to assess effects of crop phenology on ELWL, and
4. to compare grain yield of the fastest and slowest water looser genotypes in different moisture regimes.

Materials and methods

The experiment was conducted at Debre Zeit Agricultural Research Center in 1990/91 main cropping season using two sowing dates (normal and late planting), and in 1991/92 off season under two moisture regimes (stressed and non-stressed) supplied through irrigation. This gave a total for four environments representing various moisture regimes (Table 1).

Table 1

Characteristics of the test environments

Environment	Season	Moisture (mm) (rainfall/irrigation source)
E_1	Main season (normal planting)	320
E_2	Main season (late planting)	205
E_3	Off-season (optimum irrigation)	390
E_4	Off-season (stressed)	220

Twenty-one pure lines of tef having similar maturity and diverse in agronomy and morphological traits (panicle length, plant height, panicle form, etc.) were randomly chosen from tef germplasm pool, and evaluated in a randomized complete block design of three replications. Management practices were applied as per recommendation.

Data were collected on DSI (Fisher and Maurer, 1978), grain yield and ELWL at tillering (when first tiller begins to appear), stem elongation and anthesis. For ELWL measurement, six young and fully expanded leaves were sampled from each plot in a double polyethene bag, and these were brought to the laboratory enclosed in an ice-box. The samples were weighed three times: immediately after sampling, after a period of wilting and after oven drying (80 °C for 24 h). A wilting period of 2 hour in a controlled temperature chamber (30 °C, 75 ±1% RH) was used. ELWL was expressed as gram of water lost per gram of leaf dry matter per hour (g/g/h).

Results and discussion

Combined analysis of variance for all environments (Table 2) and stress environments (Table 3) showed that there were significant differences ($P<0.01$) among tef cultivars for ELWL during vegetative stage – the variance being higher during tillering stage than jointing stage. The variation among genotypes disappeared at anthesis. Therefore, this trait seems to be operative during vegetative stage (Clarke, 1987). Genotype environment interactions were found to be significant during vegetative stage when data were combined over all environments (Table 2), whereas non-significant interactions were observed when data were combined for moisture stress environments alone (Table 3). So, this trait can be used under a variety of conditions (Clarke and McCaig, 1982; Jaradat and Konzak, 1983).

Table 2

Mean squares (g/g/h) from combined analyses of variance for ELWL at all environments (E_1, E_2, E_3, E_4)

Source	Crop phenology		
	Tillering	Jointing	Anthesis
Location (L)	0.32**	0.06*	0.02
Reps/L	0.03	0.01	0.01
Genotypes (G)	0.34**	0.05**	0.01
G×L	0.22**	0.04*	0.01
Error	0.02	0.02	0.01

*, ** Significantly different at 0.05 and 0.01 levels of probability, respectively

Table 3

Mean squares (g/g/h) from combined analyses of variance for ELWL at low moisture environments (E_2 and E_4)

Source	Crop phenology		
	Tillering	Jointing	Anthesis
Locations (L)	0.33**	0.22**	0.01
Reps/L	0.03	0.03	0.01
Genotypes (G)	0.44**	0.14	0.00
G×L	0.03	0.04	0.01
Error	0.02	0.03	0.01

** Significantly different at 0.01 levels of probability respectively

Correlation coefficients between grain yield and ELWL were negative and significant (Table 4) during vegetative stage in low moisture environments, indicating that fast water loss was associated with low grain yield and slow water loss with high grain yield under moisture stress (Sandhu and Laude, 1958). Positive and significant (Table 4) correlation coefficients were found between DSI and ELWL in low moisture environments, suggesting that slow water loss was associated with

higher degree of drought resistance and fast water loss with higher degree of drought susceptibility under moisture stress. However, there was no significant correlation among ELWL, grain yield and DSI in high moisture environments. Furthermore, comparison of the yields of the five fastest and five slowest water loosers showed significant differences in ELWL measured at vegetative stage in low moisture environments and non-significant differences in high moisture environments, and during anthesis, in low moisture environments (Table 5). Where yields were different, fast water loosers gave lower yields than slow loosers, suggesting that ELWL has some promise as a selection criteria in breeding for low moisture area (Sandhu and Laude, 1958).

Table 4

Correlation coefficients among ELWL, and DSI and grain yield

Environment	Grain yield			DSI		
	Tillering	Jointing	Anthesis	Tillering	Jointing	Anthesis
Low moisture						
E_2	–0.41**	–0.28*	0.02	0.36**	0.30**	0.06
E_4	–0.41**	–0.27*	0.03	0.30*	0.30*	0.02
High moisture						
E_1	0.04	–0.09	0.56	0.20	–0.05	0.14
E_3	0.15	0.14	0.05	0.10	0.22	–0.09

*, ** Significant at 0.05 and 0.01 levels of probability, respectively

Table 5

Grain yields (kg/ha) of five fastest and five slowest excised-leaf water loosing genotypes

Environment	Tillering		Elongation		Anthesis	
	Fastest	Slowest	Fastest	Slowest	Fastest	Slowest
Low moisture						
E_2	527	1183**	465	1120**	1200	1156
E_4	455	760*	545	1018*	500	485
High moisture						
E_1	1567	1700	1860	2011	1564	1622
E_2	1611	1520	1960	2102	1727	1850

*, ** Significantly different at 0.05 and 0.01 levels of probability, respectively

Concerning the effects of crop growth stages on ELWL, there were significant differences ($P<0.05$) among growth stages (Table 6) such that ELWL was higher during tillering stage followed by jointing stage and anthesis in that order. This

finding agreed with that of Clarke (1987). However, it is not clear wether the change after anthesis is due to physiological changes or physical factors.

In conclusion, ELWL is a trait positively related to grain yield under moisture stress environments and can be used as selection criteria during vegetative stage in breeding tef for moisture stress environments. Before this, however, studies of its inheritance need to be done.

Table 6

Excised-leaf water loss (g/g/h) at various phenological stages

Crop phenology	Environment			
	E_1	E_2	E_3	E_4
Tillering	1.721a	1.558a	2.005a	1.958a
Jointing	1.314b	1.193b	1.721b	1.293b
Anthesis	1.305b	0.830c	1.406c	0.887c

Means within a column followed by a common letter are not significantly different at 0.05 level of probability using Duncan's multiple range test.

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EFFECT OF FOLIAR APPLICATION OF PROLINE ON THE SALT STRESSED RICE SEEDLINGS

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The effect of foliar application of proline on the shoot growth and solute composition of salt-tolerant (ev. Co 43) and salt-sensitive (cv. TKM 9) rice cultivars was investigated in EC 7, 10 and 15 m mohs/cm salinity levels by pot culture experiments. The exogenous application of proline had no appreciable improvement in the salt-induced inhibition of dry matter accumulation of shoot in both salt-tolerant and salt-sensitive rice cultivars. In the absence of exogenous application of proline, the endogenous proline concentrations in the leaves of Co 43 and TKM 9 gradually increased with increasing levels of Na^+ and Cl^- in the different salinity levels tested. However, the Co 43 was more efficient in maintaining high levels of proline than the TKM 9 in response to salinity. Foliar application of proline did not alter the Na^+ and Cl^- contents of leaves in both rice cultivars under saline conditions, but Co 43 showed more proline loading capacity over TKM 9 in their leaves when proline was exogenously supplied on the salt-stressed plants. The physiological significance of endogenous proline is discussed.

Keywords: rice, salt stress, proline

Introduction

Proline is a five-carbon amino acid which accumulates in plants in large quantities under water and salt stresses (Hanson and Hitz, 1982; Stewart and Larher, 1980). It exhibits properties characteristic of hydrophilic colloids. The physiological significance of proline accumulation is controversial. Proline has been assigned the role of cytosolute (Wyn Jones and Storey, 1978), nitrogen storage compound (Barnett and Naylor, 1966), energy donor (Dashek and Erickson, 1981), protective agent for cytoplasmic enzymes and cellular structure (Schobert, 1977), associated with salt tolerance (Krishnamurthy et al., 1987a, 1988), air pollution tolerance (Anbazhagan et al., 1988) and drought tolerance (Singh et al., 1973). The possible role of proline, along with some other organic solutes, is that it accumulates in the cytoplasm and lowers the solute potential there, balancing a low solute potential in the vacuole due to the accumulation of salts or other solutes present (Voetberg and Stewart, 1984). There is some evidence that salts, especially Na^+ and Cl^-, are sequestered in the vacuole (Flowers et al., 1977), and some evidence that proline is located mainly in the cytoplasm of cells in stressed tissue (Pahlich et al., 1983). However, there is limited information on the physiological function of proline in rice (Ball, 1975; Krishnamurthy et al., 1987a, 1988).

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In our previous paper, we have reported that salt-tolerant rice cultivars are more efficient in maintaining high levels of endogenous proline in their root and shoot systems than are the salt-sensitive rice cultivars, when subjected to NaCl salinity (Krishnamurthy et al., 1987a; 1988). This paper reports the effect of foliar application of proline on the shoot growth and endogenous Na^+, Cl^- and proline concentrations of salt-tolerant and salt-sensitive rice cultivars exposed to NaCl salinity.

Materials and methods

The seeds of rice cultivars Co 43 (salt-tolerant) and TKM 9 (salt-sensitive) were procured from Tamil Nadu Agricultural University, Coimbatore. The salt-tolerant and salt-sensitive groups were classified in our earlier reports on the basis of growth and grain yield (Krishnamurthy et al., 1987b; 1987c). The pot culture experiments were carried out in earthen pots filled with 7 kg of soil under net house conditions during the wet season. Three-day-old germinated seeds were sown in pots having EC 7, 10 and 15 m mhos/cm salinity levels of the soil. Salinization was imposed by adding NaCl in solution form to raise the salt concentrations of the soil to EC 7, 10 and 15 m mhos/cm levels in the pots (Krishnamurthy et al., 1989; Krishnamurthy and Bhagwat, 1989). Control pots received only tap water. The nonsaline and saline treated plants were sprayed with 4 mM L – Proline aqueous solution on seven-day-old seedlings, and the processes repeated every once seven days till the plants were 25 days old. Control plants were sprayed only with water. The shoot system was harvested at 25 days and the expanded young leaf (third) from the top was selected for the estimation of proline, sodium and chloride. Proline was determined as described earlier using ninhydrin reagent (Bates et al., 1973). Sodium was determined by flame emission spectrophotometry (Richards, 1953) and chloride was assayed by titration with mercuric nitrate (Krishnamurthy and Bhagwat, 1990). All the assays were made from 3 replicates (i.e. three independent experimental earthen pots), each containing three separate plants.

Results and discussion

Generally it was observed that the exogenous application of proline had no appreciable ameliorating effect on the salt-induced inhibition of dry matter accumulation of shoot in both salt-tolerant and salt-sensitive rice cultivars during their seedling growth stages (Table 1). However, salt-tolerant Co 43 was benefited by the exogenous application of proline and exhibited an improved fresh matter content of shoot system over that of the salt-sensitive TKM 9 under saline conditions. These findings did not support the general observation that exogenous proline helped the non-resistant varieties of barely (Singh et al., 1972) to overcome the stress effect and peas (Bar-Nun and Poljakoff-Mayber, 1977) in the effective counteracting of salinity in promoting germination. It is reasonable to assume, from present results and others, that proline probably have some specific counteracting mechanism from saline injury, depending upon the varieties and different plant species which are yet to be further investigated.

The levels of proline were markedly increased in the leaves of salt-stressed Co 43 under salinization in the presence or absence of the exogenous application of proline (Table 2). Contrary to Co 43, the salt-sensitive TKM 9 accumulated less proline in the leaves under a similar situation. In the absence of exogenous

Table 1

Effect of foliar application of proline on the vegetative growth of shoot system of rice seedlings exposed to NaCl stress

Treatment	Salinity level EC m mhos/cm	Fresh weight (mg/seedling)		Dry weight (mg/seedling)	
		Co 43	TKM 9	Co 43	TKM 9
Nil	Control	661±20	709±24	121±5	141±3
Nil	7	646±16	695±40	108±6	104±4
Nil	10	440±26	645±38	83±3	85±2
Nil	15	387±10	560±29	64±2	65±2
P	Control	800±32	720±52	123±4	144±3
P	7	749±25	698±47	113±2	109±6
P	10	668±49	650±27	95±3	90±4
P	15	585±36	565±34	79±3	68±3

P : Proline; Each value is the mean ± L.S.D. for 3 replicates eah containing 3 plants.

application of proline, the endogenous proline concentration in the leaves of Co 43 and TKM 9 gradually increased with increasing levels of Na^+ and Cl^- in all three salinity levels tested. These results agree with others, that proline concentrations were directly proportional to Na^+ concentrations (Voetberg and Stewart, 1984) and each increase in sodium concentration was balanced by an increase in proline concentration equal to about 4% of the rise in sodium. The results are consistent with the idea that proline could act as a cytoplasmic solute, accumulated in response to an extracellular accumulation of salts or an accumulation of salts in the vacuole, since a constant high proline concentration would be required to balance a constant high salt concentration (Flowers et al., 1977). The above results, and also our earlier reports on several rice cultivars, confirmed that salt-tolerant cultivars had the capacity to accumulate higher levels of proline than salt-sensitive cultivars under saline conditions (Krishnamurthy et al., 1987a; 1988).

The rate of increase in proline concentration also had a positive relationship to the salinity levels when proline was exogenously supplied (Table 2). Further, salt-tolerant Co 43 had a probable potentiality to take up and enjoy a higher amount of proline than salt-sensitive TKM 9 in their leaves, in response to foliar application of proline. With an exogenous application of proline, the endogenous proline levels of TKM 9 continued to reach 1.17μ mole $(g \text{ fresh weight})^{-1}$ at EC 7 salinity level and declined sharply thereafter. Foliar application of proline did not alter the sodium and chloride contents of both rice cultivars in all salinity levels tested. However, these findings do not support the hypothesis that proline concentrations occur directly proportional to salt concentrations in the tissue (Neales and Sharkey, 1981) and also have a linear relationship with the sodium concentrations (Voetberg and Stewart, 1984). It is reasonable to conclude that the exogenous application of proline does not effectively counteract salt injury in salt-tolerant and salt-sensitive lines of rice under a saline environment.

Table 2

Effect of foliar application of proline on the endogenous concentrations of proline, sodium and chloride in the leaves of rice seedlings exposed to NaCl stress

Treat-ment	Salinity level (EC m mos/cm)	Proline (µmol/g fresh weight)		Sodium (µmol/g dry weight)		Chloride (µmol/g dry weight)	
		Co 43	TK	Co 43		Co 43	
Nil	Control	0.85±0.09	0.41±0.06	0.15±0.01	0.25±0.05	0.29±0.05	0.34±0.02
Nil	7	1.00±0.20	0.55±0.04	0.50±0.08	0.91±0.07	0.53±0.06	1.14±0.07
Nil	10	1.23±0.20	0.64±0.12	0.67±0.09	1.39±0.04	1.11±0.04	2.07±0.09
Nil	15	1.65±0.35	0.55±0.02	1.11±0.13	1.90±0.06	1.49±0.05	3.05±0.08
P	Control	1.35±0.13	0.73±0.06	0.17±0.01	0.25±0.01	0.27±0.04	0.36±0.03
P	7	1.79±0.12	1.17±0.20	0.45±0.09	0.88±0.11	0.45±0.03	1.07±0.09
P	10	2.27±0.20	1.03±0.14	0.61±0.11	1.31±0.09	1.00±0.05	2.06±0.04
P	15	2.96±0.16	0.99±0.30	1.05±0.11	1.88±0.10	1.48±0.06	2.91±0.12

P: Proline; Each value is the mean ± L.S.D. for 3 replicates each containing 3 plants.

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RESPONSES OF SOME SALT-TOLERANT AND SALT-SENSITIVE ACCESSIONS OF PEARL MILLET (*PENNISETUM GLAUCUM* (L.) R. Br.) TO DROUGHT STRESS

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Responses to repeated cycles of drought of four salt-tolerant accessions of pearl millet (*Pennisetum glaucum* (L.) R. Br.), WIR-6-Ostistoe, KAT/PM-2, Kitui Local and Selection II and two salt-sensitive, Togu Bold Grain and Y-84 were assessed in a pot experiment under greenhouse conditions. Of all accessions only one salt-tolerant WIR-6-Ostistoe was relatively drought-tolerant, as it had significantly greater biomass production then the other accessions at 3 or 6 drought cycles. No consistent pattern of accessions was observed for leaf area ratios, relative growth rate and coefficient of shoot elongation at both drought treatments.

Increasing drought stress intensity had no significant effect on leaf diffusive resistance, stomatal indices and relative water content of all six accessions. Deposition of epicuticular wax on leaf surface of all accessions was affected differently at various water deficit treatmants. At the highest drought treatment, Kitui Local was the highest in epicuticular wax content of all accessions. Leaf soluble proteins remained unchanged due to drought in WIR-6-Ostistoe, Selection II and Togu Bold Grain, but they decreased significantly in the remaining 3 accessions. Total free amino acids of WIR-6-Ostiatoe, KAT/PM-2 and Kitui Local increased at the highest drought treatment. The shoot soluble sugars were significantly lower and starch higher in the relatively drought-tolerant WIR-6-Ostistoe compared with the other accessions. The leaf proline content of the drought-tolerant WIR-6-Ostistoe and the 2 other accessions, Selection II and KAT/PM-2, increased consistently after both drought treatments.

From this study it is clear that with some exceptions drought tolerance and salt tolerance of the pearl millet accessions examined here are two independent phenomena.

Keywords: *Pennisetum glaucum,* drought stress, drought-tolarence, salt-tolarence

Introduction

Shortage of water remains the most important factor threatening the food security of people in the developing countries. There is evidence that the problem is becoming more serious. For instance, the world's cereal production has declined in the past few years (FAO, 1988) against a requirement for an increase of about 3% (FAO, 1981) in developing countries to maintain even current nutritional levels to the year 2000 and beyond (McWilliam, 1989).

Keeping this problem in mind, scientists have recommended several strategies that can substantially overcome drought stress. One approach is to replace the conventional canal irrigation system with closed metal, concrete or plastic conduits, which will reduce water evaporation and seepage. Different management practices to store irrigation water are also underway. Yet, all these approaches are highly expensive from the viewpoint of most developing countries.

Selections of plant species/crop cultivars with considerable drought resistance have been considered as economic and efficient means to utilize drought-prone of

areas, in combination with appropriate management practices, for reducing water losses (Atsmon, 1973; Blum, 1974; Hurd, 1976; Turner and Jones, 1980; Quisenberry, 1982; William, 1989).

It is now evident that drought occurs widely in arid and semi-arid regions of the world, where salinity is also prevalent due to rapid evapotranspiration of subsoil water (Ashraf and Bokhari, 1987). One of the major objectives in this study was to assess the responses of a small number of pearl millet accessions differing in their degree of salt tolerance (Ashraf and McNeilly, 1991), since it is now generally accepted that drought tolerance and salinity tolerance share the mechanism of osmotic adjustment (Hsiao, 1973; Blum, 1985). A crop cultivar having adaptability to both salt and drought stress would be of great value for the economic utilization of semi-arid regions of the world.

Materials and methods

Seeds of 6 accessions of pearl millet (*Pennisetum glaucum* (L.) R. Br.) were obtained from different countries, i.e. WIR-6-Ostistoe from USSR, KAT/PM-2 and Kitui Local from Kenya and Selection II (developed after 2 cycles of selection at high selection pressure of salt), Y-84 and Togu Bold Grain from the Maize and Millet Research Institute, Yousaf Wala, Sahiwal, Pakistan.

All seed samples were surface sterilized in 5% sodium hypochlorite solution for 5 minutes before experimentation. About 100 seeds of each accession were sown in plastic Petri dishes. After 2 weeks, 5 seedlings of comparable size of each accession were transplanted equidistant from each other into 18 cm plastic pots containing 4.0 kg sandy clay soil, whose water holding capacity was 22.5%.

The experiment was placed in a randomized complete block design with 4 blocks. Each block contained 6 accessions and 3 drought treatments. The drought cycles were started after 2 weeks of normal growth in full strength Hoagland nutrient solution (Epstein, 1972).

The water treatments were as follows:

T_0 Watering each day to field capacity throughout the experiment.

T_1 The plants were droughted 3-times until wilting occurred and rewatered to field capacity.

T_2 The plants were droughted 6-times as T_1.

The plants were considered wilted when 2-3 leaves of a plant showed wilt. After the droughted plants had begun wilting, these plants and corresponding control plants were rehydrated by watering the soil to field capacity. At the end of drought treatments, different growth and biochemical and physiological parameters were measured.

Fresh weights of shoots and roots were recorded and then, after drying plant material at 70 °C for a week, the dry weights of shoots and roots were recorded.

Relative growth rate:

Relative growth rate is defined as the weight gain per unit of plant weight per unit time (Blackman, 1919).

For the determination of relative growth rate, plant dry weights of 3 successive harvests were recorded after every 2 weeks. Relative growth rate was determined by the following formula:

$$RGR = \frac{1}{W}\frac{dW}{dT}$$

$$(g/g\ day^{-1})$$

where

W = dry weight of the plants at first harvest

dW = difference in dry weights of plants at two harvests

dT = difference of time between two successive harvests.

Succulence

Leaves were randomly taken from each rehydrated plant after the completion of 3 or 6 drought cycles and their fresh weight was recorded, and area was measured by leaf area meter (Delta-T Devices). Then the leaves were dried at 70 °C for a week and their dry weight was recorded. The succulence was estimated by the following formula

$$\text{Succulence} = \frac{\text{Fresh wt. - Dry wt.}}{\text{leaf area } (m^2)}$$

g of water/m^2

Estimation of epicuticular wax

A half gram of leaves was randomly taken from each plant and their area was measured by leaf area meter (Delta-T Devices). The leaf samples were washed 3 times in 10 ml carbontetrachloride for 30 seconds per wash. The extract thus obtained was filtered, evaporated to dryness and the remaining wax was weighed. The wax content was expressed on the basis of leaf area only, i.e. wax content µg/cm^2.

Relative water content

Fresh leaf material of rehydrated plants was randomly taken from each plant after the completion of 3 or 6 drought cycles. Their fresh weight was recorded and leaf pieces were dipped in 10 ml distilled water in the test tubes, already labelled. These test tubes were left for 24 hours under the tube lights. After 24 hours the leaf pieces were blotted and their turgid weight was taken. The leaf material was dried at 70 °C for a week and the dry weight recorded. The relative water contents were calculated by the following formula.

$$\text{R.W.C.} = \frac{\text{Fresh wt. - Dry wt.}}{\text{Turgid wt. - Dry wt.}} \times 100$$

Leaf area ratio

For the determination of leaf area ratios of rehydrated plants after 3 or 6 cycles, the data of the following parameters were used:

i) dry weights of shoot and root of a plant

ii) leaf area of all the leaves of that plant.

Leaf area ratio was calculated with the following formula:

$$\text{Leaf area ratio, } F = s / W$$

where

s =leaf area

W =dry wt. of shoot and root of a plant

Leaf diffusive resistance

Leaf resistance of the rehydrated plants was measured after the completion of 3 or 6 cycles with an automatic parameter (MK_3, Delta-T Devices) pump rate of the instrument was adjusted at the pump-down time 2 seconds. Then

RH-range was adjusted to 40–50%. Leaf diffusive resistance data were taken 3 times a day, i.e. at 09.00, 12.00, 17.00 hours. Data recorded at these different times were pooled to calculate the mean leaf diffusive resistance per day.

Stomatal index

Stomatal index is defined as the number of stomata per unit area. Stomatal index was determined by peeling the epidermis and counting the number of stomata in a specific area by an electric microscope and micrometers. The following biochemical parameters were determined:

Total soluble sugars and starch

Soluble sugars and starch were estimated following Malik and Srivastava (1985).

For the estimation of soluble sugars and starch, 0.1 g of well-ground dry material was homogenized and centrifuged at g × 2900. The residue was retained and was repeatedly washed with 80% ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars.

The residue was used for the determination of starch, for which 5 ml of distilled water and 6.5 ml of 52% perchloric acid were added to it. The extraction of starch with perchloric acid was carried out at 0 °C for 20 minutes, then centrifuged at g × 2900 and the extract was retained. With the residue the above step was repeated using fresh perchloric acid and the extract of this step was combined with the extract of the first step, and finally the volume of each of the sugar and starch extracts was made up to 100 ml by the addition of distilled water. The extracts were treated with the anthrone reagent and the absorbance was read at 625 nm.

$$\text{Total soluble sugars or starch (glucose units) mg/g of dry wt.} = \frac{\text{Conc. of glucose soln.}}{\text{absorbance of glucose}} \times \text{absorbance of sample} \times \text{dilution factor.}$$

Soluble protein estimation

Total soluble proteins were estimated as described by Lowry et al. (1951), so that 0.2 g of fresh material were taken, homogenized in 4 ml of sodium phosphate buffer solution (pH = 7.0), and filtered, then, 0.2 ml of each sample extract was treated with Folin phenol reagent and the optical densities were read at 620 nm using spectrophotometer (Hitachi U-2000). Total soluble proteins were estimated according to the following formula:

$$\text{Totalsoluble proteins (mg/g f.wt.)} = \frac{\text{Reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

Total free amino acids

Total free amino acids were determined following Hamilton and Van Slyke (1943).

For the estimation of total free amino acids, 1 ml of each sample extract was extracted for the soluble proteins and was treated with 1 ml of 10% pyridine and 1 ml of 2% ninhydrin solution. Then the optical densities of the treated samples were read at 570 nm using spectrophotometer (Hitachi U-2000). Total free amino acids were calculated as follows:

$$\text{Total free amino acids } (\mu g/g \text{ fresh weight}) = \frac{\text{Reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

Proline contents

Proline was estimated spectrophotometrically following the ninhydrin method described by Bates et al. (1973), and 0.5 g of fresh leaf material were used in this analysis.

Statistical analysis of data

The results of all the parameters were subjected to an analysis of variance and least significant difference (LSD) was calculated following Snedecor and Cochran (1980) for comparing means.

Results

The fresh and dry weight shoot data of six accessions of millet grown in 3 or 6 cycles of drought, and their analysis of variance summaries have been presented in Figs 1 and 2, and Table 1, respectively.

Increasing drought cycles had a significant ($p \leq 0.001$) adverse effect on fresh and dry biomass of shoots and roots. Increasing drought cycles differently affected the fresh and dry matter production of shoots of all accessions ($p \leq 0.001$). The shoot fresh weight of WIR-6-Ostistoe and Y-84 decreased consistently with the increase in intensity of drought stress. The fresh and dry matter of shoots of the remaining accessions reduced uniformly after both drought treatments. After 3 drought cycles, WIR-6-Ostistoe had significantly greater ($p \leq 0.05$) and KAT/PM-2 and Togu Bold Grain lower shoot fresh weight than the other accessions. By contrast, after 6 drought cycles, Selection II had significantly greater ($p \leq 0.05$) and Y-84 lower fresh weight of shoots compared with the other accessions. WIR-6-Ostistoe again had significantly greater ($p \leq 0.05$) shoot dry biomass than all the remaining accessions after the first drought treatment. The accessions did not differ significantly after 6 drought cycles.

Increasing intensity of drought stress consistently decreased the root fresh matter of WIR-6-Ostistoe and Kitui Local (Fig. 2). By contrast, root fresh weight of all the other accessions reduced uniformly after both drought treatments. Similarly, the reduction in root dry matter production in all 6 accessions was uniform after both drought treatments. After 3 cycles of drought, WIR-6-Ostistoe had significantly greater ($p \leq 0.05$) fresh and dry biomass than the other accessions. All the accessions did not differ for root fresh and dry matter after both drought treatments.

Analysis of variance of relative growth rate in Table 3 indicates that drought stress caused significant ($p \leq 0.001$) reductions in growth rate. The growth rates of KAT/PM-2 and Togu Bold Grain (Table 2) decreased consistently, and that of

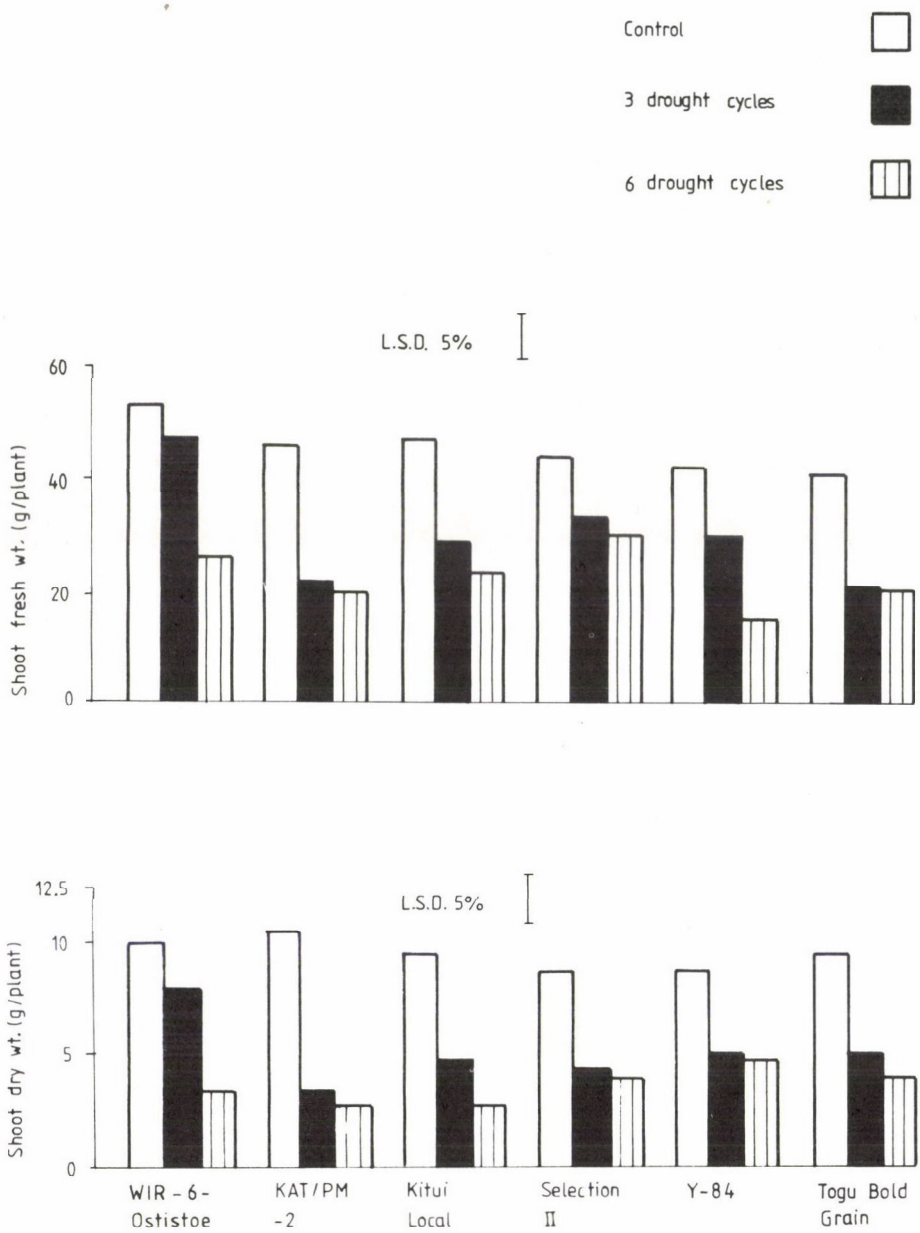

Fig. 1. Mean shoot fresh and dry weight. (g/plant) of 6 accessions of millet after the completion of 3 or 6 drought cycles

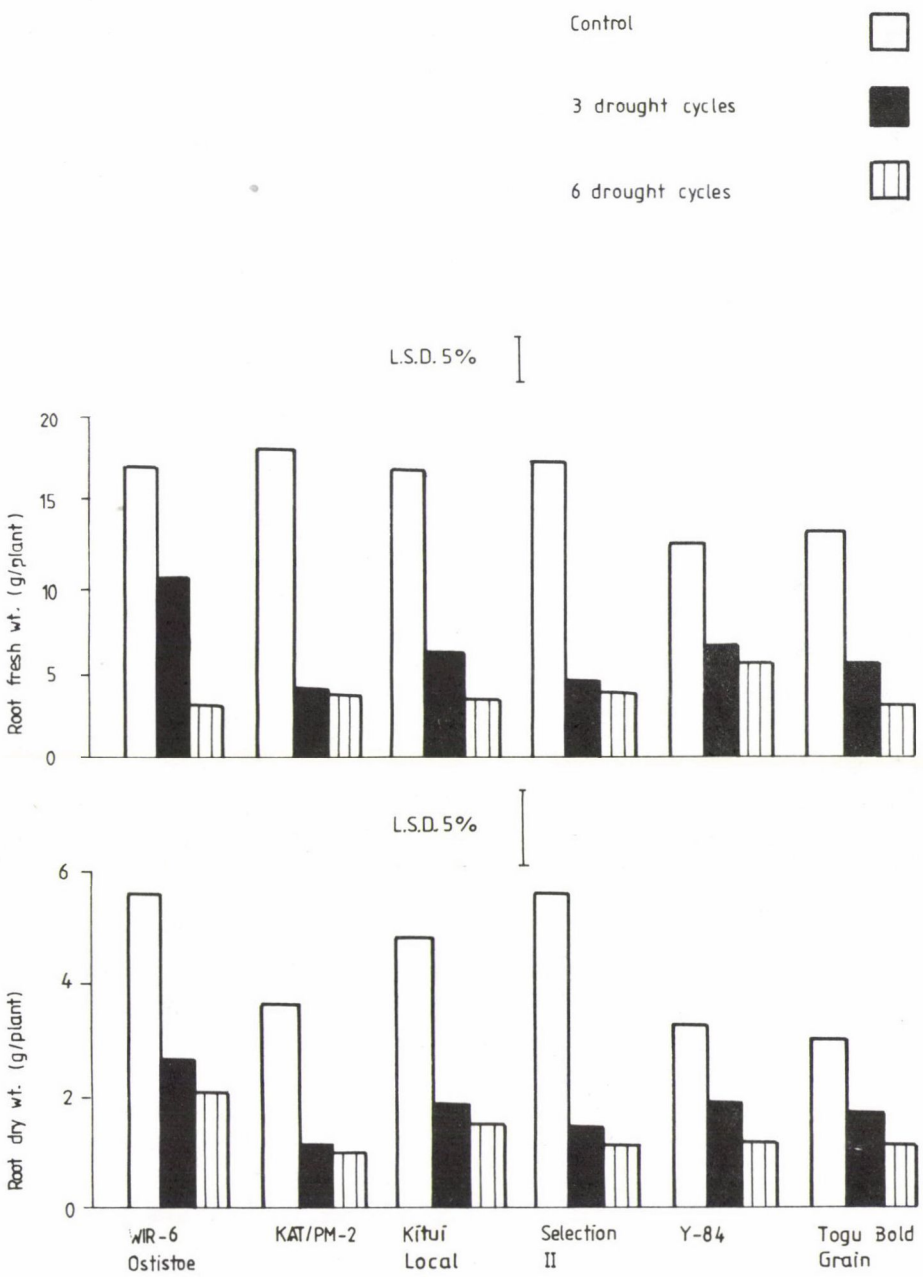

Fig. 2. Mean root fresh and dry weight. (g/plant) of 6 accessions of millet after the completion of 3 or 6 drought cycles

Table 1

Analysis of variance summaries (mean squeres) of shoot fresh wt., root fresh wt., shoot dry wt., and root dry wt. of 6 accessions of millet after the completion of 3 or 6 drought cycles

Source of variation	Degrees of freedom	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.
Blocks	3	52.1 NS	4.1 NS	5.2 NS	1.01 NS
Accessions (Acc.)	5	498.2***	36.3***	56.9***	6.19***
Treatments (T)	2	540.8***	48.9***	73.6***	7.21***
Acc. x T	10	220.9***	17.5***	24.5***	2.18**
Error	51	30.4	2.42	3.4	0.73

, * significant at 0.01 and 0.001 levels, respectively
NS, non-significant

Selection II increased significantly ($p \leq 0.05$), after the completion of 3 drought cycles, but it remained unchanged after the second drought treatment compared with the control. The relative growth rates of WIR-6-Ostistoe and Kitui Local decreased significantly ($p \leq 0.05$) only at the highest treatment, whereas that of Y-84 reduced uniformly after both treatments. Selection II was the highest in relative growth rate of all accessions at both drought treatments, whereas WIR-6-Ostistoe was the second highest after 3 drought cycles, and the lowest at the highest drought treatment. The remaining accessions did not differ significantly after experiencing both drought treatments.

The repeated drought cycles had no significant effect on succulence of all accessions but the response of all accessions to drought was different ($p \leq 0.001$) (Table 3). Kitui Local was the highest and KAT/PM-2 the lowest of all accessions after 3 drought cycles. By contrast, Selection II was the superior, and Togu Bold Grain inferior, to all accessions after 6 drought cycles.

Analysis of variance for data for epicuticular wax (Table 3) show that increasing drought stress intensity had significantly affected ($p \leq 0.01$) all accessions. The epicuticular wax content of WIR-6-Ostistoe, Selection II and Togu Bold Grain increased after 3 cycles, whereas it remained unchanged after the completion of 6 drought cycles. Although statistically insignificant, the epicuticular wax content of Kitui Local consistently increased after both drought treatments. The epicuticular wax content of KAT/PM-2, Kitui Local and Y-84 increased significantly after 6 drought cycles. Togu Bold Grain was the highest and Y-84 the lowest in wax deposition on leaves of all accessions after the completion of 3 drought cycles. By contrast, after the highest drought treatment, Kitui Local was the highest and KAT/PM-2 and Y-84 the second highest of all accessions in epicuticular wax content.

The imposition of repeated drought cycles had no significant effect on leaf relative water content on all accessions and the response of different accessions to drought stress was significantly different. WIR-6-Ostistoe was the lowest in relative water content of all accessions after both treatments. Kitui Local was the highest in relative water content of all accessions after 6 drought cycles.

The increasing intensity of drought differently affected the leaf area ratios of all 6 accessions (Table 4). Leaf area ratios of KAT/PM-2, Kitui Local and Y-84 increased after 3 drought cycles and decreased after the completion of 6 drought cycles. There was a consistent increase in leaf area ratio of Selection II at different drought cycles. By contrast, the leaf area ratio of WIR-6-Ostistoe decreased at both drought treatments. The leaf area ratio of Togu Bold Grain increased significantly at the highest drought treatments. Togu Bold Grain was the lowest and Y-84 the highest of all accessions after 3 drought cycles. Selection II was the highest, and Togu Bold Grain the second highest of all accessions after 6 drought cycles. At the same treatment, WIR-6-Ostistoe, KAT/PM-2 and Kitui Local had significantly lower leaf area ratios than did the other accessions.

Leaf diffusive resistance data (Table 5) show that increasing drought cycles had no significant effect on all 6 accessions and the response of all accessions to increasing drought intensity was also insignificant.

The increasing drought stress had no significant effect on stomatal indices of all 6 accessions, but the response of different accessions to drought intensity was significant ($p \leq 0.05$). After the completion of 3 drought cycles, there were no significant differences among all 6 accessions, whereas after 6 cycles of drought Kitui Local and Selection II had significantly greater ($p \leq 0.05$) and Y-84 and KAT/PM-2 lower stomatal indices than the other accessions.

The mean data for total soluble sugars of shoots (Table 6) show that the increasing drought intensity had a significant effect ($p \leq 0.05$) on all accessions. Soluble sugars of shoots of Selection II and Y-84 remained unaffected, whereas those of WIR-6-Ostistoe and Kitui Local decreased and of Togu Bold Grain increased after 6 drought cycles. There was a consistent increase in shoot soluble sugars of KAT/PM-2 at both treatments. After 3 drought cycles, KAT/PM-2 and Y-84 were the highest and WIR-6-Ostistoe the lowest of all the other accessions. After the second drought treatment, KAT/PM-2 was superior and WIR-6-Ostistoe inferior to all the other accessions in shoot soluble sugars.

Soluble sugars of roots (Table 7) show that there was no significant effect of repeated drought cycles on all the 6 accessions. KAT/PM-2 was the highest and Kitui Local the second highest of all accessions after 3 drought cycles. After the imposition of second drought treatment, Selection II was the highest and Y-84 the lowest of all accessions.

The starch of shoots (Table 7) was significantly ($p \leq 0.01$) affected by the repeated drought cycles, as it increased after both drought treatments in all accessions except Togu Bold Grain, in which it remained unaffected. WIR-6-Ostistoe, KAT/PM-2, Kitui Local and Y-84 had significantly greater ($p \leq 0.05$) shoot starch at the first drought treatment. After 3 drought cycles, WIR-6-Ostistoe and Kitui Local had significantly greater ($p \leq 0.05$) shoot starch than all the other accessions. By contrast, after the completion of 6 drought cycles, Selection II was the highest and Y-84 the lowest in shoot starch of all 6 accessions.

Data for root starch (Table 7) show that there was a significant ($p \leq 0.05$)

Table 2

Mean relative growth rate, succulence, epicuticular wax and relative water content of 6 accessions of millet after the completion of 3 or 6 drought cycles

Accessions	Relative growth rate ($g/g\ days^{-1}$)			Succulence ($g\ of\ water/m^2$)			Epicuticular wax ($\mu g/cm^2$)			Relative water content (%)		
	Drought cycles											
	0 (control)	3	6	0 (control)	3	6	0 (control)	3	6	0 (control)	3	6
WIR-6-Ostistoe	68.53 ab x	62.92 ad x	10.00 a y	127.87 a x	160.40 ad y	159.55 ac y	106.17 a y	158.20 a y	96.36 a x	64.57 ac x	58.88 a x	67.24 a x
AT/PM-2	64.10 abc x	44.26 bd y	25.27 bd z	129.74 a x	98.40 b y	169.23 ac x	144.88 b x	112.02 bc x	197.62 b y	64.47 ac x	69.84 abc x	75.66 ab x
Kitui Local	52.09 c x	49.34 bd x	20.97 abd y	203.50 b xy	218.38 c x	181.68 ab y	102.24 a x	134.61 ab x	419.20 c y	76.67 ab xy	66.93 ab x	87.86 b y
Selection II	78.02 b x	107.98 c y	71.05 c x	279.60 c x	172.07 d y	204.20 b z	104.72 a x	149.46 a y	105.46 a x	69.86 ab x	79.00 bc x	74.78 ab x
Y-84	77.05 b x	41.66 bd y	34.53 d y	169.10 d x	141.78 a y	183.61 ab x	107.33 a x	83.88 c x	163.94 b y	77.77 b x	79.78 c x	73.76 a x
Togu Bold Grain	76.54 b x	50.31 d y	25.38 d z	135.15 a x	163.48 ad x	145.38 c x	109.04 a x	256.03 d y	121.27 a x	52.57 c x	75.16 bc y	70.97 a y
	$LSD_{5\%} = 14.9$			$LSD_{5\%} = 28.9$			$LSD_{5\%} = 36.4$			$LSD_{5\%} = 12.3$		

Means with the same letters in each column and each row do not differ significantly

Table 3

Analysis of variance summaries (mean square) of relative growth rate, succulence, epicuticular wax and relative water content of 6 accessions of millet after the completion of 3 or 6 drought cycles

Source of variation	df	Relative growth rate	Succulence	Epicuticular wax	Relative water content
Blocks	3	156.9 NS	582.7 NS	907.6 NS	58.7 NS
Accessions (Acc.)	5	1291.5***	5163.6***	10093.8***	966.6***
Treatments (T)	2	1804.8***	818.1 NS	3393.9**	137.5 NS
Acc. x T	10	803.1***	2511.8***	3454.4***	301.6**
Error	51	111.0	417.6	662.5	75.6

, * significant at 0.01 and 0.001 levels, respectively
NS, non-significant

effect of drought stress on all 6 accessions. There was a uniform decrease in root starch in Y-84 and Togu Bold Grain, whereas in Selection II there was a uniform increase in the root starch. The root starch of KAT/PM-2 and WIR-6-Ostistoe remained unaffected at both treatments. The root starch of Kitui Local increased at the highest drought treatment. Selection II was the highest, WIR-6-Ostistoe and Togu Bold Grain the lowest of all accessions after 3 drought cycles. By contrast, Y-84 and Togu Bold Grain were the lowest in root starch of all accessions after the completion of 6 drought cycles.

Soluble proteins (Table 8) of fresh leaf material remained the same in WIR-6-Ostistoe, Selection II and Togu Bold Grein. The other 3 accessions had a consistent decrease in soluble proteins after the repeated cycles of drought. Togu Bold Grain was the highest and Kitui Local the lowest in soluble proteins of all accessions after 3 drought cycles. After 6 cycles of drought, WIR-6-Ostistoe and Togu Bold Grain had significantly greater ($p \leq 0.05$) soluble proteins than the other accessions.

Total free amino acids of WIR-6-Ostistoe, KAT/PM-2 and Kitui Local decreased at the first treatment (Table 8) but increased at the highest drought treatment, whereas those of Y-84 remained unchanged after both drought treatments. By contrast, free amino acids of Selection II and Togu Bold Grain increased only at the first drought treatment. Selection II and Togu Bold Grain were the highest in free amino acids of all accessions after 3 drought cycles. By contrast, WIR-6-Ostistoe was the highest after the highest drought treatment.

The leaf proline content (Table 9) of Selection II, WIR-6-Ostistoe and Kitui Local increased consistently after both drought treatments and that of KAT/PM-2 remained unaffected. By contrast, the proline content of Y-84 and Togu Bold Grain increased after 3 and 6 drought cycles respectively. Togu Bold Grain was the lowest in proline content at 3 drought cycles, and WIR-6-Ostistoe, and Kitui Local at 6 drought cycles of all accessions. Selection II was the highest in proline accumulation after 6 drought cycles.

Table 4

Mean leaf area ratio, leaf diffusive resistance and stomatal index of 6 accessions of millet after the completion of 3 or 6 drought cycles

Accessions	Leaf area ratios			Leaf diffusive resistance (s/cm)			Stomatal index		
	Drought cycles								
	0 (control)	3	6	0 (control)	3	6	0 (control)	3	6
WIR-6-Ostistor	54.42 a x	49.55 ac x	13.75 a y	9.94	6.94	7.05	354.0 a x	271.25 a y	306.50 a z
KAT/PM-2	34.85 bc x	55.90 ab y	13.80 a z	8.30	7.37	7.01	271.5 b x	247.75 a x	271.25 b x
Kitui Local	37.32 bc x	56.70 ab y	18.53 a z	5.35	5.63	4.17	295.0 bc x	271.25 a x	342.25 c y
Selection II	47.07 ab x	58.77 ab x	108.90 b y	6.71	7.12	4.98	306.7 c x	247.75 a x	342.00 c x
Y-84	33.80 c x	62.75 b y	32.75 c x	5.0	5.75	5.09	236.0 d x	259.50 a x	247.75 b x
Togu Bold Grain	41.55 abc x	37.02 c x	84.47 d y	5.01	5.63	9.20	271.25 b x	247.75 a x	330.55 ac y
	$LSD_{5\%} = 12.8$			$LSD_{5\%} = NS$			$LSD_{5\%} = 26.8$		

Means with the same letters in each column and each row do not differ significantly
NS, non-significant

Table 5

Analysis of variance summaries (mean square) of leaf area ratios, leaf diffusive resistance and stomatal index of 6 accessions of millet after the completion of 3 or 6 drought cycles

Source of variation	df	Leaf area ratio	Leaf diffusive resistance	Stomatal index
Blocks	3	107.9 NS	1.88 NS	214.6 NS
Accessions (Acc.)	5	698.7 ***	3.09 NS	2763.4 ***
Treatments (T)	2	142.0 NS	3.01 NS	493.6 NS
Acc. x T	10	512.7 ***	2.98 NS	1165.5 *
Error	51	81.92	1.66	359.2

*, *** significant at 0.05 and 0.001 levels, respectively
NS, non-significant

Discussion

One of the major purposes of the present study was to draw parallels between the degrees of drought tolerance and salt tolerance of 6 accessions of millet which differ in their responses to salt, because it is now well evident that drought and salinity share an osmotic effect on plant growth (Maas and Nieman, 1978; Greenway and Munns, 1980; Rains, 1981; Wyn Jones, 1981). In a previous study it was found that, of the 6 accessions used in this study, WIR-6-Ostistoe, KAT/PM-2, Kitui Local and Selection II were relatively salt-tolerant and Y-84 and Togu Bold Grain salt-sensitive (Ashraf and McNeilly, 1991). However, the results for plant biomass of the 6 accessions, after imposition of repeated cycles of drought, clearly show that different accessions had different responses to drought. For instance, only one salt-tolerant accession WIR-6-Ostistoe showed a positive correlation between salt tolerance and drought tolerance, as it was superior to all accessions in biomass production after the completion of 3 or 6 drought cycles. By contrast, the responses of all the remaining accessions to drought were almost uniform in relation to biomass production, showing a negative correlation between their salt tolerance and drought tolerance.

The important role of stomatal diffusive resistance and stomatal index in influencing gas exchange through the regulation of water vapours and CO_2 diffusion has been advocated (Radin and Ackerson, 1981; Baker, 1984), since these 2 factors are mainly responsible for controlling optimum levels of different water relation parameters (Hsiao, 1973; Hanson, 1984; Parsons, 1982; Morgan, 1984). The insignificant effect of repeated drought cycles on stomatal diffusive resistance can be related to the inconsistent data for stomatal indices and relative water content of all the accessions. These results are in contrast to the argument of Sinclair and Ludlow (1985), that relative water content is a stable variable and has a close relationship with stomatal diffusive resistance. But the results for stomatal diffusive

Table 6

Mean soluble sugars and starch of shoots and roots of 6 accessions of millet after the completion of 3 or 6 drought cycles.

Accessions	Soluble sugars (mg/g D.wt.)						Starch (mg/g D.wt. glucose units)					
	Shoots			*Roots*			*Shoots*			*Roots*		
	Drought cycles											
	0(control)	3	6	0(control)	3	6	0(control)	3	6	0(control)	3	6
WIR-6-Ostistoe	40.19 a x	36.09 ac x	27.13 a y	23.92 a x	21.16 a x	29.45 ad x	46.87 a x	80.83 ab y	62.47 a z	65.28 ab x	52.98 ac y	67.02 a x
KAT/PM-2	38.40 a x	56.45 b y	73.92 b z	18.71 a x	46.92 b y	30.56 ad z	46.03 a x	72.32 ac y	67.77 ab y	65.69 ab x	63.00 bc x	70.18 a x
Kitui Local	41.93 ab x	50.32 bcd y	39.61 c x	36.49 b x	34.08 c xy	26.11 ac y	54.72 ab xz	88.16 b y	62.74 ab z	64.08 ab x	61.76 bc x	71.03 a y
Selection II	43.58 ab x	42.42 cd x	50.48 d x	21.69 a x	23.34 a x	106.41 b y	61.31 bc x	69.33 c xy	73.43 b y	58.33 a x	69.69 b y	69.73 a y
Y-84	50.26 b x	55.25 b x	54.23 d x	24.50 a x	28.20 ac x	18.11 c y	50.70 ab x	68.40 c y	51.85 c x	68.80 b x	63.32 bc x	57.21 b y
Togu Bold Grain	40.41 a x	45.85 d x	54.54 d y	21.83 a x	22.90 a x	37.25 d y	65.68 c x	63.70 c x	69.28 ab x	67.28 ab x	57.12 c y	53.56 b y
	$LSD_{5\%} = 8.4$			$LSD_{5\%} = 9.2$			$LSD_{5\%} = 10.7$			$LSD_{5\%} = 9.6$		

Means with the same letters in each column and each row do not differ significantly

Table 7

Analysis of variance summaries (mean squares) of soluble sugars and starch of shoots and roots of 6 accessions of millet after the completion of 3 or 6 drought cycles

Source of variation	df	Soluble sugars of shoots	Soluble sugars of roots	Starch of shoots	Starch of roots
Blocks	3	66.2 NS	76.2 NS	97.3 NS	91.2 NS
Accessions (Acc.)	5	794.6***	961.7***	914.6***	872.6***
Treatments (T)	2	113.5*	89.6 NS	279.9**	176.7*
Acc. x T	10	256.5***	306.2***	471.9***	366.7***
Error	51	35.3	42.6	57.3	45.2

*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively
NS, non-significant

resistance can be related to those of Shimshi and Ephrat (1975) who found a negative relationship between stomatal diffusive resistance and plant dry weight in wheat. The inconsistent results for stomatal indices of drought-tolerant and drought-sensitive accessions support the findings of Gummuluru et al. (1989), and Clarke and Mc Caig (1982) who did not find any difference in stomatal indices of drought-tolerant and drought-sensitive wheat lines.

Data for epicuticular wax content show that there was no consistent relationship between the degree of drought tolerance and deposition of wax on their leaf surfaces. For instance, Selection II was as good as other relatively poor accessions at varying drought treatments, but it was the highest in deposition of wax on its leaf surface of all accessions at the highest drought treatment. By contrast, at the same treatment the drought-tolerant accession WIR-6-Ostistoe was the lowest in epicuticular wax content, thus showing a negative relationship between drought tolerance and epicuticular wax desposition. These results do not conform with those of Johnson et al. (1983) and Jordan et al. (1984) who found a positive correlation between drought tolerance and deposition of wax on leaf surface of wheat and sorghum, respectively.

The accumulation of considerable amounts of different types of organic osmotica in plants subject to water deficit conditions is a well-known phenomenon (Hsiao, 1973; Turner, 1979, 1986; Turner and Jones, 1980; Thomas, 1987; Ashraf and Mehmood, 1990). It is widely accepted that plants tolerant to severe drought conditions usually accumulate more organic osmotica such as soluble sugars, organic acids, free amino acids, proline and other such compounds than the drought-sensitive plants do (Levitt, 1972; Hsiao, 1973; Hanson, 1984). The drought-tolerant accession WIR-6-Ostistoe had significantly greater total free amino acids at the highest drought treatment but had a moderate amount of proline and lower amount of soluble sugars at both drought treatments compared with the relatively drought-sensitive accessions. Thus free amino acids could be used as selection criterion for drought-tolerance in millet. In view of these data it is not possible to use soluble

Table 8

Mean soluble proteins, total free amino acids and proline content of 6 accessions of millet after the completion of 3 or 6 drough cycles.

Accessions	Soluble proteins (mg/g Fresh wt.)			Free amino acids (µg/g Fresh wt.)			Proline (µg/g Fresh wt.)		
	Drought cycles								
	0 (control)	3	6	0 (control)	3	6	0 (control)	3	6
WIR-6-Ostistoe	0.43 ac	0.45 abc	0.50 a	56.46 a	51.28 a	297.56 a	1.37 a	4.17 ac	3.46 a
	x	x	x	x	x	y	x	y	y
KAT/PM-2	0.58 abc	0.34 ab	0.16 b	82.42 a	32.15 a	96.71 bc	6.55 b	5.95 ab	5.88 b
	x	y	y	x	y	x	x	x	x
Kitui Local	0.78 b	0.3 a	0.16 b	73.98 a	29.79 a	116.45 b	1.39 a	6.48 b	3.70 a
	x	y	y	xy	x	y	x	y	z
Selection II	0.39 c	0.43 ab	0.23 b	50.12 a	228.63 b	49.04 cd	2.04 a	6.93 b	8.03 c
	x	x	x	x	y	x	x	y	y
Y-84	0.56 bc	0.58 bc	0.21 b	44.13 a	45.68 a	36.95 d	3.76 a	6.23 b	4.02 ab
	x	x	y	x	x	x	x	y	x
Togu Bold Grain	0.47 c	0.68 c	0.50 a	25.47 a	288.83 c	22.02 d	1.55 a	2.99 c	5.12 b
	x	x	x	x	y	x	x	x	y
		$LSD_{5\%}$=0.24			$LSD_{5\%}$=48.4			$LSD_{5\%}$=2.1	

Means with the same letters in each column and each row do not differ significantly

Table 9

Analysis of variance summaries (mean squares) of proteins, free amino acids and proline of 6 accessions of millet after the completion of 3 or 6 drought cycles

Source of variation	df	Proteins	Free amino acids	Proline
Blocks	3	0.38 NS	926.3 NS	1.4 NS
Accessions (Acc.)	5	0.384***	28963.4***	40.1***
Treatments (T)	2	0.153***	13626.5***	18.2***
Acc. x T	10	0.179	10841.4***	11.5***
Error	51	0.029	1171.3	2.2

, * significant at 0.01 and 0.001 levels, respectively
NS, non-significant

sugars as selection criterion since the relatively drought sensitive KAT/PM-2 had significantly greater soluble sugars than the relatively tolerant WIR-6-Ostistoe.

It is interesting to note that all 4 originally salt-tolerant accessions had significantly higher free amino acids than did the salt-sensitive accessions at the highest drought stress treatment. These results conform with those of another study (Ashraf and Nusrat, unpublished data) in which responses of these accessions to salt stress were examined and a positive correlation was found between free amino acid accumulation and degree of salt tolerance. In view of the data for soluble sugars it is impossible to use them as selection criteria since the relatively drought-sensitive KAT/PM-2 had significantly greater soluble sugars than did the relatively tolerant WIR-6-Ostistoe.

The results for leaf soluble proteins show that increasing drought stress intensity caused a significant reduction in soluble proteins of KAT/PM-2, Kitui Local and Y-84, whereas the protein content of the remaining accessions remained unchanged. These results support the findings of Vyas et al. (1985), who also found a significant reduction in protein content with increasing drought stress. However, these results disagree with those of Wadleigh and Richards (1951) and Eck and Musick (1979) who reported that increasing drought stress intensity caused an increase in protein content in different crops.

The crucial role of proline in regulating turgor in plants subject to drought and saline conditions has long been recognized by many investigators (Greenway and Munns, 1980; Wyn Jones, 1981; Rains, 1981; Turner, 1981). Although leaf proline content of all accessions, except KAT/PM-2, increased with the increase in the intensity of drought, the maximum increase was observed in Selection II, which was lower in performance than WIR-6-Ostistoe. The negative relationship between proline content and drought tolerance of the different accessions conforms with the early findings of Moftah and Michel (1987) and Ashraf (1989) who found negative correlations between proline content and salt tolerance of soybean and blackgram, respectively.

Altogether, it can be concluded that, with some exceptions, drought tolerance and salt tolerance of the small number of accessions examined here are two independent phenomena. Each accession used its own specific mechanism to resist severe drought conditions. Therefore, from this study it is difficult to choose a single parameter as the selection criterion in breeding for drought-tolerant pearl millet, as has been suggested for the case of salt tolerance (Ashraf and McNeilly, 1987). The variation in whole plant performance to drought would thus seem to provide the best means of selection for enhanced drought tolerance.

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QUALITY CHANGES OF APPLES DURING STORAGE
Part 1. Pectic constituents and textural changes

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The effects of harvesting time on the quantitative and qualitative changes in the composition of pectic substances of Idared and Redspur apple varieties grown in Hungary were investigated during storage.

The Idared variety has 15% higher firmness than Redspur. A decrease of firmness was observed during the first six months, but there was an increase in the seventh month. The apples picked at an optimum date had the most stable firmness during storage.

The inverse connection between the firmness and dry matter content showed that the most important factors of the firmness are the cell wall structure and osmotic pressure of tissue osmotics. The decrease of the dry matter content shows the high rate of water formation by carbohydrate metabolism. A softening of apple tissue was accompanied by a decline in the alcohol insoluble solid content. The deterioration of stored apples verified that the middle date was the optimum in regard to more long-term storage.

Keywords: pectin, apple storage, quality changes

Introduction

Apple *(Malus domestica)* fruit is know as the primary popular fruit in Europe and in many other regions worldwide. Hungary is a big producer of apples (1 million tons/year) used either as fresh fruit or processed to fruit juices or concentrates. Independent of the mode of use, shorter or longer storage of the fruits is needed. The effect of the time of harvesting on the apple storage quality is of great interest.

The main parts of apple cell walls are the micro-fibrillar structures composed from cellulose and the matrix containing hemicellulose, pectic substances and some minor components such as glycoproteins and minerals. The softening of fruits during ripening is primarily connected with changes of pectic substances forming an "intercellular cement" in the fruit tissue. The changes are complex; nevertheless, the main processes are the hydrolysis of protopectin, the decrease of degree of esterification and, in the last storage period, the decrease of the total amount of pectic substances caused by an enzyme system (Bartley, 1976).

The simplest way to follow the changes of pectic substances is to measure the amount of soluble pectic substances (Testoni and Zerbini, 1989). It can be generally

stated that the unriped fruits contain small amounts of soluble pectin, but this increases greatly with ripening (Huber, 1984). Nevertheless, soluble pectin with carboxyl groups can be rendered insolube by crosslinking adjacent polymers with divalent cations, such as calcium or magnesium (Eskin, 1979).

It was also observed that during ripening an increase of activity of hydrolytic enzymes, degrading pectic substances, appeared (Knee, 1975; Bartley, 1976). As a consequence of the degradation of pectic substances, changes occur in the cell wall structure and a softening of the fruits can be observed (Stow, 1988). This softening continues also during storage periods (Morris and Morris, 1989). During ripening, structural changes may be observed in the middle lamella and primary cell wall. It was also found that the permeability of cell membranes increases during storage. These changes are connected mainly with the lipids of membranes.

In the framework of this paper we will discuss only the results of our research work related to the changes of pectic substances texture, and firmness in connection with the dry matter content in two apple varieties (Idared and Redspur) grown in Hungary.

We tried to discuss the effect of harvesting date (to determine the optimum value regarding storage changes, deterioration), storage time and variety on the firmness of flesh tissue, texture and determining composition factors such as pectin composition, dry matter content, and spotting, rotting and browning.

Materials and methods

An a*b*c factorial design was made in two seasons. The factors and their levels were as follows: variety (2 levels), picking date (3 levels), storage period (4 levels). Apple fruits of cultivars were obtained from the Ráckeve Experimental Farm, Hungary. The fruits of Idared variety were picked in two maturity degree on October 7 and 23, and that of Redspur variety were done on September 29 and October 7 in 1988, whereas in 1989 three maturity degrees of both varieties picked on September 22, 30 and October 12 were tested.

All the fruits were stored in one side controlled atmosphere 18% O_2 and 2.0–2.5% CO_2 at 2.5–3.0 °C and 90–92% RH. The samples of fruits were stored out after 5, 6 and 7 months in both seasons.

The total soluble solid (TSS) was determined by refractometer, expressed as a percent of the fresh weight at room temperature (23 °C).

The firmness was determined by penetrometer type FT-327 (N/cm²) on the equator opposite sides of the fruit after removing 2 cm² peel disks.

The dry matter content was determined by drying 100 g of fresh fruit at 60 °C for 24 hours (expressed in %).

The alcohol-insoluble solids (AIS) were prepared according to the Ruth and Lavee method (1971). Fifty grams of cutical tissue from three randomly chosen apple fruits were blended with 200 cm^3 boiling acetone for 5 min and boiled for an additional 20 mins. After filtering on a Büchner funnel, the precipitate was washed three times with 70% ethanol and twice with acetone. The eluates were discarded and the colorless powder obtained in the Büchner funnel was dried, weighted, ground, and calculated as a percentage.

Fractionation. The AIS preparations were separated into three types of pectic substances by successive extractions with distilled water (WP), 0.75% ammonium oxalate (AOP), and 0.1 N sodium hydroxide (SP) according to Roo and Bruemmer (1981). Each type was analyzed by the colorimetric reaction between carbasole and the anhydrogalacturonic acid of the pectin (McCamb and McCready, 1952).

Damage values were determined by sensorical evaluation, which counted the rotted, browned, and spotted ones of a hundred apples.

The determinations were conducted in triplicate on each sample. A statistical analysis was carried out, according to Snedecor and Cochran (1967), using a computer program.

Result and discussion

The characterization of new cultivars in Hungary is of great interest. These results would be the basis of further investigations of storage ability and characteristics of some popular varieties in EC. The changes of the firmness of investigated apples are summarized in Fig. 1a–d and in Table 1. As seen in Fig. 1a and Fig. 1b, Idared variety had generally 15% higher firmness than Redspur variety. During the storage a decrease of firmness was observed in both varieties. The increase was found after six months in the second season only. The changes in the firmness may be due to the movement of calcium in cell walls. This process may assist solubilization of pectin during ripening and depolymerization of cell wall polymers (Huber, 1984). A comparison of average values of all samples harvested at different times showed the same tendency (Fig. 1c and 1d).

Table 1

Averages of firmness and dry matter content as a function of variety, harvesting date storage period. Results of 4 factorial designs

	Firmness (N/cm^2)		Dry matter (%)	
	87/88	88/89	87/88	88/89
Variety				
Idared	7.31	7.42	12.35	13.67
Redspur	6.36	6.67	13.62	15.03
Harvesting date				
I	–	6.99	–	14.06
II	6.24	6.99	13.46	15.97
III	6.43	7.14	12.52	13.03
Storage period				
0 month	8.00	8.41	13.81	16.09
5 month	6.31	6.56	13.10	15.31
6 month	6.20	6.33	12.78	14.58
7 month	6.84	6.87	12.26	11.42

It seems that the harvest date has no direct effect, but the interaction between the storage period and harvest date showed a significant difference. It means that the apples picked at the optimum date had the lowest firmness before storage and the highest firmness after long-term (7 months) storage. This fact shows that this picking date was the best in regard to firmness after storage.

Concerning the dry matter content (DMC), it can be stated that the Idared variety had lower DMC (12.4%, 13.6%) than Redspur (13.7%, 15.0%) in the two seasons, respectively (Fig. 2a, b). There is an inverse connection between the firmness and DMC. This shows that the cause of firmness changes is not the change of DMC, but the structure of the cell wall and the osmotic pressure, which is antagonistic to the DMC. As for the maturity progress in both seasons, the dry matter

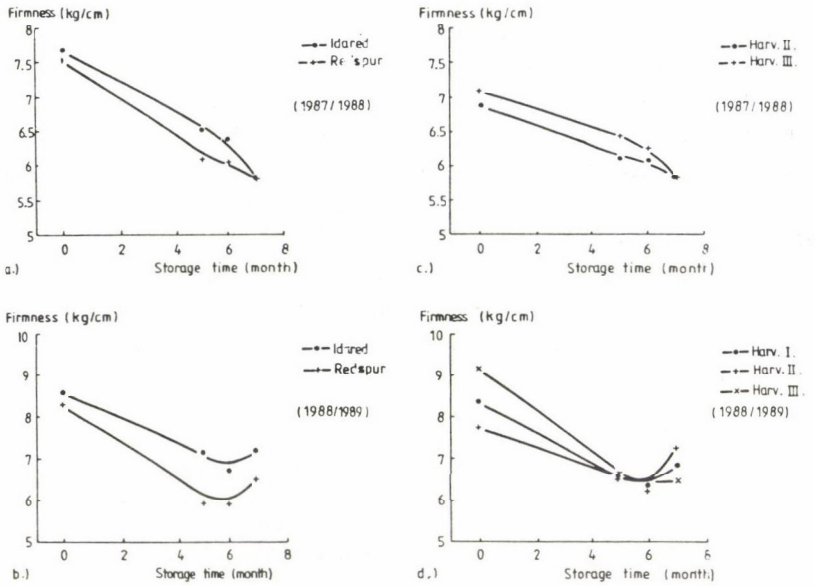

Fig. 1. Changes in firmness during storage in two seasons

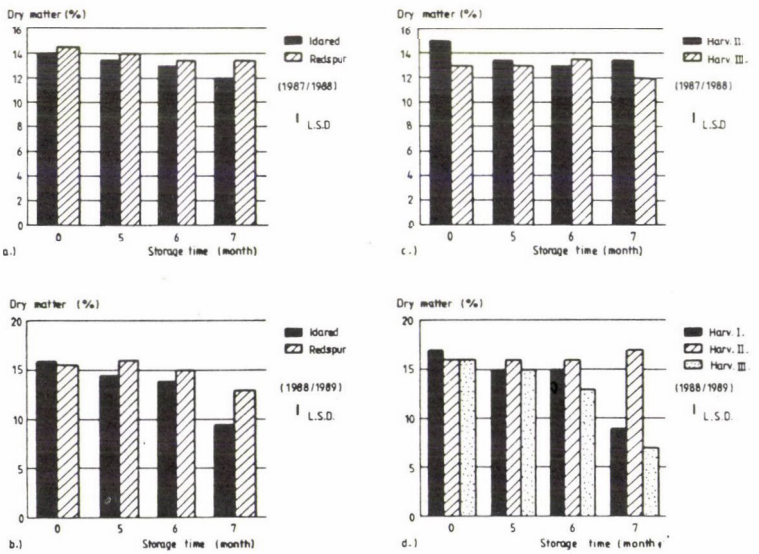

Fig. 2. Changes in dry matter content during storage in two seasons

content showed a decrease from 13.5% to 12.5% and from 14.0% to 13.0%. However, there is a relatively high level of DMC at the optimum picking date in 1989.

After 7 months of storage the dry matter content decreased from 13.8% to 12.3% and from 16.1% to 11.4% in the two seasons, respectively. The changes of individual varieties are shown in Fig. 2a–d.

During the storage there are two processes that mainly effect the water content. The first of them is the metabolism which increases the water quantity because of the oxidation of carbohydrates; second is the mass reduction by water loss. This change shows the higher rate of water formation by carbohydrate metabolism.

The varieties and harvest dates as well as the storage period caused significant effects on the dry mattes content in both seasons.

The two varieties differed in their AIS content before storage, and this difference changes just the opposite of that during storage. This is due to the fast decrease in Redspur variety. Most remarkable is the connection between the firmness and AIS. The changes of AIS are similar to that in firmness in both seasons. The slight decrease in the first season and the decrease-increase in the second one shows the close connection between these two parameters. In addition to this, the date of picking affected the WP fraction but not the others. The WP content at optimum picking date is 30–40% higher than that of apples harvested 10 days later.

The AIS and WP are lower, but AOP is higher, and the SP had a discontinuous trend by the advance of maturity. The AIS and WP content decreased as ripeness advanced. On the other hand, there was a positive trend for the AOP with the ripening progress. The AOP was formed from the WP. These data agree with the results of Shewfelt et al. (1971) observed in peaches and those of Robertson and Swinburne (1981) measured in kiwi fruit.

Softening of apple flesh, as indicated by the decline in resistance to penetrometer force, was accompanied by a decline in the AIS and increase in WP recovered from the fruit. The decrease in AIS also reflects the conversion of pectic substances to alcohol-soluble compounds, whereas AIS declined from 1.78% to 1.66% andfrom 2.95 to 2.39% after 7 months of storage in the two seasons, respectively (Table 2).

The results suggest that there is a significant inverse relationship between the firmness of the apple fruit and WP. Such a relationship was suggested by Robertson and Swinburne (1981) and Bartley (1976), and confirms the widely accepted view that during the ripening of fruits, enzymes dissolve protopectin to form a high-methoxyl, water-soluble pectin with an associated decrease in firmness.

The relationship between firmness and pectin content was also calculated. It was found that the relationship between firmness and AIS, WP can be quantified with a 0.59, –0.37 correlation coefficient. Whereas the relationships between firmness and other pectin fractions are not strong, as in the case of AOP, there is no such a relationship as in the case of SP.

The data also show that a correlation exists between the firmness and the dry matter content. A stepwise variable selection was calculated for the pectic constituents and the dry matter content versus the firmness. The relationships may be explained by the following equation (F: firmness):

F=5.37+0.94*AIS–0.16*WP c^2=0.435
F=11.07–0.95*DMC c^2=0.333

There is a good correlation between the predicted values of the firmness and the measured AIS and WP. In addition to the investigation of firmness and pectic substances the degree of deterioration (scald, browning, other diseases) has also been studied (Table 3). The deterioration of stored apples verified that the second date was the optimum in regard to longer-term storage. The differences are well seen after 7 months, and the damage (sum of browning and rotting) is significantly lower at harvest date II at than that at earlier and later dates. Although the effect of harvest date on the firmness after storage is small, the degree of deterioration is highly dependent on picking time.

Table 2

Averages of the pectic constituents as a function of variety, harvesting date, storage period

	Pectic constituents (mg/g fresh weight)			
	AIS	WP	AOP	SP
1987/88				
Variety				
Idared	1.67	4.23	2.86	2.20
Redspur	1.60	3.78	1.70	2.98
Harvesting date				
II	1.60	4.62	2.22	1.94
III	1.67	3.39	2.34	3.24
Storage period				
0 month	1.78	3.51	2.07	2.87
5 month	1.59	3.69	2.33	2.82
6 month	1.56	3.94	2.57	2.43
7 month	1.60	4.89	2.12	2.24
1988/89				
Variety				
Idared	2.62	3.20	2.30	3.13
Redspur	2.36	4.28	1.74	4.26
Harvesting date				
I	2.58	3.67	1.60	4.00
II	2.53	4.64	2.17	3.78
III	2.37	2.91	2.29	3.32
Storage period				
0 month	2.95	2.53	1.65	4.39
5 month	2.38	3.86	2.45	3.68
6 month	2.25	4.45	2.00	2.99
7 month	2.39	4.13	1.98	3.72

Table 3

Deterioration of apples during storage

Samples		Spotting % s	Rotting % r	Browning % b	Damage % r+b
Idared harvest I	5 month	0.2	0.4	5.6	6.0
	6 month	0.0	1.6	6.5	8.1
	7 month	0.0	5.2	10.0	15.2
Idared harvest II	5 month	0.0	3.3	0.9	4.2
	6 month	1.6	1.9	1.9	3.8
	7 month	0.2	5.4	0.2	5.6
Idared harvest III	5 month	0.0	3.9	0.8	4.7
	6 month	15.9	5.9	2.3	8.2
	7 month	27.9	6.9	3.5	10.4
Redspur harvest I	5 month	3.4	0.8	0.2	1.0
	6 month	8.5	0.8	0.0	0.8
	7 month	25.2	4.2	0.0	4.2
Redspur harvest II	5 month	0.0	0.4	0.2	0.6
	6 month	0.0	0.8	0.0	0.8
	7 month	0.0	1.9	0.0	1.9
Redspur harvest III	5 month	0.0	1.4	0.0	1.4
	6 month	0.0	5.2	0.0	5.2
	7 month	0.0	6.7	0.0	6.7

Conclusions

It could be concluded that, under the experimental conditions of this study, with apple varieties which substantially differ in TSS, dry matter contents undergo the same general patterns of firmness and pectic substance changes as ripeness advances. Increased evidence is presented for the close relationship among the decreasing firmness, decreasing level of AIS and increasing level of WP.

In addition to these general conclusions, the higher firmness in the second season is caused by higher dry matter content, higher AIS content and lower WP, but the other pectic fractions (AOP, SP) have no effect on the changes of firmness. The higher AIS caused the harder fruit flesh in the Idared variety, compared to Redspur. The lower dry matter is not connected with the higher flesh firmness of the Idared. This change explains the better quality of Idared variety after storage. The differences of firmness at harvest disappeared during 6 months of storage. This observation suggests the picking ripeness has an effect on firmness, AIS, WP, DMC and an expressed effect on the deterioration after storage.

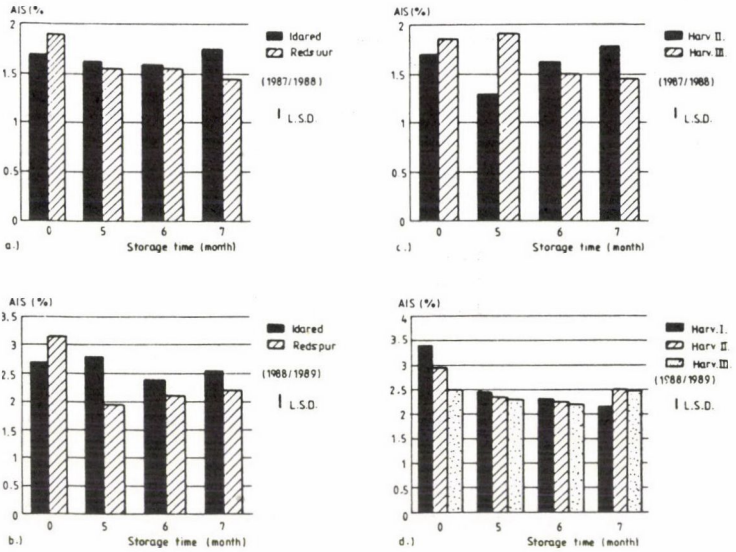

Fig. 3. Changes in AIS (pectin) content during storage in two seasons

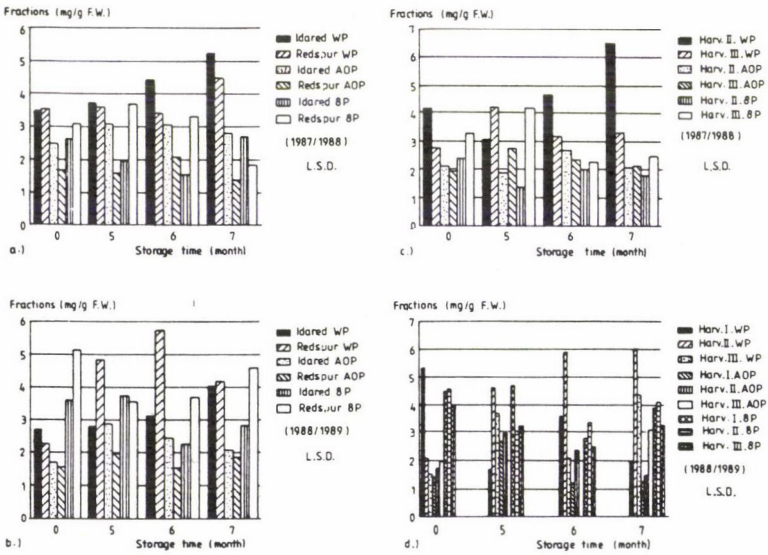

Fig. 4. Changes in rate of pectin fractions during storage in two seasons

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Plant cultivation

EFFECT OF N-FERTILIZATION ON N-CONTENT IN VEGETATIVE PARTS OF WHEAT DURING GRAIN DEVELOPMENT

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In a harvesting experiment changes in the nitrogen content at various N-fertilizer levels (0–200 kg/ha) were followed in the vegetative plant parts most important from the point of view of N-translocation. In the period between milky- and full ripening a considerable proportion of the N-assimilates of flag-leaf blade, flag-leaf sheath and uppermost internode was depleted. N-fertilization increased significantly the nitrogen content in these plant parts only up to the rate of 120 kg/ha N, and N-mobilization was highest also with this quantity of nitrogen. The decrease in the nitrogen content of the vegetative parts was 65–71% of the value obtained in milky ripening. The role of the uppermost internode in the N-metabolism is supposedly just as important as that of the flag-leaf blade in the grain development period examined. Namely, the decrease in its N-content generally exceeded that of the flag-leaf blade. The correlations between the nitrogen contents of the vegetative parts and that of the grain ranged from 0.56* to 0.86***.

Keywords: grain development, N-content, N-fertilization, vegetative parts, winter wheat

Introduction

As proved by numerous experiment results, a considerable proportion of the nitrogen incorporated in the vegetative parts of wheat before flowering is reutilized in the grains through translocation (Van Sanford and MacKown, 1987; Debreczeni, 1989; Clarke et al., 1990). According to Waldren and Flowerday (1979) some two-thirds of N contained in the leaves is translocated to the grains. Smith et al. (1991) have reported that 78% of the N assimilated by wheat from foliar applied N at heading had been incorporated into the heads by the time of harvest.

Although N–fertilization can considerably increase both the amount of N available for redistribution and translocated from the vegetative parts into the grains (Spiertz and Ellen, 1978; Papakosta and Gagianas, 1991), Harms and Nowak (1990) could not detect any qualitative differences in nitrogen remobilization in well fertilized and nitrogen deficient wheat plants: in both cases, the grain N mainly originated from flag-leaf, stem and glumes. In an experiment by Simpson et al. (1983) under N–deficient conditions 40% of the nitrogen required for grain development came from the leaves, 23% from the glumes and 23% from the stem.

The N–translocation from the vegetative parts could be detected in wheat plants even after cutting them at the beginning of grain development, that is in plants under N–stress (Berecz and Debreczeni, 1990). This observation may be connected

with the fact that N-deprivation during grain filling positively influences the N– remobilization from the vegetative parts into the developing grains (Van Sanford and MacKown, 1987).

However, not too much information is available in the literature on the extent to which these processes take place during grain development, and on the changes occurring at various N-fertilization levels in the quantity of nitrogen mobilized from the vegetative parts. Therefore joining in a harvesting study built on a N-fertilization experiment, we examined the trend of nitrogen contained in the major vegetative parts, since these data may help in interpreting the results of nitrogen fertilization, and may even be useful for breeders.

Materials and methods

The experiment was carried out at Keszthely, in a Ramann brown forest soil of medium N-status, in 1991. The small plot experiment arranged in random block design with 4 replications included the following treatments: 80, 120, 160 and 200 kg/ha N, respectively, distributed in spring (Feekes 2-3). For the harvesting study built on the N-fertilization experiment whole plant samples were cut on 5 occasions from the beginning of milky ripening. The water contents of grain at the different dates of sampling are shown in Table 1.

Table 1

Water contents of grain (%) pertaining to the different treatments and sampling dates, respectively

Treatments	Sampling dates				
N, kg/ha	27.06	04.07	11.07	18.07	26.07
0	57.0	47.0	28.9	22.0	20.8
80	60.4	51.1	35.5	22.2	20.4
120	60.0	50.5	37.6	22.4	18.7
160	60.5	50.7	38.9	23.3	19.4
200	59.6	50,6	39.2	22.6	19.2

The wheat plants cut above ground level from a 2 m² area were sheaved and stored until the last date of sampling; then they were threshed, and from 100 plants per sheaf the flag-leaf blade, the flag-leaf sheath and the uppermost internode were removed. These vegetative parts were weighed, ground, and determined for nitrogen content with Kjeldahl's method modified by Lengerke et al. (1974).

Results and discussion

The N-content of the vegetative parts examined showed a considerable decrease in the course of ripening (Table 2). This decrease may mainly be due to the translocation of N-assimilates from the vegetative parts to the generative parts, i.e. to the grains. Similar results have previously been reported by McMullan et al. (1988). The degree of the decrease, however, was considerably affected by the N supply of plants. It was of the slightest degree (18%) in the control samples in the

Table 2

Nitrogen content in the vegetative parts of wheat at various rates of N-fertilization (N, mg/100 vegetative plant parts)

Treatments	Flag-leaf blade					Flag-leaf sheath					Uppermost internode				
N, kg/ha	27.06	04.07	11.07	18.07	26.07	27.06	04.07	11.07	18.07	26.07	27.06	04.07	11.07	18.07	26.07
0	55	62	44	46	43	41	30	34	36	36	106	79	75	77	85
80	132	113	95	65	65	68	58	41	38	32	164	138	93	95	97
120	287	125	130	98	79	139	61	65	71	46	296	140	134	153	78
160	233	157	156	106	107	94	78	72	53	60	238	155	132	111	72
200	220	167	124	153	127	86	76	63	64	62	203	172	127	135	90
L.S.D. 5%	72		70	37	37	24	27	20	25	20	55	38	26	40	
P%	0.1	10	NS	0,1	1	0.1	5	1	10	5	0.1	1	0.1	5	NS

NS: Non-significant

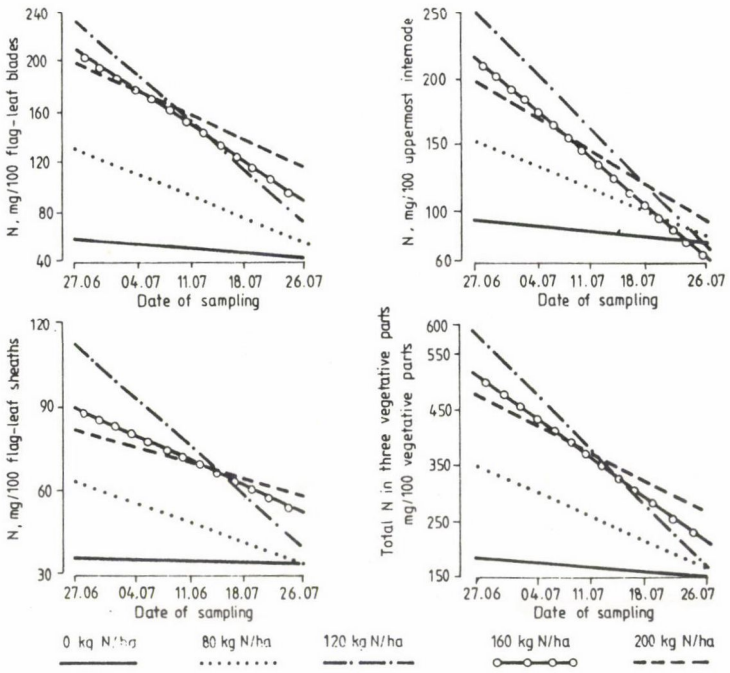

Fig. 1. Regression lines between the N- content of vegetative parts and the sampling date

average of the three vegatative parts. The N-fertilization resulted in a considerable increase in the extent of N-depletion (48% at 80 kg/ha N- dose and 71% at 120 kg/ha N- dose), the further increase in N levels, however, did not bring about a proportionally greater increase in it (53% at 160 kg/ha N dose and 42% at 200 kg/ha N dose). Increased N- translocation due to N-fertilization was also reported by Spiertz and Ellen (1978) and Papakosta and Gagianas (1991).

In the average of the N-treatments, the N-loss from the different vegetative plant parts shows a similar tendency, though differences can be detected in their extent. It amounted to 48% from the flag-leaf blade, to 39% from the flag-leaf sheath and to 48% from the uppermost internodium. A similarly high rate of depletion of the protein content of flag-leaf and uppermost internode was reported by Noaman and Taylor (1990).

It is obvious that in samples taken from the plot not given N the decrease was of a much lower extent in all three vegetative parts, and – according to the results of analyses – it came to an end sooner. Further, our results show that in an absolute sense the smallest quantity of N was removed from the leaf sheath. The uppermost internode, on the other hand, may have a great importance comparable to that of the flag-leaf from the point of view of N-translocation in the grain development period examined. Noaman and Taylor (1990) also found the role of the uppermost internode to be important in the N-metabolism of wheat.

In 15 of the 20 cases examined the reduction of N-content could be described

by a linear regression significant at least at P=1%. The rise of the straight lines, and accordingly the relative decrease calculated from the values of the regression lines, and even the absolute decrease were highest in the 120 kg/ha N–treatment, and – according to the results of analyses – lasted up to the state of full maturity (Fig. 1, Table 3).

Table 3

Relative decrease (%) in the nitrogen content of vegetative parts calculated from the values of regression lines

Treatments N, kg/ha	Flag-leaf blade	Flag-leaf sheath	Uppermost internode	Three vegetative parts
0	28	5	18	19
80	43	46	46	54
120	69	65	71	71
160	58	42	70	61
200	42	29	54	46

Nitrogen fertilization was found at each date of sampling to increase the N-content in the three vegetative plant parts, though it was only at the first or at most the second date of sampling that this increase was significant even with the 80 kg/ha quantity of N applied (Table 2). N–doses larger than 120 kg/ha resulted in no case in statistically proved increase in the N-content of the vegetative parts concerned. Similar results have been found when investigating the effect of N-fertilization on the grain yield; it increased up to 120 kg/ha N–dose, but further increase in N–levels did not bring about significant changes in the grain yield (Ragasits, 1988; Berecz, 1989).

In 9 out of 15 cases, the N-content of the plant parts examined at different stages of grain development showed a positive correlation with the pertaining grain nitrogen contents, significant at least at P=5% (Table 4).

With the exception of a single case, statistically proved positive correlation could be indicated between the quantity of nitrogen fertilizer and the N-content of the three vegetative parts examined in each stage of grain development (Table 4).

Since the quantity of remobilized vegetative nitrogen is greatly influenced by varietal, meteorological and environmental factors (Van Sanford and MacKown, 1987; Debreczeni, 1989), we continued the investigations in 1992 with further 3 cultivars, extending the examinations to include the rachis and the glume among the plant parts.

Table 4

Correlation coefficients between the N-content of vegetative parts (N, mg/100 vegetative parts), grain N-content (mg N/grain) and N-doses (kg/ha), respectively

Vegetative parts	Vegetative N-content and grain N-content					Vegetative N-content and N-doses				
	27.06	04.07	11.07	18.07	26.07	27.06	04.07	11.07	18.07	26.07
Flag-leaf blade	0.12 NS	0.56*	0.62**	0.56*	0.55*	0.71***	0.67**	0.55*	0.84***	0.80***
Flag-leaf sheath	–0.10 NS	0.42 NS	0.60**	0.86***	0.34 NS	0.53*	0.74***	0.65**	0.52*	0.67**
Uppermost internode	0.06 NS	0.66**	0.57*	0.85***	015 NS	0.57*	0.80***	0.69***	0.54*	–0.11 NS

*,**,***: Significant at the 5, 1 and 0.1% probability levels, respectively
NS: Non-significant

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EFFECT OF POTASSIUM FERTILIZATION ON THE YIELD AND BAKING QUALITY OF WHEAT

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In an experiment carried on since 1953 on brown forest soil (Ramann) at Keszthely, the effect of potassium on the yield and baking parameters of the wheat variety Mv-8 was studied in 1987–1989.

Under the conditions of the experiment the potassium fertilizer did not significantly increase the grain yield of the wheat. The thousand-grain-weight and hectolitre weight were, though, significantly increased by the potassium fertilizer, but the various rates of fertilization did not show demonstrable differences as regards their effects.

The highest gluten content was obtained with 80 kg/ha K_2O, and, with the exception of gluten spreading, the other quality parameters too showed the most favourable trend at this rate of potassium fertilization.

Keywords: baking quality, potassium, yield, wheat

Introduction

In comparison to nitrogen and phosphorus nutrition, the effect of potassium fertilizers on the quality of wheat has been dealt with very few publications. The authors have generally reported a favourable effect of potassium nutrition on quality. Hojjati and Maleki (1972) found the lysine content of the grain protein to increase under the influence of potassium fertilizer. According to Haeder and Mengel (1974) the crude protein content of the wheat grain can be increased by improving the potassium supply. Koch and Mengel (1977) studying the subject arrived at the conclusion that potassium promoted the translocation of amino acids from the vegetative plant parts to the grain. Kolbe and Müller (1983) observed a slight decrease in the endosperm proteins in response to potassium nutrition.

On the basis of several authors' experiences Erdei and Szániel (1975) point out that potassium nutrition has only an indirect effect on the quality of wheat. Douglas and Dyson (1985) found a significant negative correlation between the potassium content and the baking quality of the wheat grain. Otherwise, in a 10-year experiment by Douglas (1987), he found that potassium had no valuable effect on the baking quality of wheat. As seen from the literary data, uniform opinion has not been formed of the effect of potassium on the quality of wheat. This justifies us in having taken the question under close examination.

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Materials and methods

The experiment has been carried on since 1963 at Keszthely. The soil of the experimental area is a brown forest soil (Ramann) of nearly neutral chemical reaction, poorly supplied with organic matter and phosphorus and moderately well with potassium. In 1963 the soil analysis revealed an available ammonium lactate-soluble (AL) K_2O-content of 110 ppm.

Each treatment was given a nutrient supply of 174 kg/ha N and 140 kg/ha P_2O_5. In the experiment wheat and maize are grown alternately every two years. The plots of 48 m² are arranged in a random block design. In 1987–1989, Mv-8 was the variety examined. Quality testing was carried out according to the relevant Hungarian standards.

Results

Over an average of three years, the potassium fertilization increased the yield of wheat, though this increase was not significant. The highest yield increase was attained (0.46 t/ha) with the 160 kg/ha dose, and a further increase in the rate of fertilization did not result in a surplus yield (Table 2). Our results agree with those obtained by Kadlicskó and Krisztián (1988) in two brown forest soils of similar type.

In response to the treatments, statistically proved differences were shown in the thousand-grain-weights. In comparison to the plots not given potassium fertilizer since 1963 (control) the after-effect of potassium (2. treatments) resulted in a significant increase in thousand-grain-weight. While with potassium supplied at a rate of 80 kg/ha, a further demonstrable increase was attained, the 160 kg/ha dose only resulted in a tendency-like increase.

The hectolitre weight showed a significant increase as a response to potassium treatment compared to the control, but the various doses did not differ as regards their effects.

In the gluten content, differences appeared under the influence of the treatments (Table 3). In comparison to the control, the after-effect of potassium increased the gluten content by 0.9% and the 80 kg/ha dose by 1.1%. The further increase of potassium supply resulted in a significant decrease in the gluten content.

While in the other quality parameters no significant differences were obtained, in the farinographic value, water uptake and Zeleny's number similar changes were observed as in the gluten content. Similarly to the gluten content, the values of the latter three quality parameters were the highest with the 80 kg/ha rate of potassium nutrition. On the 240 kg/ha level, the values reflected a poorer quality compared to the control. Gluten spreading only shows minor differences which, however, cannot be explained by the effect of treatments.

On the basis of the results of the experiment it can be stated that, with the yield and baking parameters of wheat taken into consideration, potassium fertilization at a rate higher than 80–100 kg/ha on brown forest soils is not reasonable.

Table 1

The treatments of the experiment and soil available potassium content

Treatment	K_2O, kg/ha		AL-K_2O, ppm
	1963–1973	1974–1990	1990
1	–	–	110
2	216	–	136
3	216	80	163
4	216	160	210
5	226	240	250

Table 2

Effect of potassium fertilization on the yield, thousand-grain-weight and hectolitre weight of wheat

Treatment	Yield (t/ha)	Thousand-grain-weight (g)	Hectolitre weight (g)
1	3.73	32.7	75.7
2	3.80	34.1	76.3
3	4.00	35.1	77.7
4	4.27	35.4	77.7
5	4.27	34.7	77.7
L.S.D.$_{5\%}$	NS	0.9***	1.6^{+}

$^{+}$, ***: Significant at the 10 and 0.1% probability levels, respectively.
NS: Non-significant

Table 3

Effect of potassium fertilization on the baking quality of wheat

Treat-ment	Gluten		Farinograph		Water uptake (%)	Zeleny's number
	%	spreading (mm)	value	group		
1	30.1	6.0	52.8	B-2	62.4	36
2	31.0	5.7	56.8	B-1	61.8	36
3	31.2	5.9	58.7	B-1	63.0	39
4	28.9	4.7	52.6	B-2	62.8	38
5	28.3	5.9	50.7	B-2	59.8	32
L.S.D. $_{5\%}$	1.2**	NS	NS	NS	NS	NS

**: Significant at the 1% probability level
NS: Non-significant

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FIELD RESPONSE OF WHEAT TO ZINC APPLICATION IN SOILS OF SEMIARID REGION IN PUNJAB, INDIA

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The results of field experiments conducted at 30 locations on different soils of semiarid region to study the response of wheat to zinc application and critical deficiency level of Zn are reported. The sites were selected on the basis of their varying degree of Zn deficiency. Zn was incorporated in the soil at the rate of 0 and 5.6 kg Zn ha^{-1} as zinc sulphate. Soil application of 5.6 kg Zn ha^{-1} significantly increased the grain yield of wheat in deficient soils. The average response of 0.3 t ha^{-1} was observed with the application of 5.6 kg Zn ha^{-1}. Soil extractable Zn was significantly related with Bray's per cent grain yield. Both the graphical and statistical methods of Cate and Nelson (1965) suggested the critical deficiency level to be 0.76 mg kg^{-1} soil of DTPA extractable Zn. This level gave a predictability value of 64%. The influence of soil characteristics on the DTPA-Zn and grain yield indicated that as the organic content in soil increased and the soil texture became finer, the DTPA extractable Zn increased. The grain yield in control plots also increased with the increase in DTPA-Zn in soil but the grain response to applied Zn decreased with the increase in DTPA-Zn in soil.

Keywords: wheat, response of Zn, semiarid region, critical level

Introduction

Zinc deficiency in field crops constitute a major soil fertility problem in many states of India and for that reason application of Zn to various crops has become a common practice. In Punjab state, about 47% of soils are deficient in Zn (Nayyar et al., 1990). The DTPA extraction of soil has been shown to be a useful method for monitoring Zn deficiency in neutral and alkaline calcareous soils (Lindsay and Norvell, 1978; Singh and Takkar, 1981; Singh and Shukla, 1985). Most of the investigations have been made in pot culture under green house conditions which differ greatly from the field. Moreover, critical level of soil Zn which separate deficient from non-deficient soils, or the concentration below which deficiency occurs, also vary with crop and the soil.

The present investigation was, therefore, carried out on farmers' fields in alkaline soils of semiarid region to study:

a) the response of wheat to Zn application;
b) the critical deficiency level of Zn in these soils and
c) the effect of soil properties on DTPA extractable Zn and crop yield.

Materials and methods

Field experiments were conducted at 30 locations (with a range of DTPA extractable Zn) in the semiarid region of the Punjab State. The area lies between latitudes 29° 56' 13" and 30° 31' 30" N, and longitudes 75° 33' and 76° 19'E. The wheat variety WL-2265 was grown as a test crop. The experiments were laid out in a randomized block design with a plot area of 50 sq. metre for each treatment. A basal dose of 120 kg N, 26 kg P and 33 kg K ha^{-1} was applied as urea, single super phosphate and muriate of potash respectively to all the plots. The whole of phosphorus and potassium and half of nitrogen was applied at sowing and the remaining 60 kg N ha^{-1} was applied with the first irrigation i.e. one month after seeding.

The Zn fertilizer treatments consisted of i) 0.0kg Zn (control) and ii) 5.6 kg Zn ha^{-1} as zinc sulphate. From each experimental site, representative soil samples were collected before fertilization at the sowing of wheat crop. Soil samples were taken with a tube auger so as to get a uniform core from the surface to the plough depth (0–15 cm) from 6–8 spots at random from each site. The soil samples were air dried in shade, well mixed and a composite sample of about half kg was taken. The soil samples were analysed for texture, pH, organic carbon and available phosphorus by standard procedures (Jackson, 1967). These were analysed for their initial available Zn content by extraction with DTPA solution (0.005 M diethylenetriamine penta acetic acid + 0.1 M triethanol amine + 0.01 M $CaCl_2$, with a pH 7.3) using a soils: solution ratio of 1:2 and a shaking time of 2 hours (Lindsay and Norvell, 1978). Zinc in the filterates was determined by atomic absorption spectrophotometry.

The crop was grown to maturity and the grain yield of the control as well as of Zn treated plots was recorded. The critical deficiency level of Zn in soil was determined by the procedures of Cate and Nelson (1965, 1971).

Bray's per cent yield was calculated as:

$$\frac{\text{yield without Zn application}}{\text{yield with 5.6 kg Zn ha}^{-1}\text{ level}} \times 100$$

Results and discussion

The soils under study were coarse to fine textured, ls to sicl; alkaline in reaction, pH 7.5 to 8.2; low to medium in organic carbon, 0.14 to 0.8%; and adequate in available P, 5.0 to 34.7 mg kg^{-1} soil. DTPA extractable Zn varied from 0.38 to 3.00 mg kg^{-1} soil with a mean value of 0.72.

Response of wheat to Zn application

The application of 5.6 kg Zn ha^{-1} level increased the grain yield of wheat over control (Table 1). The increase in grain yield in different sites was in the range of 0.10 to 0.76 t ha^{-1}. Response was variable between the soils but, in majority of the soils under study, Zn application significantly increased the wheat yield indicating the necessity for Zn fertilization to maintain the soil Zn status at a sufficient level for better crop production. Yield in the absence of applied Zn ranged from 2.7 to 5.2 t ha^{-1} as compared to 3.1 to 5.5 t ha^{-1} in Zn treated soils. The mean grain yield on soil deficient in Zn ($\leq$0.76 mg Zn kg^{-1} soil) was 3.7 t ha^{-1} and that under Zn sufficient soil (> 0.76 mg Zn kg^{-1} soil) it was 4.2 t ha^{-1}, while increase in Zn sufficient soils was from 0.06 to 0.63 t ha^{-1} with a mean value of 0.26 t ha^{-1}. In the present study, an average response of 0.3 t ha^{-1} was observed with the application of 5.6 kg Zn ha^{-1} as Zinc Sulphate.

Table 1

Some physical and chemical charasteristics of the experimental soils and wheat grain yield in different locations

Soil No.	Textural class	pH	EC (dS m^{-1})	Organic carbon (%)	P (mg kg^{-1} soil)	DTPA Zn (mg kg^{-1} soil)	Grain yield (t ha^{-1}) control	Grain yield (t ha^{-1}) 5.6 kg Zn ha^{-1}	Bray's per cent yield
1	ls	7.8	.42	.50	5.6	1.40	4.9	5.1	97.0
2	ls	7.9	.48	.64	7.8	1.50	5.2	5.5	95.6
3	sicl	7.7	.60	.68	10.1	1.32	4.9	5.1	96.0
4	sicl	7.8	.36	.39	6.7	0.80	5.2	5.5	95.1
5	cl	7.5	.48	.70	20.8	2.00	4.0	4.2	95.2
6	sl	8.0	.46	.81	11.2	1.16	4.7	4.9	95.9
7	cl	7.6	.57	.80	7.8	2.50	3.9	4.1	93.9
8	sl	7.9	.48	.62	31.3	0.68	4.9	5.3	92.0
9	ls	7.9	.24	.35	18.5	0.48	3.2	3.9	80.9
10	sicl	7.9	.66	.56	5.6	0.56	3.9	4.4	90.2
11	cl	7.5	.56	.86	6.7	1.43	3.2	3.3	96.1
12	sl	7.8	.48	.47	6.1	1.00	3.3	3.6	90.3
13	ls	8.2	.21	.14	6.7	0.72	3.1	3.6	87.4
14	ls	7.9	.36	.21	7.8	0.42	2.7	3.1	89.3
15	ls	7.8	.31	.42	7.8	0.50	3.0	3.4	88.4
16	ls	8.1	.23	.16	11.2	2.00	3.4	3.6	95.3
17	sicl	7.7	.70	.77	19.0	0.46	3.5	4.6	84.8
18	ls	7.8	.43	.43	17.9	0.58	3.7	4.0	93.7
19	sicl	7.6	.34	.73	34.7	0.62	3.5	3.9	88.7
20	ls	8.0	.22	.14	12.3	0.38	3.3	3.7	88.5
21	sicl	7.8	.46	.55	22.4	0.62	5.0	5.2	95.6
22	sicl	7.9	.52	.54	6.7	0.64	4.7	5.3	89.2
23	sicl	8.3	.49	.71	6.2	3.00	4.7	4.8	98.7
24	sl	7.7	.47	.48	35.8	0.54	3.8	4.2	90.3
25	sil	8.2	.42	.73	31.0	2.40	4.3	5.0	87.3
26	sicl	7.7	.53	.59	11.2	2.20	4.1	4.2	94.2
27	sil	8.1	.44	.46	9.0	0.82	3.6	3.9	91.9
28	ls	7.6	.33	.43	9.0	1.04	3.8	4.3	88.8
29	ls	7.6	.35	.24	5.6	0.80	3.9	4.2	92.7
30	ls	7.6	.35	.18	5.0	0.48	3.2	3.9	80.6
Mean		7.8	.43	.50	13.3	1.11	4.0	4.3	91.4
L.S.D.$_{5\%}$		0.2	.13	.21	9.0	0.72	0.7	0.7	4.5

Soil critical deficiency level

The critical deficiency level of DTPA extractable Zn was calculated by plotting Bray's per cent grain yield against DTPA-Zn according to the graphical method of Cate and Nelson (1965). This value was also computed by using the statistical method of Cate and Nelson (1971). Both the approaches gave the same

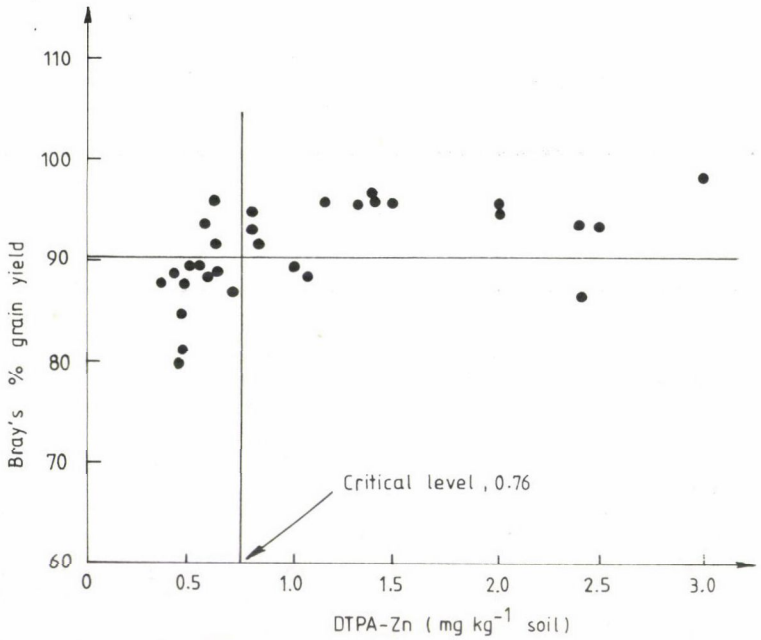

Fig. 1. Critical deficiency level of Zn in soil

critical deficiency level of 0.76 mg Zn kg^{-1} soil (Fig. 1) below which economic response of wheat to Zn fertilization can be expected. Several workers have reported different critical levels of Zn in soils and crop. Rathore et al. (1978) reported 0.46 mg kg^{-1} DTPA extractable Zn for wheat as the critical level in calcareous alkaline soils of Madhya Pradesh, while Sakal et al. (1979) suggested 0.54 mg kg^{-1} critical value in coarse textured soils of Bihar state. Takkar (1982) reported that the critical level of Zn increased as the soil texture became finer.

The probability of the percentage response of wheat to Zn application in soils testing less than the critical level of 0.76 mg kg^{-1} soil was 64% and in soil testing greater than 0.76 mg kg^{-1} soil was 12% (Table 2).

Table 2

Effect of Zn application on the grain yield of wheat. Results are mean values of all soil treatments

Zn status of soil	DTPA extractable Zn (mg kg^{-1} soil)	No. of soils	Grain yield (t ha^{-1}) control	Grain yield (t ha^{-1}) 5.6 kg Zn ha^{-1}	Yield increase (t ha^{-1})	Per cent soils responding to Zn
Deficient	≤0.76	14	(3.7) 2.7–5.0	(4.2) 3.1–5.3	0.5	64 –
Sufficient	>0.76	16	(4.2) 3.2–5.2	(4.5) 3.3–5.5	0.3	12 –

Table 3

Influence of soil characteristics on the DTPA-Zn and wheat grain yield

Soil parameter	No. of soils	DTPA-Zn (mg kg^{-1} soil)	Grain yield without Zn (t ha^{-1})	Grain yield response (%)
Texture				
Coarse	12	0.86	3.6	14.4
Fine	18	1.26	4.2	7.1
EC (dS m^{-1})				
≤0.40	11	0.75	3.5	14.3
>0.40	19	1.30	4.2	7.0
Organic carbon (%)				
≤0.50	15	0.80	3.6	11.1
>0.50	15	1.40	4.3	7.4

Relationship of soil characteristics with Zn availability and crop yield

The data on available Zn and grain yield response were grouped according to important soil characteristics (Table 3). As the soil texture became finer and soil organic carbon content in soils increases, the DTPA extractable Zn increased and the wheat grain response to applied Zn decreased. In the coarse textured soils, the average grain response to Zn application was 14.4% and in fine textured soils it was only 7.1% Similarly, the average grain response to Zn application was 11.1 and 7.4% in soils containing organic matter less and greater than 0.50%, respectively. The incidence of Zn deficiency will apparently be serious on alkaline, coarse textured soils, low in organic matter. From the results obtained, it appears that coarse textured soils, low in organic matter, responded to Zn fertilization better than fine textured soils rich in organic matter.

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PRESOWING SEED TREATMENT WITH PYRIDOXINE INCREASED GROWTH AND GRAIN YIELD OF TRITICALE

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The grains of two varieties of triticale namely, Tigre "S" and Muskox "S" were soaked in graded aqueous solutions of pyridoxine hydrochloride for 8 h prior to their sowing in sand culture. The plants raised from pyridoxine treated grains exhibited higher rate of root and shoot growth, sampled at 45, 60, 75, 90 and 105 days after sowing. The leaves of these plants were also characterised with increased nitrate reductase activity and the contents of pyridoxine, chlorophyll, nitrate, nitrogen, phosphorus and potassium. Net assimilation rate increased up to day 90. At harvest, the grain and straw yield also improved. The seeds of Tigre "S" treated with 0.1% of the pyridoxine solution proved better than the other cultivar.

Keywords: pyridoxine, triticale, nitrate reductase, net assimilation rate, nitrate, nitrogen, phosphorus, potassium, growth, yield

Introduction

The rate limiting step in the conversion of inorganic nitrogen to organic nitrogen is the reduction of nitrate to nitrite. The initial process is regulated by the substrate (NO_3) induced enzyme, nitrate reductase (Candella et al., 1957).

Pyridoxine regulates transamination (Lehninger, 1982), synthesis of auxin (Moore and Shaner, 1967) and the activity of α-amino-laevulinic acid synthetase (Kikuchi et al., 1958). At the level of the root, the vitamin is suggested to increase respiratory activity, thereby stimulating the active uptake of ions (Rao et al., 1987; Samiullah et al., 1988).

It was, therefore, considered imperative to study the effect of pyridoxine, applied through grain soaking, on the growth of two varieties of triticale whose seeds possessed different levels of native vitamin content.

Materials and methods

Healthy grains of two varieties of triticale (Tigre "S" and Muskox "S") were surface sterilised with 0.01% $HgCl_2$ followed by soaking in 0.00 (T_1), 0.001 (T_2), 0.01 (T_3) and 0.1 (T_4) % aqueous solution of pyridoxine hydrochloride, for 8 h. Treated grains were sown in 9" earthen pots, lined with polythene sleeves, containing acid-washed sand. Thinning was done after a week and only three plants were left in each pot. 500 ml of full nutrient solution (Hewitt, 1966) was supplied to each pot every day in the morning (Table 9). Each treatment was represented by 25 pots.

The sampling was done at 45, 60, 75 (tillering stage), 90 (heading stage) and 105 days after sowing (milky grain stage). At every sampling, 4 pots from each treatment were taken out randomly and all twelve plants from each treatment were grouped and divided into three replicates of four plants each. The root and shoot were analysed for their growth characteristics. The leaves were subjected to chemical analysis for the contents of pyridoxine, chlorophyll, nitrate, total

nitrogen, phosphorus, potassium and nitrate reductase activity. The plants in the remaining 5 pots, out of 25 per treatment, were allowed to grow up to maturity and studied for different yield characteristics, at harvest (145 DAS). Net assimilation rate (NAR) was computed for 30–45, 45–60, 60–75, 75–90 and 90–105 days after sowing.

Table 9

Quantity of the nutrients supplied to each pot per day (g)

$Ca(No_3)_2$, anhydrous	0.328
KNO_3	0.202
$Mg\ SO_4.7H_2O$	0.184
$NaH_2PO_4.2H_2O$	0.104
$FeC_6H_5O_7.3H_2O$	0.0149
$MnSO_4.4H_20$	0.00111
$CuSO_4.5H_2O$	0.0012
$ZnSO_4.7H_20$	0.0014
H_3BO_3	0.0093
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0004

Leaf-nitrate reductase activity was estimated by the method of Jaworski (1971). Nitrate content was determined following the method of Johnson and Ulrich (1950) and the method of Mackinney (1941) was used for determining total chlorophyll content. Pyridoxine content and total nitrogen was analysed by the method of Hochberg et al. (1944) and Lindner (1944), respectively. The procedure adopted by Fiske and Subba Row (1925) was followed for the estimation of phosphorus. The potassium content in the digested leaf material was estimated flame photometrically. The net assimilation rate was calculated according to the formula worked out by Milthorpe and Moorby (1979).

Results and discussion

The plants developed from treated seeds maintained significantly higher level of pyridoxine (Fig. 1b), chlorophyll (Fig. 1a), nitrate (Fig. 2b), percent nitrogen (Fig. 2c), phosphorus, potassium (Fig. 3a, b) and the activity of nitrate reductase (Fig. 2a) in the shoot, over the control at all stages of growth. The nitrate, total nitrogen, phosphorus, potassium and chlorophyll contents increased up to day 75, but declined at the next stages of growth, irrespective of the treatment. However, the activity of nitrate reductase decreased with the increase in the age of the plants from 60 to 105 days after sowing (Fig. 2a). The net assimilation rate exhibited a linear progressive increase up to heading stage (90 DAS) only (Fig. 2d). This favourable effect of the treatment was further reflected in the growth of the root and shoot as exhibited by their fresh and dry weight and the production of tillers and leaves in the aerial part (Tables 1–6). At harvest, almost all the ear characteristics (Table 7) and seed and straw yield per pot (Fig. 4) were enhanced significantly by the treatment. In general, the cultivar Tigre "S" responded best to the soaking treatment in 0.1% solution (T_4).

Although most of the seeds possess an ample amount of vitamins, varying rates of their accumulation in different varieties during their formation exert varying effects both on germination and on response reactions of the seeds to various influences (Strogonov and Genkel, 1976).

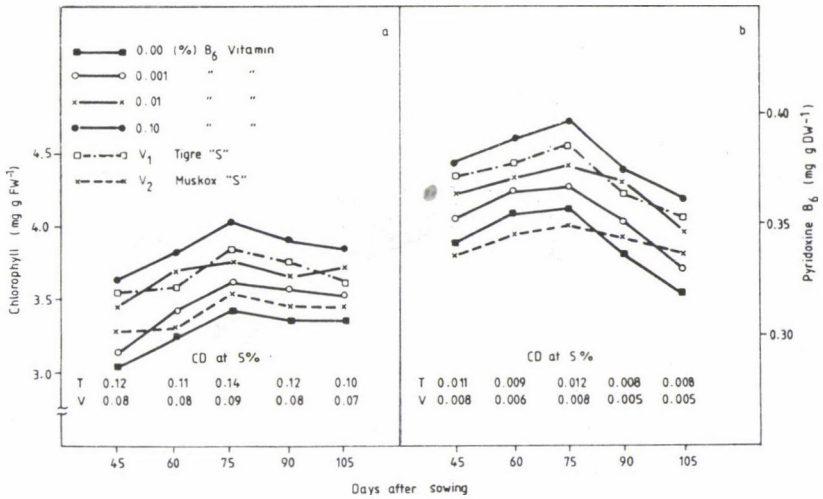

Fig. 1. Effect of grain treatment with pyridoxine on leaf-chlorophyll (a) and pyridoxine (b) content in two varieties of triticale. (CD – Critical difference, T – treatment, V – Variety)

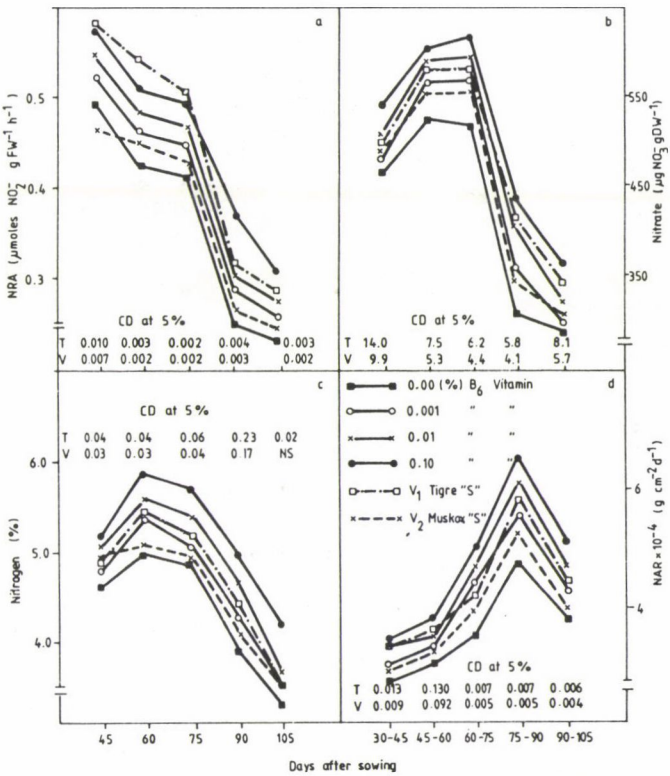

Fig. 2. Effect of grain treatment with pyridoxine on leaf nitrate reductase activity (NRA) (a), nitrate (b), percent nitrogen (c) and Net assimilation rate (NAR) (d) in two varieties of triticale. (N – Nitrogen, CD – Critical difference, NS – Non-significant, T – Treatment, V – Variety)

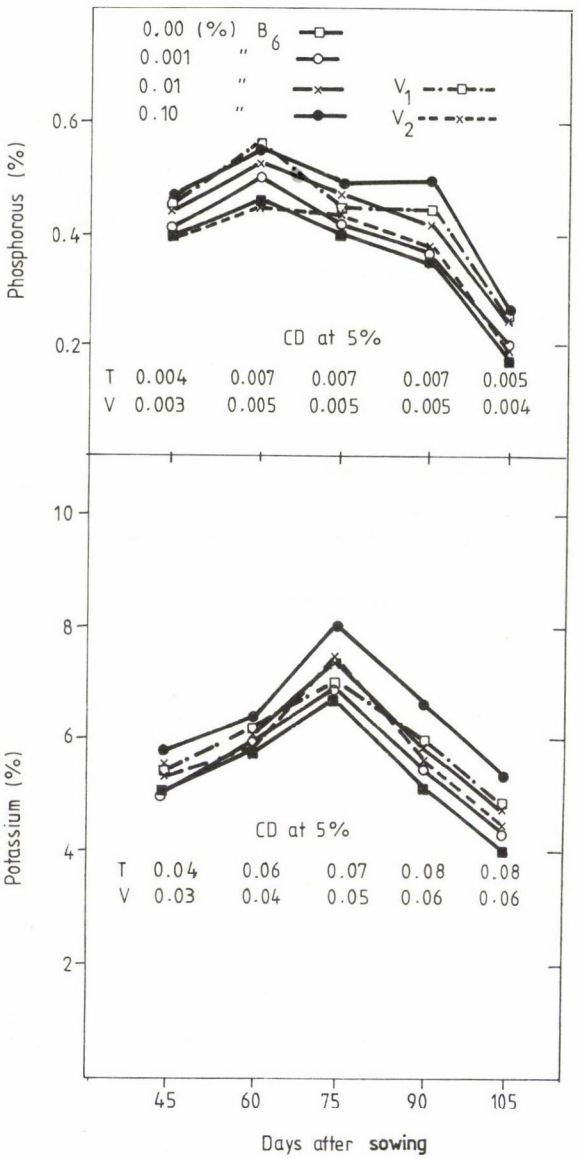

Fig. 3. Effect of grain treatment with pyridoxine on per cent phosphorus (a) and potassium (b) content in the shoot of two varieties of triticale. (V_1 – Tigre "S", V_2 – Muskox "S", CD – Critical difference, T – Treatment, V – Variety)

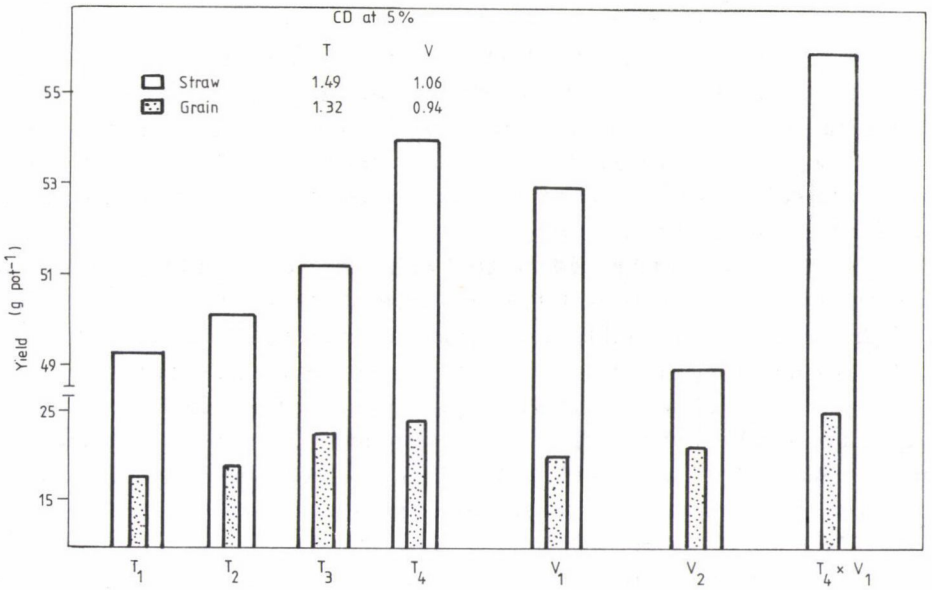

Fig. 4. Effect of grain treatment with pyridoxine on grain and straw yield per pot (g) in two varieties of triticale. (Per cent pyridoxine solution 0.00 (T_1), 0.001 (T_2), 0.01 (T_3), and 0.1 (T_4); V_1 – Tigre "S", V_2 – Muskox "S")

Pyridoxine treatment of the grains not only stimulated the germination processes in the seeds (Haque et al., 1988) but the resulting plants possessed longer roots with more fresh and dry weight (Tables 1–2) and (Haque, 1989). It may, therefore, be proposed that the plants raised from the grains treated with the B_6 vitamin had more soil exploring capacity, thus removed larger quantities of nitrogen, phosphorus and potassium (Figs 2c and 3a–b) from the culture medium. In addition to this, Kodandaramaiah (1983) and Samiullah et al. (1988) are of the opinion that pyridoxine either alters the permeability of the root cells or acts as co-enzyme of certain carrier proteins, responsible for nutrient uptake across the membrane. The increase in the activity of nitrate reductase (Fig. 2a) is simply the repercussion of the high nitrate content (Fig. 2b) because it is a substrate-induced enzyme (Candella et al., 1957). An increase in NRA in legumes has also been reported (Samiullah et al., 1988). Pyridoxal phosphate, the active form of pyridoxine, is also known to be directly involved in the synthesis of amino acids as a co-factor of transaminases or aminotransferases (Lehninger, 1982). Nitrogen assimilation, as also exhibited by high nitrogen content (Fig. 2c) was, therefore, favoured by the treatment. Higher protein level in association with additional amounts of porphyrin in whose synthesis pyridoxal phosphate is involved as a co-factor of α-aminolaevulinic acid synthetase (Kikuchi et al., 1958) might have resulted in higher chlorophyll content (Fig. 1a). It is supported by the findings of Kozhin and Kravtsov (1973) and Kodandaramaiah and Rao (1984).

The cumulative effect of an increased level of chlorophyll and leaf area (not

reported here) exerted a marked effect on the plant to produce more photosynthates as expressed by net assimilation rate (Fig. 2d). These plants also possessed significantly better meristematic activity which is evident from higher tiller and leaf number (Tables 3–4). The higher rate of cell division together with increased net assimilation rate would have resulted in more fresh and dry weight (Tables 5–6). In other plants also, shoot growth was favourably affected by pyridoxine at early stages of growth, irrespective of its way of application (Barbieri, 1959; Ovcharov and Kulieva, 1968; Samiullah et al., 1988).

The plants raised from the seeds treated with pyridoxine exhibited higher rate of meristematic activity and dry matter production at all the stages of growth studied (Tables 1–6). This acquired ability was further reflected in the ear characteristics (Table 7). The cumulative effect of significantly enhanced ear number per plant, ear weight per plant, spikelet and grain number per ear and 1,000 grain weight resulted in increased grain and straw yield (Fig. 4), because ear characteristics determine the seed yield of the plants (Murthy and Sethi, 1961). In other crops also, a favourable effect of pyridoxine treatment has been reported (Kudorev and Pavlov, 1965; Ahmad et al., 1982; Samiullah et al., 1988). The seed and straw yield was positively correlated ($P < 0.01$) with growth and chemical characteristics in the plants (Table 8). This indicates that the treatment increased "nutrient use efficiency" of the resulting plants which finally enhanced biological yield, at harvest.

Table 1

Effect of grain treatment with pyridoxine on fresh weight of root per plant (g) in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	1.56	1.48	1.52	2.71	2.55	2.63	5.92	5.09	5.51	11.09	10.18	10.64	15.87	12.37	14.12
T_2(0.001)	1.90	1.67	1.79	3.28	2.80	3.04	7.65	6.35	7.00	14.14	11.35	12.75	19.32	15.42	17.37
T_3(0.01)	3.02	1.87	2.45	3.70	2.79	3.25	8.39	6.67	7.53	15.01	12.88	13.95	21.47	15.89	18.68
T_4(0.1)	2.03	2.11	2.07	4.10	3.67	3.89	8.54	6.81	7.68	17.71	14.20	15.96	21.97	16.21	19.09
Mean	2.13	1.78		3.45	2.95		7.63	6.23		14.49	12.15		19.66	14.97	
Critical difference at 5%															
Treatment			0.25			0.24			0.09			0.64			0.24
Variety			0.18			0.17			0.12			0.46			0.16
Treatment × Variety			0.35			0.34			0.24			0.91			0.32

V_1 – Tigre "S", V_2 – Muskox "S"

Table 2

Effect of grain treatment with pyridoxine on dry weight of root per plant (g) in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	0.296	0.274	0.285	0.509	0.453	0.481	1.136	1.014	1.075	2.442	2.233	2.338	3.778	3.104	3.441
T_2(0.001)	0.361	0.290	0.326	0.611	0.490	0.551	1.461	1.226	1.364	3.127	2.484	2.806	4.817	3.868	4.343
T_3(0.01)	0.391	0.312	0.352	0.655	0.527	0.591	1.604	1.345	1.475	3.285	2.655	2.970	3.243	4.114	4.679
T_4(0.1)	0.375	0.333	0.354	0.702	0.569	0.636	1.631	1.360	1.496	3.542	2.849	3.196	5.304	4.343	4.824
Mean	0.356	0.302		0.619	0.510		1.458	1.246		3.099	2.555		4.786	3.857	
Critical difference at 5%															
Treatment			0.001			0.006			0.003			0.126			0.110
Variety			0.001			0.004			0.002			0.089			0.077
Treatment × variety			0.001			0.008			0.004			0.178			0.154

V_1 – Tigre "S", V_2 – Muskox "S"

Table 3

Effect of grain treatment with pyridoxine on tiller number per plant in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	3.33	3.00	3.17	6.00	6.00	6.00	6.33	6.00	6.17	7.00	7.00	7.00	8.33	7.00	7.67
T_2(0.001)	2.67	3.00	2.84	5.67	5.33	5.50	7.33	6.67	7.09	8.00	7.00	7.50	9,33	7.33	8.33
T_3(0.01)	3.00	3.00	3.00	5.67	7.33	6.50	7.33	8.00	7.67	8.00	9.00	8.50	9.33	10.00	9.67
T_4(0.1)	3.00	3.00	3.00	6.00	6.67	6.34	9.33	8.00	8.67	10.33	9.00	9.67	11.33	10.67	11.00
Mean	3.00	3.00		5.83	6.33		7.58	7.17		8.33	8.00		9.58	8.75	
Critical difference at 5%															
Treatment			Non-significant			0.71			0.55			0.24			0.60
Variety			Non-significant			0.50			0.39			0.17			0.43
Treatment × variety			Non-significant			Non-significant			0.78			0.34			0.85

V_1 – Tigre "S", V_2 – Muskox "S"

Table 4

Effect of grain treatment with pyridoxine on tiller number per plant in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	9.00	8.00	8.50	14.00	12.33	13.17	21.33	18.67	20.00	26.00	22.67	24.34	20.33	19.00	19.67
T_2(0.001)	11.00	9.00	10.00	15.67	13.00	14.34	24.33	20.33	22.33	29.67	24.33	27.00	23.67	21.00	22.34
T_3(0.01)	13.00	11.00	12.00	16.67	15.00	15.84	27.00	23.00	25.00	32.67	27.00	29.84	26.67	22.67	24.67
T_4(0.1)	13.67	12.00	12.84	19,00	16.00	17.50	30.00	25.00	27.50	35.00	29.00	32.00	30.00	24.00	27.00
Mean	11.67	10.00		16.34	14.08		25.67	21.75		30.84	25.75		25.17	21.67	
Critical difference at 5%															
Treatment			0.24			0.38			0.51			0.51			0.46
Variety			0.17			0.27			0.36			0.36			0.32
Treatment × variety			0.34			0.53			0.72			0.72			0.64

V_1 – Tigre "S", V_2 – Muskox "S"

Table 5

Effect of grain treatment with pyridoxine on fresh weight of shoot per plant in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	4.45	4.98	5.22	12.35	9.72	11.04	21.92	19.06	20.49	35.36	31.08	33.22	59.36	54.03	56.70
T_2(0.001)	6.75	5.31	6.03	14.63	11.11	12.87	26.52	20.02	23.27	43.64	37.10	40.37	62.67	56.87	59.77
T_3(0.01)	7.81	5.76	6.79	15.76	12.34	14.05	27.96	24.33	26.15	49.40	42.11	45.76	65.77	60.22	63.00
T_4(0.1)	6.92	6.12	6.52	15.97	12.72	14.35	30.47	25.64	28.06	53.12	44.29	48.71	70.00	62.12	66.06
Mean	6.73	5.54		14.68	11.47		26.72	22.26		45.38	38.65		64.45	58.31	
Critical difference at 5%															
Treatment			0.18			0.19			1.05			1.44			1.72
Variety			0.13			0.19			1.05			1.44			1.72
Treatment × variety				0.26			0.25			1.57			2.02		2.42

V_1 – Tigre "S", V_2 – Muskox "S"

Table 6

Effect of grain treatment with pyridoxine on dry weight of shoot per plant (g) in two varieties of triticale (Mean of three replicates)

Treatments (% aqueous solution of vitamin B_6)	Sampling time (days after sowing)														
	45			60			75			90			105		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1(0.00)	1.07	0.97	1.02	2.38	2.00	2.19	4.54	3.41	3.98	7.94	6.12	7.03	11.80	9.43	10.62
T_2(0.001)	1.29	1.01	1.15	2.70	2.17	2.44	5.21	4.06	4.64	10.11	7.97	9.04	15.06	11.82	13.44
T_3(0.01)	1.40	1.11	1.26	2.91	2.33	2.62	5.51	4.37	4.94	10.88	8.58	9.73	16.27	12.86	14.57
T_4(0.1)	1.33	1.15	1.24	2.95	2.44	2.70	5.81	4.66	5.24	11.65	9.21	10.43	18.04	13.97	16.01
Mean	1.27	1.06		2.73	2.24		5.27	4.13		10.15	7.97		15.29	12.02	
Critical difference at 5%															
Treatment			0.01			0.08			0.24			0.54			1.01
Variety			0.01			0.12			0.17			0.38			0.71
Treatment × variety			0.01			Non-significant			Non-significant			0.77			1.42

V_1 – Tigre "S", V_2 – Muskox "S"

Table 7

Effect of presowing seed treatment with pyridoxine on some yield characteristics in triticale

Treatments (% aqeous solution of vitamin B_6)	Ear number/plant			Ear length (cm)			Spikelet number/ear			Seed number/ear			Ear weight/plant (g)			1.00 grain weight (g)		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (0.00)	12.33	10.33	11.33	16.74	16.43	16.59	15.84	14.55	15.21	28.00	25.00	26.50	59.33	56.33	57.83	41.14	30.72	35.93
T_2 (0.001)	12.33	10.33	11.33	16.80	16.40	16.60	16.34	15.05	15.70	28.06	25.67	26.84	59.33	56.00	57.67	42.03	38.71	40.37
T_3 (0.01)	12.67	11.33	12.00	17.02	16.86	16.94	17.87	16.62	17.25	33.67	26.67	30.17	62.33	60.33	61.33	42.99	40.83	41.91
T_4 (0.1)	15.33	12.67	14.00	18.61	17.10	17.86	19.74	18.31	19.03	34.67	31.00	32.84	69.00	63.33	66.17	46.28	43.97	45.13
Mean	13.17	11.17		17.29	16.70		17.45	16.13		31.09	27.09		62.50	59.00		43.11	40.56	
Critical difference at 5%																		
Treatment			0.71			0.73			0.68			0.18			1.45			1.55
Variety			0.50			0.52			0.48			0.84			1.05			1.10
Treatment × variety			N.S.			1.04			N.S.			1.67			2.10			N.S.

V_1 – Tigre "S", V_2 – Muskox "S"
N.S. – Non-significant

Table 8

Correlation between straw and seed yield with various growth and chemical characteristics

Parameters	Correlation coefficient (r)									
	Straw yield					Seed yield				
	Days after sowing									
	45	60	75	90		45	60	75	90	105
Growth characteristics										
Tiller number plant^{-1}	–	–	0.80**	0.83**	0.87**	–	–	0.73**	0.73**	0.77**
Leaf number plant^{-1}	0.93**	0.91**	0.92**	0.88**	0.89**	0.86**	0.95**	0.94**	0.96**	0.92**
Shoot length plant^{-1}	N.S.	N.S.	0.60*	0.81**	N.S.	N.S.	N.S.	N.S.	0.62*	N.S.
Shoot fresh weight plant^{-1}	0.73**	0.77**	0.88**	0.90**	0.89**	0.79**	0.92**	0.94**	0.89**	0.97**
Shoot dry weight plant^{-1}	0.73**	0.76**	0.77**	0.82**	0.84**	0.85**	0.91**	0.92**	0.92**	0.93**
Root fresh weight plant^{-1}	0.64*	0.87**	0.77**	0.89**	0.74**	0.50*	0.85**	0.86**	0.92**	0.90**
Root dry weight plant^{-1}	0.77**	0.83**	0.79**	0.81**	0.79**	0.86**	0.95**	0.83**	0.92**	0.88**
Net assimilation rate	0.86**	0.81**	0.71**	0.78**	0.83**	0.86**	0.74**	0.76**	0.75**	0.82**
(Days interval)	(30–45)	(45–60)	(60–75)	(75–90)	(90–105)	(30–45)	(45–60)	(60–75)	(75–90)	(90–105)
Chemical characteristics of leaves										
Nitrate reductase activity	0.63*	0.74**	0.88**	0.84**	0.91**	0.93**	0.95**	0.96**	0.96**	0.96**
Nitrate content	0.97**	0.91**	0.92**	0.90**	0.94**	0.86**	0.85**	0.95**	0.94**	0.84**
Nitrogen content	0.96**	0.89**	0.92**	0.90**	0.87**	0.83**	0.73**	0.81**	0.86**	0.66*
Phosphorus content	0.86**	0.75**	0.94**	0.93**	0.88**	0.96**	0.95**	0.70*	0.89**	0.96**
Potassium content	0.94**	0.93**	0.96**	0.95**	0.94**	0.77**	0.93**	N.S.	0.81**	0.89**
1,000 Seed weight (at harvest)			0.92**					0.94**		

* Significant at 5%, ** Significant at 1%, N.S. Non-significant

Table 9

Quantity of the nutrients supplied to each pot per day (g)

$Ca(NO_3)_2$, anhydrous	0.328
KNO_3	0.202
$MgSO_4.7H_2O$	0.184
$NaH_2PO_4.2H_2O$	0.104
$FeC_6.H_5O_7.+H_2O$	0.0149
$MnSO_4.4H_2O$	0.00111
$CuSO_4.5H_2O$	0.0012
$ZnSO_4.7H_2O$	0.0014
H_3BO_3	0.0093
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0004

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EFFECT OF PLANT DENSITY AND PLANT DISTRIBUTION WITHIN THE ROW ON GRAIN YIELD AND STANDING ABILITY FOR MAIZE

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Altough the maize (*Zea mays* L.) response to plant density, depending on genotypes, has been one of the main contributors to yield gain, knowledge of reactions among hybrids to uneven plant distribution within the row, are lacking. Thus, a 3-year research program was conducted under source limit continental climate, in Hungary, to discover if there are differences among the hybrids in yield and standing ability responses, and the basis of these differences. For this purpose four hybrids were chosen; two of them have short growing seasons, the others have long ones. One of each group is plant density tolerant, and the other is sensitive. In terms of sink-source relationship, a tolerant hybrid belongs to source limit, and a sensitive hybrid to sink limit groups. The rest belong to the provisional. Because there was no observable effect caused by unevennesss in terms of lodging and stalk break, so with these features only the plant density, effects were investigated. It was found that the hybrid yield responses to density and unevenness depend also on sink-source relationship. The sink limit genotype is more tolerant than the source limit hybrid. In the case of same level of sink-source relationship, the plant density tolerant genotype adapts itself better to the unevenness tha ependently from yield responses to plant density and sink-source relationship. If the elements of stalk break, as rind puncture and stalk diameter are of low levels influenced by plant density, the stalk becomes more similar to the ring shape to compensate for the weakness of rind puncture. No correlation was observed between root and stalk strength. These findings would be good for improving a new breeding strategy.

Keywords: *Zea mays* L., genotypes, plant density, uneven plant distribution, sink-source relationship

Introduction

The plant density was one of the most effective contributors of maize (*Zea mays* L.) yield gain during the last fifty years (Cardwell, 1982). At the same time the uneven plant distribution within the row, associated to the high density, is a substantial decreasing factor. The standing ability is one of the most important features for effective machine-harvest, and that is why its lack is also a decreasing factor.

The different responses of hybrids to density in grain yield are well known (Early et al., 1966; Rutger, 1971; Cornelius and Byars, 1976). Over the yield, Bunting (1973), Pintér et al. (1987) and Pintér (1980) found differences among genotypes in sensitivity to plant density. This was characterized by the plant density (PS), in which no significant yield decrease ($P_{0.05}$) was observed related to maximum yield (Y_{max}). According to the PS values, tolerant and sensitive genotypes were found (Pintér et al., 1990).

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The standing ability depends on lodging/root strength and stalk break/stalk strength. Although these features are first of all genetically controlled (Zuber and Loesch, 1966; Martin and Russel, 1984), their plant density interaction is well known (Thompson, 1963; Wilcoxson and Covey, 1963; Zuber and Dicke, 1964).

Mock and Heghin (1976) and Krall et al. (1977) found yield decrease as a response to uneven plant distribution within the row. The yield decreases were controlled by genotype. If certain genotypes are sensitive to plant density, they have higher yield decrease to uneven distribution than the tolerant types (Pintér et al., 1978). A similar result was found in the case of lodging (Pintér, 1980).

However, many findings are on plant density responses both on yield and on standing ability, and some contributors dealt with the response to unevenness. Still, our knowledge is weak on combined influence, including different stresses. So two questions are raised: i) are there differences between tolerant and sensitive genotypes both in yield and in standing ability responses to unevenness with increasing plant density? ii) if the differences occur, what is their basis?

Materials and methods

A 3-year (1988–1990) field research program was conducted on Research Station, Pannon University, Georgicon Faculty, Keszthely, Hungary (46.6 north latitude and elevation 170 m) under source limit growing area, while the Corn Belt in the United States is known as a sink limit region (Daynard et al., 1977).

The different genotypes were chosen by the results of Hungarian trials, according to the following standpoints; a) hybrids must be as similar as possible in developing and growing features, because in one hand all stresses affect them in the same developing stage, so, this effect does not influence the response of genotypes to uneven plant distribution; on the other hand the different growing, e.g. plant height or leaf area, does not influence the clear genotype response; b) genotypes should differ in tolerance to plant density; c) the hybrids must vary in terms of sink-source relationship, because the source limit genotype differs from sink limit type in stress tolerance (Tollenaar, 1977). In these respects, P 3965 and P 3978; P 3732 and Sze DC 488 hybrids have been chosen for the short and long seasons, respectively. The first genotypes of both groups are plant density tolerant, while the second ones are sensitive. Regarding the grain sink, P 3965, and P 3732, Sze Dc 488, and P 3978 are source limit, and provisionals, and sink limit, respectively, or in terms of stover-ear compensation (Zimmer and Wermke, 1986) A, A-B and C types, respectively. The P 3965, P 3978, P 3732 and Sze DC 488 hybrids were bred by Pioneer Hi-Bred International, Des Moines, Iowa, USA and Cereal Reseach Institute, Szeged, Hungary, respectively.

The field trials were set up on silty-clay soil under a conventional tillage system with 100, 150 and 90 kg ha^{-1} N, P_2O_5 and K_2O, respectively given in fall, with the exception of N, whose first half was given in autumn, the rest in spring time before planting. The experiment was chemically weed and pest controlled.

In addition to the above-mentioned main characteristics of the Hungarian continental climate, the weather is changeable with large differences between day and night temperatures, as is also common in the whole Carpathian Basin. Weather and soil condition of the plots met the requirements of plants during the growing season. No stresses originated from water and nutrients supply, or weed- and pest control etc. were observed. That is why the tendencies due to the applied treatments, as well as the yearly results, were evaluated together.

The experiment was laid out as a split-plot design with four replicates. The plot size was 20 m^2. The row spacing was 70 cm. Seven-meter-long plots included 4 rows. To avoid border effect, 1.0 m from both the front and end of plots, and two outside rows were removed. The plots were double planted by handgun on the 23th and 30th April, and 2nd May, 1988, 1989, and 1990, respectively. The applied treatments where; a) plant densities: 4; 6; 8; 10; 12; 14 plants m^{-2}; b) unevenness, achieved by planting and thinning according to a special research map, in cv percent of plants spacing within the row: 0.0 as check, 45.0 and 90.0.

During the growing season data of 50% silking, plant height, leaf area, root strength and rind puncture were

registered. At the grain ripening stage/black layer formation, the lodging, stalk break and stalk diameter at second internode from the soil level were measured. The harvest was done by hand after the black layer formation. The plant height from soil surface to first tassel branch and leaf area, measured by Li-3000 A (Li-Cor, Inc., Nebraska, USA) were registered just after the silking. For root strength and rind puncture measurements, different plots with only plant density treatments were planted. They were measured in the same time just after the silking. At first the rind puncture was measured on 2nd internode from soil surface (Colbert and Zuber, 1978) afterwards the stem was cut and the root was pulled out characterizing the root srength (Penny, 1981). If the stalk has less than a 45 ° angle to the soil level, it is considered to be lodged. In the case of stalk, a break below the ear was considered as broken stalk. The stalk diameter was characterized by the average of thinnest and thickest diameters of 2nd internode from the soil surface.

The length of season, as a character of developing was measured by growing degree days (GDD) with 10 °C daily average, based on hourly registered thermal unit, accumulated from emergency to 50% silking. Less than 5 °C and more than 28 °C were neglected from calculations, because Stevens et al. (1986) using thermal unit accumulation from 5 to 28 °C found better correlation to the stress than using the range of 10 °C to 30 °C (Cross and Zuber, 1972). The grain yield (in 850 g kg^{-1} dry matter content) response of different genotypes to unevenness was evaluated by the highest yield (Y_{max}), plant density required to Y_{max} (PD_{max}), and (PS) (Pintér et al., 1990). Presuming that the average of features for standing ability does not show clearly the effect of unevenness, its cv-values were computed too. Besides the stalk diameter the stem shape was also evaluated, characterized by thin thick^{-1} diameters. Response of hybrids to plant density in feature of standing ability was characterized by both computed average values at PD_{max} used linear correlation equation and the b-value of linear relationship between plant density treatments and corresponding features. Replications were investigated separately, so it was possible statistically to compare these values.

Results and discussion

The results are separated to check whether the hybrids are suitable for this investigating purpose, and to evaluate the responses to plant density and unevenness in both yield and standing ability.

Characterization of genotypes

Although the genotypes were chosen by the result of preliminary trials, their fitness to this research was checked again. This process was done according to growing, developing, and sink-source relationship, and susceptibility of yield to plant density. Because the growing, developing, and sink-sources relationship are influenced by plant density, the comparisons of hybrids were not carried out at the same density, but at the genetically adequate plant density levels/PD_{max}. (This idea was also followed at LAI, grain to leaf area unit, and at features of standing ability comparisons.) At the same time yield susceptibility to plant density was investigated in even distribution, involving all plant density treatments.

Regarding the two ripening groups, the plant height as a character for growing, was quite similar for long season hybrids (Table 1). Although within the short season group, there is a significant, but not substantial difference between the two hybrids. In developing features, the hybrids within the groups are similar. Consequently the required similarity of chosen hybrids fits very well to the purpose of investigation. Thus, all stresses appear at the same developing stage, or the dissimilar growing does not influence the genotype responses.

In terms of required dissimilarity, first the even distribution (cv=0.0) of PS

was investigated (Table 2). In the case of both groups, the tolerant and sensitive hybrids also differ significante. Secondly the hybrids also differ terms of sink-source relationship (Table 3). According to grain to leaf area unit, three groups could be separated. One tolerant is source limit, the other is provisional. One sensitive is sink limit, the other is provisional. Because the sink-source relationship is a character for stress tolarence (Tollenaar, 1977), this collection is suitable to investigate a) whether the tolerant hybrid is able to compensate the stress caused by unevenness, if it is source limit; b) whether the sensitive hybrid suffers from stress, if it is sink limit. In other words, this collection is fit for the investigation of the characters that are important to compensate for the stress caused by unevenness.

Table 1

Growing and developing characteristics of investigated hybrids at optimum plant density for grain yield

Genotypes		Differences
Tolerant	Sensitive	(Tolerant-sensitive)
Growing/plant height from soil level to first tassel branch (cm)		
	Short season hybrids	
168.8	163.3	5.5*
	Long season hybrids	
177.8	176.5	1.3
Developing/GDD (daily average 10 °C)		
	Short season hybrids	
110.4	104.6	5.8
	Long season hybrids	
139.2	133.8	5.4

The reliability of differences was checked by F-test, * = $P_{0.05\%}$

Responses to plant density and unevenness

The first hypothesis was, if a genotype is tolerant to the stress caused by plant density, it would be also tolerant to a harder stress caused by plant density plus unevenness. Secondly, that a higher level of tolerance depends on the sink-source relationship.

In term of Y_{max} the tolerant genotypes show different responses to unevenness (Table 2), one of them has strong tolerance, but the other has a high level sensitivity. Because the susceptibility is linked to high PD_{max} at even plant distribution, unevenness seems to be the reason. But the LAI of two hybrids is the same at PD_{max}

Table 2

Effect of plant density and plant distribution within the row on the highest yield (Y_{max}/g m^{-2}) plant density required to Y_{max} (PD_{max}/plants m^{-2}) and sensitivity to dense characterized by plant density interval for high yield (PS/plants m^{-2})

Degree of uneven plant distributions (cv for plant distance)	Genotypes						Differences (Tolerant-sensitive)		
	Tolerant			Sensitive					
	Y_{max}	PD_{max}	PS	Y_{max}	PD_{max}	PS	Y_{max}	PD_{max}	PS
				Short season hybrids					
0.0	1.701a[§]	9.2a	2.4a	1.741a	7.2a	1.6a	-40c	2.0**	0.8***
45.0	1.513cd	6.6d	1.4c	1.784a	6.4ab	1.2ab	-271***[§§]	0.2	0.2*
90.0	1.527cd	6.6d	1.3cd	1.645b	6.5ab	1.3ab	-118*	0.1	0.0
				Long season hybrids					
0.0	2.140a	6.9a	1.9a	1.968a	5.8a	1.2a	172*	1.1***	0.7***
45.0	2.136a	7.2a	1.8a	1.881b	5.8a	1.2a	255**	1.4***	0.6**
90.0	2.156a	6.7ab	1.5ab	1.792c	5.4ab	1.4a	364***	1.3***	0.1

[§] The same letter within the section of column indicates no significant differences at P–0.05, done by Duncan (1955) test.
[§§] The reliability of differences were checked by F-test, at P – 0.05, P – 0.01 and P – 0.001 *, **, ***, respectively.

(Table 3), that is why another motive may be the basis. Because the hybrid, with high level sensitivity is source limit, the reason for it is that this type of genotype is tolerant enough for plant density, but in the case of a higher level stress it becomes sensitive. The two sensitive hybrids present similar responses to unevenness, only the sink limit one has a slightly higher tolerance than the provisional one.

Table 3

Classification of genotypes in term of sink-source relationship at PD_{max}

Hybrid	PD_{max}	LAI	Grain to leaf area unit (g 1000 cm^{-2})	Sink-source relationship
		Short season		
Tolerant	9.2	4.6a*	358.7c	source limit
Sensitive	7.2	3.2b	568.2a	sink limit
		Long season		
Tolerant	6.9	4.5a	485.5b	provisional
Sensitive	5.8	3.8b	497.8b	provisional

* The same letter within the section of column indicates significant differences at P – 0.05, done by Duncan (1955) test.

Except for tolerant source limit genotype, the PD_{max} of uneven treatment was roughly as high as that of even distribution. In the case of exception, the PD_{max} decreases substantially.

In respect of changing of PS there are also two groups similar to the above tendencies. In the case of tolerant source limit hybrid, the PS decreases significantly on influence of unevenness, but the others do not show this tendency.

The second hypothesis seems to support that a high level tolerance to the stresses caused by both plant density and unevenness depends on the sink-source relationship. Consequently, only the long growing season hybrids are suitable to investigate the clear genotype effect, because both of them are similar or provisional in terms of sink-source relationship.

In the case of long growing season groups, if the unevenness increases, the difference between tolerant and sensitive hybrids in Y_{max} also increases. The basis of this fact is, that the Y_{max} of tolerant hybrid does not change, but at the same time that of sensitive hybrid decreases significantly. The differences in PD_{max} between the two types of hybrids are independent from unevenness. At the same time the sensitivity (PS) of two different types becomes similar with increasing unevenness. However, the responses of plant density tolerant and sensitive genotype to unevenness in PD_{max} and PS are similar, but that of in Y_{max} quite different. Comparing similar genotypes in term of sink-source relationship, the first hypothesis even seems to be proved. The tolerant genotype becomes also tolerant to a harder stress in the same level as at even distribution. At the same time the sensitive one becomes more sensitive to the stress caused by both density and unevenness.

Table 4

Lodging (L) and stalk break (B) in per cent affected by both plant density and plant distribution

Plant density (plants m-²)	Hybrids: Short season, Tolerant L	Tolerant B	Sensitive L	Sensitive B	Long season, Tolerant L	Tolerant B	Sensitive L	Sensitive B	All together L	All together B
					Even plant distribution (cv=0.0)					
4.0	8.8a*	0.1a	8.8a	1.3a	0.1a	1.3a	12.1a	1.3a	7.5a	1.0a
6.0	9.2a	1.7a	34.2bc	0.8a	6.5a	0.8a	31.7cd	0.8a	20.4bc	1.1a
8.0	28.8bc	0.1a	21.3b	0.6a	2.5a	1.3a	51.3ef	1.9a	26.0cd	1.0a
10.0	21.0b	0.5a	20.0b	0.5a	15.5ab	0.5a	21.8ab	1.0a	19.6bc	0.6a
12.0	42.2cd	2.6a	24.6b	0.9a	13.8ab	1.3a	38.7d	1.3a	29.8cd	1.5a
14.0	26.9bc	2.2a	28.5bc	1.9a	63.2fg	0.7a	71.9hi	1.5a	47.6fg	1.6a
					Medium uneven plant distribution (cv=45)					
4.0	3.8a	1.3a	9.3a	0.1a	0.1a	0.1a	22.9a	0.1a	9.0a	0.3a
6.0	15.8b	0.9a	2.5a	0.1a	2.5a	0.8a	46.7cd	0.9a	16.9ab	0.7a
8.0	32.5bc	1.3a	8.1a	1.3a	6.3a	0.1a	54.5ef	0.7a	25.4bc	0.8a
10.0	29.5bc	0.5a	6.6a	0.1a	9.3a	0.1a	35.7bc	0.1a	20.3b	0.2a
12.0	33.6bc	0.1a	24.6bc	0.9a	16.4ab	0.4a	45.9cd	0.4a	30.1bc	0.4a
14.0	54.6cd	2.0a	25.9bc	1.5a	63.4gh	0.7a	56.9ef	1.1a	50.1de	0.8a
					High uneven plant distribution (cv=90)					
4.0	13.8a	0.1a	10.0a	1.3a	0.1a	0.1a	28.2a	0.1a	13.0a	0.3a
6.0	31.3bc	0.1a	9.3a	0.1a	0.8a	0.1a	43.3gh	0.1a	21.1ab	0.0a
8.0	40.6cd	0.6a	28.3bc	0.6a	21.7bc	0.6a	43.3gh	0.6a	33.5b	0.6a
10.0	13.6a	1.0a	34.8bc	0.1a	13.6b	0.1a	58.0hi	1.1a	30.4b	0.5a
12.0	28.8bc	0.9a	41.5c	4.2a	28.8c	0.1a	65.2ij	0.9a	37.3bc	1.5a
14.0	56.1cd	1.5a	31.5bc	0.4a	56.1fg	0.1a	54.9h	0.7a	40.6cd	0.7a

* The same letter within the section of column indicates no significant differences at P – 0.05%, done by Duncan (1955) test.

Standing ability

Standing ability involves partly stalk break, partly lodging. They are depending on one hand on their parameters, on the other hand on leaf, stalk, tassel, ear areas, which are exposed to the windpressure, as well as to macro- and microclimate. While from the elements of macroclimate the wind is one of the most important, many micro-ecosystem features would also be important e.g. standing ability of bordered plots, the direction of wind etc. Most of the recently mentioned features are known as bases of investigating error.

However, the lowest density treatments caused the lowest lodging, and lodging seems to be increased by unevenness, but no consequent effects caused by increasing unevenness are registered (Table 4). In respect of stalk break neither genotype, nor plant density nor unevenness effects are presented. These facts are mainly due to macro- and microclimate and ecosystem that affect the standing ability in a large part. That is why, instead of lodging and stalk break, their elements are investigated. Because the evenness had no consequent effect on lodging, only the plant density treatments were investigated.

The choice of investigated elements is based on the earlier findings. While the lodging is practically fully controlled by root strength, the stalk break is affected by the some elements. It depends on stalk diameter, health of pith tissue and the rind strength (Daynard et al., 1979; Zuber et al., 1980). Because the health of pith tissue depends on the sink-source relatonship (Zuber and Loesh, 1966; Tollenaar, 1977) and the investigated hybrids differ in this term, the clear effect of plant density is not shown by the present collection of genotypes. For these reasons the measurement of the health of pith tissue was neglected from the presented research program.

The basic question is, whether the tendency of differences among the hybrids is similar to that of shown by grain yield? In the case of root strength, although there are differences between hybrids (Table 5), the tendency both in average at PD_{max} and in sensitivity characterized by b-value of linear correlation is not similar to that of in grain yield. The rind puncture shows also a genotype effect, but it is not compared to the yield pattern. In respect of stalk diameter (neither the parameter at PD_{max} density, nor b-values of linear correlation) hybrids do not differ within the groups.

Having no similar pattern in elements of lodging and stalk break to that of grain yield, a question is raised, whether the variability of plants in the population is influenced by genotypes. Except for one case, the higher plant density shows a higher variability, but the differences among the hybrids are not significant (Table 7).

Table 5

Response to the plant density in features of standing ability

Hybrids	Parameter at PD_{max} plant density	β-values of linear correlation	r^2
	Root strength/pull (MJ plant^{-1})		
	Short season		
Tolerant	106.5b+	–4.42b	94++
Sensitive	112.8a	–5.66a	97
	Long season		
Tolerant	114.6a	–5.10a	96
Sensitive	106.6b	–4.71a	94
	Rind puncture (MJ mm^{-2})		
	Short season		
Tolerant	4.5a	–0.13a	95
Sensitive	4.0b	–0.10b	99
	Long season		
Tolerant	4.9a	–0.14a	96
Sensitive	4.7a	–0.14a	97
	Stalk diameter (mm)		
	Short season		
Tolerant	24.0a	–0.78a	98
Sensitive	22.8a	–0.78a	93
	Long season		
Tolerant	25.1a	–0.88a	98
Sensitive	25.5a	–0.75a	96

+ The same letter within the section of column indicates no significant differences at P – 0.05 done by Duncan (1955) test
++ All r^2 are significant at $P_{0.001}$

Some differences were observed in stalk shape (Table 6). If the plant density increases, the stalks form increasing changes to ring shape, but the differences among the hybrids in this respect are not compared to that of yield.

Table 6

Effect of plant density on stalk shape indicated by thin thick^{-1} diameters

Plant density (plants m^{-2})	Hybrids			
	Short season		Long season	
	Tolerant	Sensitive	Tolerant	Sensitive
4.0	0.83b+	0.76d	0.87a	0.86b
6.0	0.86ab	0.83c	0.88a	0.89ab
8.0	0.87a	0.85bc	0.89a	0.92a
10.0	0.87a	0.86ab	0.88a	0.90a
12.0	0.88a	0.87a	0.89a	0.91a
14.0	0.89a	0.87a	0.89a	0.91a

+ The same letter within the section of column indicates no significant differences at P – 0.05, done by Duncan (1955) test.

Table 7

The variability for features of standing ability affected by plant density

Plant density (plants m^{-2})	Hybrids											
	Short season		Long season		Short season		Long season		Short season		long season	
	tolerant	sensitive										
			T	S	T	S	T	S	T	S	T	S
	Stalk diameters (mm)				Rind puncture (NJ mm^{-2})				Root strength (NJ plant^{-1})			
4.0	6.07d*	8.02a	5.65c	5.90cd	8.46c	8.91c	6.91d	6.96e	16.48e	15.15e	14.16c	18.40e
6.0	8.87bc	7.20a	5.27c	6.52c	8.63c	8.61c	9.70c	9.63d	18.81d	19.06d	15.98c	20.68d
8.0	7.67c	7.87a	5.62c	5.80d	10.63b	9.83c	11.20b	11.00c	21.61c	22.11c	20.80b	21.85cd
10.0	10.07ab	7.87a	6.80b	8.32b	13.89a	13.09b	13.21a	10.88c	25.33b	21.61c	22.18ab	23.28c
12.0	11.37a	8.97a	7.90a	8.90ab	14.36a	14.55a	11.51b	12.53b	29.03a	28.78b	24.45a	30.13a
14.0	10.12ab	9.25a	7.95a	9.37a	14.00a	15.10a	12.33ab	14.03a	29.61a	30.98a	24.01a	27.11b

* The same letter within the column indicates no significant differences at P – 0.05 done by Duncan (1955) test.

In general the responses of genotypes to the plant density in standing ability and their elements differ from that in yield. Among these elements there are some connections. If both the rind puncture and stalh diameter are low, the shape of stalk becomes significantly similar to ring, with higher plant density till 10.0 plants m^{-2}. If only the rind puncture is relatively low, the stalk shape becomes significantly more ring-shaped with higher plant density till 6.0 plants m^{-2}. In the case of high rind puncture and thick stalk, the stalk shape does not change with the plant density. After these tendencies the above- mentioned three groups were observed.

No relationship existed between root and stalk strength.

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PHOSPHORUS MANAGEMENT PRACTICES ON GROWTH AND YIELD OF SOYBEAN (*GLYCINE MAX* (L.) MERRILL)

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This field experiment was conducted in 1987 to evaluate the efficiency of rock phosphate in comparison with levels of single super phosphate, and combined effect of phosphate solubilizing microorganism on the availability of phosphorus from rock phosphate. The experimental station is located at a longitude of 77° 35' E, lattitude of 12°, 58' N and altitude of 930 metres above mean sea level. The soil is red sandy loam in texture with slightly acidic pH and the crop was raised under irrigated condition. The maximum seed yield of 2453 kg/ha was obtained with 80 kg/ha P_2O_5 as single superphosphate, while the next higher yield of 2380 kg/ha was obtained with 40 kg/ha P_2O_5 as single superphosphate + 40 kg/ha P_2O_5 as rock phosphate treated with *Aspergillus awamori*. The seed yield of soybean with these two treatments are significantly more than the seed yield reported from other treatments. The yield components like number of pods/plant (35.13 to 40.93), weight of pod/plant (29.93 to 32.40 g), number of seeds/pod (3.73 to 3.83) and 100-seed weight (11.98 to 12.38 g) and seed weight/plant (13.63 to 14.26 g/plant) were also more with 80 kg/ha P_2O_5 as SSP and 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* i.e., T_9 compared to other treatments.

Keywords: phosphorus management, rock phosphate, phosphate solubilizing microorganisms, *Aspergillus awamori,* soybean yield, irrigated condition

Introduction

Phosphorus is one of the critical nutrient elements for increasing crop growth and yield. The necessity and significance of nutrient phosphorus is keenly felt since most of the soils are deficient in phosphorus and hence much attention has being given to fertilization during the recent past. The efficiency of phosphatic fertilizers can be increased through several techniques. The rock phosphate is found to be more efficient than other phosphates in acid soils (Natarajan et al., 1983; Mengel, 1986). Recent research data have also shown that the efficiency of rock phosphate can be increased either by mixing the organic measures or phosphate mineralising micro-organisms (Patil et al., 1979; Anon, 1981). Compared to other phosphatic fertilizers, rook phosphate is cheaper and hence a proper utilization of this resource is considered important in view of the increased cost of synthetic phosphatic fertilizers (Geethakumari, 1981). Research information on the use of rock phosphate as a phosphatic fertilizer have been generated but it was felt that the available information is very meagre and, as such, generating further information is greatly relevant under the present energy crisis. With this view, the present study was initiated to assess the response of soybean to different phosphorus management practices on red sandy loams of Bangalore.

Materials and methods

A field experiment was conducted during *Kharif* (rainy season) 1987 at the Agronomy Field Unit, Main Research Station, University of Agricultural Sciences, Bangalore, India. The experimental station is located at a longitude of 77° 35'E, latitude of 12° 58'N and altitude of 930 metres above mean sea level. The soils of the experimental site is red sandy loam in texture with 31.63% sand, 6.88% silt and 22.37% clay at 0–15 cm soil depth.

The site was acidic in reaction (pH 6.2) with EC of 0.09 to 0.13 m.mhos/cm at 25 °C, and with medium fertility having 278, 33 and 150 kg/ha of available N, P_2O_5 and K_2O, respectively. The methods used for characterizing organic carbon, available N, P_2O_5 and K_2O were Walkey and Black (Wet oxidation method) method, alkaline permanganat method, Brace method and neutral normal ammonium acetate extraction method, respectively (Jackson, 1973).

There were nine treatments viz., T_1 – No Phosphorus, T_2 – 40 kg/ha P_2O_5 as SSP, T_3 – 40 kg/ha P_2O_5 as Rock phosphate (RP), T_4 – 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori*, T_5 – 80 kg/ha P_2O_5 as SSP, T_6 – 80 kg/ha P_2O_5 as RP, T_7 – 80 kg/ha P_2O_5 as RP treated with *Aspergillus awamori*, T_8 – 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP and T_9 – 40 as RP treated with *Aspergillus awamori*.

The commercial single superphosphate (SSP) containing 16% P_2O_5, available in the market was used. Whereas Mussorie rock phosphate (RP) supplied by Pyrites and Phosphate Chemicals Limited, containing 22.0% P_2O_5, 38.50% CaO, 5.00% MgO, 4.00% sulphide sulphur, 0.10% sulphate sulphur and 1.14% organic carbon was applied.

The *Aspergillus awamori* fungus (a phosphorus-solubilizing heterotrophic fungus known to metabolize insoluble phosphorus was isolated from the rhizosphere of the plant grown in rock phosphate deposited area) was used to treat the rock phosphate as per the treatments, which were laid out in a Randomized Block Design and replicated thrice. The size of the experimental plots was 3.0×5.0 m² and in all there were 27 plots. The soybean-Hardee seeds were sown on 31st July 1987 following 30 cm × 10 cm spacing.

Immediately after sowing, the *Aspergillus awamori* culture was broadcasted at the rate of 375 g/ha in the seedline on the respective treatments and covered with a thin layer of soil. The blade hoe was drawn manually when the crop was 25 days old, to control the weeds and to stir the soil. One spray of monocrotophos and mancozeb was given on the 40th day after sowing as a prophylactic measure against insects and diseases. The irrigation was given throughout the crop period at 50% available soil moisture depletion, by taking rainwater into account and following the surface method of irrigation.

Results and discussion

Plant growth is dependent on the rate of accumulation of dry matter during growth. The daily rate of dry matter accumulation varied due to different phosphorus management practices. Ramakrishnegowda (1981), working on different cowpea genotypes, reported that there was a rapid increase in the dry matter production up to the flowering stage and subsequently the growth rate was very low. Geethakumari (1981) observed that three-fourths of the total dry matter was produced in pulses from the beginning of bud initiation to 10% flowering.

The data on dry matter accumulation is given in Table 1. The highest daily rate of dry matter accumulation of 2.33 g/day/plant, during 45 to 60 days after sowing, was obtained with 80 kg/ha P_2O_5 as SSP followed by 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* (2.21 g/day/plant). The lower daily rate of dry matter accumulation (1.04 g/day/plant) was obtained with no phosphorus treatment. The reasons for a higher daily rate of dry matter accumulation, with 80 kg/ha P_2O_5 as SSP and 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori*, may possibly be due to higher dry matter production through increased plant height, more number of branches and physiologically active green leaves retained for a longer period; in turn, higher leaf activity might have encouraged increased photosynthetic activity.

Table 1

Effect of phosphorus management practices on dry matter accumulation (g/plant), plant height, number of branches and leaf area during different growth stages

Treatment details		Dry matter accumulation (g/plant)				Plant height (cm)			Number of branches/plant			Leaf area (cm^2/plant)		
		Days after sowing												
		30	45	60	110	45	60	110	45	60	110	30	45	60
T_1	Control (no phosphorus)	1.40	1.70	15.62	14.73	19.20	23.98	29.69	1.73	2.23	2.03	207.53	395.52	416.98
T_2	40 kg/h P_2O_5 as SSP	3.67	4.30	28.35	27.43	25.56	33.95	39.30	2.66	3.10	3.63	326.83	485.72	560.58
T_3	40 kg/h P_2O_5 as RP	1.66	2.30	17.25	17.16	20.30	25.96	30.02	1.86	2.33	2.97	229.65	405.28	434.21
T_4	40 kg/h P_2O_5 as RP +*Aspergillus awamori*	2.72	2.89	18.83	18.81	20.50	28.12	34.23	1.97	2.53	3.10	262.73	445.96	475.52
T_5	80 kg/h P_2O_5 as SSP	6.38	7.98	34.96	37.00	29.86	45.24	43.83	3.66	4.00	4.40	390.66	617.56	643.06
T_6	80 kg/h P_2O_5 as RP	2.49	3.38	23.16	21.13	21.65	29.61	35.78	2.21	2.80	3.26	271.27	463.35	522.95
T_7	80 kg/h P_2O_5 as RP +*Aspergillus awamori*	2.96	3.94	24.68	24.83	24.30	31.66	37.91	2.53	3.06	3.51	301.39	465.20	521.52
T_8	40 kg/h P_2O_5 as SSP +40 kg/h P_2O_5 as RP	4.26	5.19	31.30	30.16	25.98	35.88	40.45	2.96	3.46	3.73	340.63	491.42	580.29
T_9	40 kg/h P_2O_5 as SSP +40 kg/h P_2O_5 as RP +*Aspergillus awamori*	5.41	5.73	33.22	344.90	27.15	36.75	41.80	3.43	3.80	4.20	362.15	586.01	612.33
F-Test		**	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D.$_{5\%}$		0.85	1.41	3.19	2.35	5.07	4.32	3.73	0.61	0.84	0.57	16.51	15.07	22.81

The increase in the different vegetative parts during early stages through the efficient utilisation of nitrogen by the crop in the presence of adequate phosphoric acid was also reported by Venugopalan and Morachan (1974). Singh and Singh (1968) noticed a steady increase in the dry weight of pulses as the amount of phosphorus increased. A continuous supply of phosphorus during the period of crop growth from higher level of water soluble phosphate and combined use of water soluble and rock phosphate treated with *Aspergillus awamori* mineralization process was reported by several workers (Vidyasekharan et al., 1973). Ahmed and Jha (1977), while reviewing the effect of rock phosphate treated with *Aspergillus awamori,* stated that mineralization of insoluble phosphate was enhanced with the production of organic acids, and thereby they release slowly.

The efficiency of rock phosphate can be improved and made comparable to superphosphate by partial acidulation or by using it in conjunction with super phosphate in the ratio of 60:40, especially for potatoes and wheat, as reported by Marwaha and Kumar (1981). The efficacy of rock phosphate in neutral and alkaline soils could be considerably increased by mixing it with pyrite in a suitable proportion. They also suggested the possibility of using a mixture of rock phosphate and super phosphate as another alternative for increased efficacy of various combinations of rock phosphate and low grade pyrite, or super phosphate on neutral soils (Gupta and Mishra, 1978; Mishra et al., 1983).

Poojari et al. (1988) also reported that in neutral to calcareous soils, an application of rock phosphate and super phosphate mixture in the ratio of 1:2 or 1:3 was comparable with super phosphate at the same rate of application in a Rice - groundnut cropping sequence.

The economic yield, as the part of the biological yield of the crop and dry matter production, is important for determination of the grain yield (Donald and Hamblin, 1962). Assessments of total dry matter production during the growth period under different phosphorus management practices (Table 1) indicated that higher total dry matter of 37.00 g/plant was obtained with 80 kg/ha P_2O_5 as SSP, followed by 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* (34.90 g/plant); and the lower dry matter (14.73 g/plant) was obtained with no phosphorus treatment. In the present study, plants with no phosphorus treatment recorded low plant growth rates and resulted in early senescence and quick drying of leaves, which in turn reduced the size of the photosynthesizing surface, causing reduction in crop growth due to the lower rate of assimilation. This consequently reduced the total straw yield production and, thus, the seed yield.

Since the production of a number of branches and leaf area and plant height (Table 1) can be taken as an indication of the total dry matter production of the plant during the vegetative phase, these growth components can be considered for substantiating the straw yield variations.

The maximum number of branches per plant was attained at harvest and at this period the treatment with 80 kg/ha P_2O_5 as SSP i,e., T_5 (4.4 branches/plant) and 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* i.e. T_9 (4.20 branches/plant) recorded the higher number. The lowest branch number at

harvest was recorded with no phosphorus i.e. T_1 (2.03 branches/plant) and 40 kg/ha P_2O_5 as RP i.e., T_3 (2.97 branches/plant). A similar trend was observed with plant height.

The additional straw yield in crop plants due to more branches per plant, which in turn provide for greater photosynthetic activity, was also reported by Loomis and Williams (1973). A maximum leaf area (634.06 cm²/plant) was recorded at 60 days after sowing with 80 kg/ha P_2O_5 as SSP i.e. T_5, followed by 40 kg/ha as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* i.e. T_9 (612.33 cm²/plant) and the low leaf area (416.98 cm²/plant) was recorded with no phosphorus treatment i.e. T_1.

A similar trend in the leaf area was noticed at an early stage of plant growth i.e. at the 30th and 45th days after sowing. The higher leaf area, with 80 kg/ha P_2O_5 as SSP and 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori,* may be due to more leaves, which in turn produced the higher assimilating area to have more photosynthesis. In soybean the increase in leaf area index due to phosphorus application was reported by Tarila et al. (1977) in cowpea.

A maximum seed yield of 2453 kg/ha was obtained with 80 kg/ha P_2O_5 as SSP i.e. T_5, followed by 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori* i.e. T_9 (2380 kg/ha). Both were found statistically on par (Table 2).

The lower seed yield of 1491 kg/ha was obtained with no phosphorus i.e. T_1, followed by 40 kg/ha P_2O_5 as RP i.e. T_3 (1526 kg/ha). The higher seed yield, with 60 kg/ha P_2O_5 as SSP and 40 kg/ha P_2O_5 as SSP + 40 kg/ha P_2O_5 as RP treated with *Aspergillus awamori,* might have been due to an adequate supply of phosphorus throughout the growth period. However, the increase in available phosphorus during the crop growth period was also observed whenever the rock phosphate was treated with solubilizer, as reported by Srikantha (1987). However, Mishra et al. (1980) reported that the advantage of using superphosphate in combination with rock phosphate lies in the fact that an adequate supply of readily available phosphorus in early stages of plant growth helps in its proper establishment, which can later utilize rock phosphate phosphorus much more efficiently during the remaining growth period.

Mishra et al. (1986), working on potato, observed that an application of single superphosphate alone was on par with a mixture of rock phosphate and single super phosphate in 1:1 ratio on the total phosphorus basis. Munerasiadiqui et al. (1986) reported that the effectiveness of Mussorie rock phosphate alone was very poor. However, in combination with single superphosphate in the ratio of 50:50 on an equivalent P_2O_5 basis, it was considered superior.

Table 2

Effect of phosphorus management partices on yield and its components

Treatment details		Biological yield (kg/ha)	Straw yield (kg/ha)	Seed yield (kg/ha)	No.of pods/ plant	Pod length (cm)	No.of seeds/ pod	Pod weight (g/plant)	Seed weight (g/plant)	100 seed weight (g)
T_1	Control (no phosphorus)	3277	1786	1491	210.30	3.26	3.00	12.40	7.30	10.10
T_2	40 kg/h P_2O_5 as SSP	4255	2299	1956	29.70	3.26	3.50	23.23	10.05	11.23
T_3	40 kg/h P_2O_5 as RP	3350	1824	1526	22.00	3.83	3.06	13.63	7.90	10.30
T_4	40 kg/h P_2O_5 as RP +*Aspergillus awamori*	3481	1851	1630	23.68	3.31	3.20	15.26	8.26	10.50
T_5	80 kg/h P_2O_5 as SSP	5247	2794	2453	4.93	3.87	3.83	32.40	14.26	12.38
T_6	80 kg/h P_2O_5 as RP	3690	1972	1718	26.36	3.46	3.33	17.30	8.96	10.70
T_7	80 kg/h P_2O_5 as RP +*Aspergillus awamori*	3781	1981	1800	28.66	3.45	3.43	20.80	9.30	10.93
T_8	40 kg/h P_2O_5 as SSP +40 kg/h P_2O_5 as RP	4675	2542	2133	30.53	3.80	3.51	25.70	11.73	11.50
T_9	40 kg/h P_2O_5 as SSP +40 kg/h P_2O_5 as RP +*Aspergillus awamori*	5130	2750	2380	35.13	3.83	3.73	29.93	13.63	11.98
F-Test		**	**	**	**	NS	NS	**	**	**
$L.S.D._{5\%}$		565.00	363.91	227.80	3.17	-	-	2.25	1.07	0.61

NS: Non-significant

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SPREAD OF CLRV IN AN OLD WALNUT PLANTATION IN TRANSDANUBIA AND THE EFFECT OF THE ROOTSTOCK ON THE TREE DECLINE

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The paper reports the results – originated from an old Transdanubian walnut plantation – on studying effects of the rootstocks for the spreading time of the virus and for the decline of trees.

The study was performed in four years (1981, 1982, 1989 and 1991) by using two methods: visual observation and ELISA.

The initial infection in 1981 and 1982 was 24.2% and 48.4%, respectively, and by 1989 it increased to 96.8%. On the one hand, there was no difference in the extent of infection between the trees of the two rootstocks but there was a great one, on the other hand, in the ratio of the dead trees. Trees on *Juglans nigra* died in 1981: 6.7%, in 1982: 13.3%, in 1989: 50% and in 1991: 63.2%. Death of trees on rootstocks *Juglans regia* started in 1982 with 3.3% and in 1991, only 16.7% of the trees died despite the fact that the condition of the infected trees was very bad.

It was found that with the increase in the number of infected trees the extent of infection in the plantation increases drastically. The extent of total tree decline on rootstock *J. nigra* is higher, because of the effect of the blackline, than on rootstock *J. regia*. *J. nigra* is a hypersensitive rootstock. Performance of trees of longer life on infected rootstock *J. regia* is greatly decreased and these trees provide a source of infection for a long time in the plantation.

Keywords: cherry leaf roll virus, walnut, survey, effect of rootstock, *Juglans regia, J. nigra,* hypersensitivity, ELISA

Introduction

Virus symptoms on walnuts were first observed in Bulgaria. On the basis of the symptoms Christoff (1958) called this disease walnut line pattern. The next data come from Italy, where Savino et al. (1976) reported on two diseases which were thought different: walnut ringspot and walnut yellow mosaic.

From these latter viruses it rapidly turned out to be caused by two strains of the cherry leaf roll virus (CLRV) and they were called CLRV-WYM and CLRV-WRS strains (Savino et al., 1977; Quacquarelli and Savino, 1977). In England, CLRV strains different from the Italian strains were found on walnut (Cooper 1979; 1980). The virus also occurs on walnut trees in Czechoslovakia (Novak and Lanzova, 1981) and in France (Delbos et al., 1983).

As the incompatibility (so not as an infectious disease) of *J. regia* and some walnut rootstocks (*J. hindsii,* Paradox, *J. nigra*), the walnut blackline disease was known in the 1920s in the states of Oregon and California. Similar incompatibility was recorded in 1954 in France and in 1965 in England (cit. Mircetich et al., 1980a).

In recent years it has also been shown that this walnut disease has been caused by one strain of the CLRV (Mircetich et al., 1980b; 1980c).

In Hungary the virus symptoms on walnut leaves were first observed in 1958 and called, after Christoff (1958), walnut line pattern (Németh,1979).

The identification of the virus was made with herbaceous indicator plants and ELISA in 1982, and the Hungarian CLRV isolates were called CLRV-WH (Németh et al., 1982).

Further research in Hungary included development of virus diagnostic methods both for testing the bearing trees and seeds (Kölber et al., 1983; Kölber and Németh, 1983) as well as a survey for the national spread of the virus.

This paper reports on the spread of the virus in a walnut plantation of 30 years in Transdanubia (the first germplasm collection containing the selected Hungarian varieties), as well as on the effect of rootstock on tree decline.

Materials and methods

A) The studied plantation and the varieties

The plantation was established in 1959–1961 on medium heavy soil in Transdanubia.

The propagation material was taken from the grafts of mother trees of regional varieties originating from the first selection of the major Hungarian walnut growing areas, with 2 trees per variety.

Table 1

Varieties in the plantation in the order of their establishment

30 TKSz	40 TKSz	102 TKSz
47 TKSz	31 TKSz	92 TKSz
E 7917	33 TKSz	E 7908
121 TKSz	Eszterházi 2	98 TKSz
610 TKSz - Tahi	M 27	44 TKSz
103 TKSz	97 TKSz	37 TKSz
101 TKSz	66 TKSz	E 7900
28 TKSz	35 TKSz	51 TKSz
104 TKSz	A 117	32 TKSz
94 TKSz	123 TKSz	T 34
Fertődi 51	96 TKSz	*J. regia praepartiensis*
Dömsödi 8	E 7912	

The trees stand on *J. regia* and *J. nigra* rootstocks.

B) Dates and methods of investigations

Investigations were made in four years (1981, 1982, 1989 and 1991) by using two methods: visual observation and ELISA.

Visual observations

Visual observations were made three times a year: infection of trees was determined twice between May and July on the basis of leaf symptoms, and once in August on the basis of leaf, graft union and fruit symptoms. Symptoms on the infected trees can be summarized as follows:

First symptoms of the disease: poor terminal growth, yellowing and drooping leaves, premature fall of leaves. In the following phase the apical shoots decline, leading finally, to the death of the tree (Fig. 1). In the case of CLRV infected English walnut trees propagated on *J. nigra* the typical blackline symptom can be found. A narrow strip of dead cambial tissue becomes visible at the junction of the scion and rootstock and profuse suckers grow from the rootstock (Fig. 2). In certain years symptoms appear on the leaves, also: chlorotic and necrotic ringspots, rings and line pattern accompanied in some cases by rusty necrosis (Fig. 3). In some cultivars on the green hull (Fig. 4) and stone fruits, characteristic bumps develop.

ELISA

ELISA test procedures followed those developed by Clark and Adams (1977) with some modifications. The conjugation of CLRV antiserum (prepared against "CLRV 57 Isolat" in Aschersleben, Germany) was made with Hungarian horseradish peroxidase enzyme by the Human Institute for Serobacteriological Production and Research (Budapest). To evaluate the results, extinction values above 0.100 were taken as positive. The plant material was ground in PBS-Tween buffer containing 2% PVP 30 (MW 44000) and 0.1% ovalbumin.

Results and discussion

On the basis of visual observations and ELISA tests made during 4 years the following results were obtained:

The initial infection in 1981 was 24.2%, in 1982: 48.4% and by 1989 it increased to 96.8% (Fig. 5). On the one hand, there was no difference in the extent of infection between the trees of the two rootstocks; there was a great one, on the other hand, in the ratio of the dead trees. Trees on *J. nigra* rootstocks died in 1989: 6.7%, in 1982: 13.3%. in 1989: 50%. and in 1991: 63.2%. Death of trees on rootstock *J. nigra* started in 1982 with 3.3%, and in 1991, only 16.7% of the trees died (Fig. 6), despite the fact that the condition of the infected trees was very bad.

The following consequences can be drawn from the above results:

– with the increase in the number of infected trees, the extent of infection in the plantation increases drastically,

– the extent of total tree decline on the hypersensitive rootstock *J. nigra* is higher, because of the effect of the blackline, than on rootstock *J. regia*. Performance of trees of longer life on infected rootstock *J. regia* is, however, greatly decreased and these trees provide sources of infection for a long time in the plantation.

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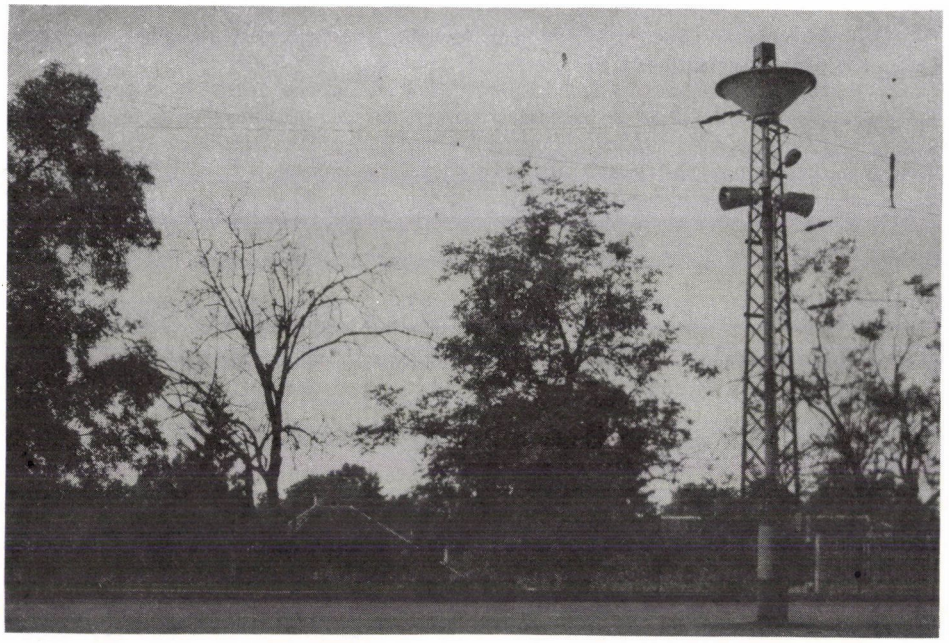

Fig. 1. English walnut trees infected with CLRV-WH at different phases of the disease. Left: healthy tree

Fig. 2. Typical blackline symptoms on English walnut tree grafted on *J. nigra* rootstock at the junction of the scion and rootstock

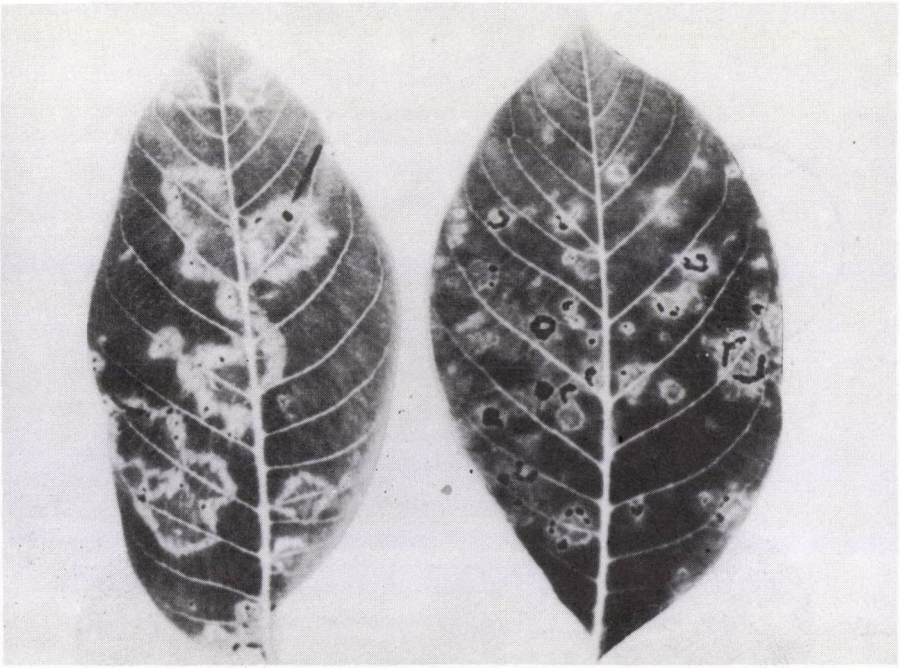

Fig. 3. Chlorotic rings and line pattern and necrotic ringspots, rings on leaves of English walnut trees infected with CLRV-WH

Fig. 4. Characteristic bumps on the green hull of the fruit of English walnut tree infected with CLRV-WH

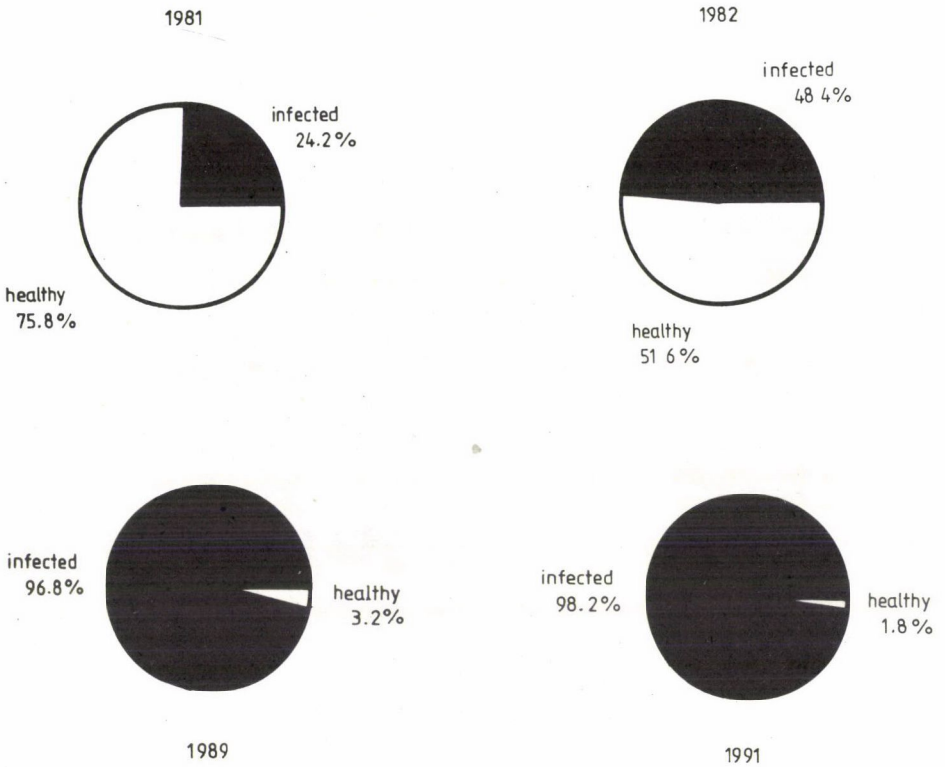

Fig. 5. Spread of CLRV-WH in a Transdanubian walnut orchard planted in 1959/61

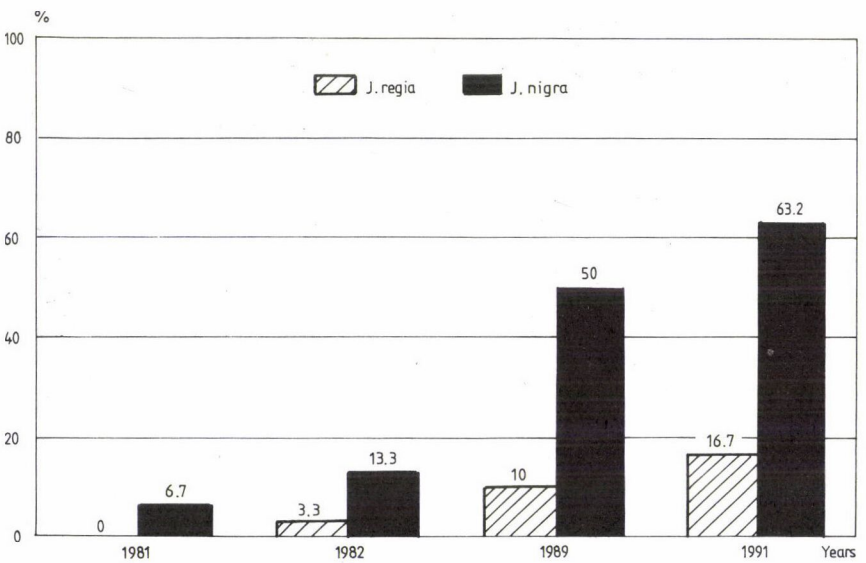

Fig. 6. Percentage of CLRV-WH-killed trees on *J. nigra* and *J. regia* rootstocks

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Plant genetics and breeding

RELATIONSHIP BETWEEN FERTILITY AND SEED CONTENT IN APPLE VARIETIES

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Between 1988 and 1990 the relationship between fruit set and seed content in 14 apple varieties was studied in apple orchards of the Debrecen State Farm. We examined the number of seeds in the case of natural self-pollination, artificial self-pollination and free pollination, in various cross combinations and in three periods of fruit development.

The seed content in the fruit of apple varieties was found to fluctuate from year to year, though to a lesser extent than the fruit set.

In the case of both natural and artificial self-pollination, low seed content was observed. In the case of open pollination, the number of seeds in apples mostly ranged from 5 to 7. Among the triploid varieties *Red Winesap* excelled in seed content, while the varieties Mutsu and *Red Jonagold* contained a very small number of seeds.

On examining the seed content of the cross-combinations we found a very close positive correlation between the seed content and the fruit set. With low seed content the fruit set is of small extent. A medium seed content (5–7 full seed/fruit) is sufficient for the fruit to develop, and in this case, under the influence of other factors the fruit set data may show a wide scatter.

In the case of cross-pollination those pollen donors are considered good the pollen of which induces the development of many seeds in the fruit primordium of the mother variety. In this regard the varieties *Gloster* and *Redspur Delicious* were outstanding. Seedless (parthenocarpic) fruits were found in those variety combinations in which the triploid Mutsu and Red Jonagold varieties were the pollen donors.

Keywords: fruit set, seed content, self-pollination, cross-pollination, open pollination, apple varieties, diploid, triploid

Introduction

The fruit setting and pollinating ability of the fruit varieties are genetically determined properties characteristic of the variety, diversely influenced by ecological factors, year, growing site, conditions of pollination and fruit set, condition of the tree, and combinations of pollen donors. In order to increase the efficiency of fruit growing it is important to know these factors that influence the fruit set, and their effects.

The relationship between the seed content of the apple and the way of the fruit set was recognized as early as at the beginning of the century (Alderman, 1918; Auchter, 1921), and so was the influence of the seed content on the fruit drop (Brittain, 1933).

According to Davary-Nejad and Nyéki (1990) the seed content of the apple is also influenced by the ploidy level of the pollen donor. Davary-Nejad (1990) and Davary-Neyad et al. (1993) found that a more intensive bee visiting increased the extent of fruit set and the seed content of fruit.

The larger the necessary number of seed/fruit, the higher are the density of flowers and the proportion of flowers setting seed. In this respect further progress may be represented by the use of the Index-V (Máthé, 1977), which through the numerical expression of the dynamic of flowering has proved suitable for application to the comparison of apple (*Malus domestica* L.), varieties (Máthé et al., 1993) chamomile (*Matricaria chamomilla L.*) – Máthé et al. (1985) and paprika (*Capsicum annuum* L.) – Máthé and Bahadli (1989). In the case of abundant fruit yield fruits containing fewer than three seeds drop (Teskey and Shoemaker, 1972).

In the initial phase of fruit development the number of seeds has a favourable effect on the size of fruit (Deveronico and Marro, 1980).

In apple trees fruit is only formed when at least a certain number of ovules become fertile. The hormone action required for this is induced by the seed set, and this in turn is influenced by the hormones present in the seed.

Materials and methods

Our investigations were made between 1988 and 1990 in apple orchards planted in three districts of the Debrecen State Farm: Pallag, Tamási puszta and Gut. Most of the apple varieties were planted in 1977 and 1978 with MM 106 rootstock at a spacing of 6 × 4 and 7 × 4 m, respectively. They were trained to free spindle crown form. The orchards were regularly and skilfully tended, irrigation was not carried out.

The fertility studies were made after the method of Rudloff and Schandrel (1950), worked out in detail by Gyúró et al. (1981) for the observation of farm-size orchards.

To examine the self-fertility (autogamy), 20–30 cm long branches in red bud stage were covered with parchment bags in parts of the crown towards the four compass points. Under 10 paper bags, the fruit set in 100-400 flowers was evaluated. We examined the natural self-fertility of the trees (without artificial pollination), and the fruit set of bagged flowers pollinated by their own pollen (geitonogamy) too.

In the different pollination combinations, the extent of fruit set was assessed at three dates, the first being when the non-fertilized fruit primordia dropped. This took place about 3–4 weeks following mass blossoming, varying from year to year.

The second occasion of fruit set evaluation was after June drop, while the third occasion was at the harvest-ripe stage of the fruit. In the ripe apples we counted the *germinable* seeds (containing embryo) and the *empty* ones (only consisting of seed-coat) per carpel. In each fertility examination the evaluation of fruit set and seed content was made as described above.

For the observation of fruit set in the free-standing flowers 200–500 flowers on 10 branches per variety were marked out in the four cardinal points of the crown, at a height of 1.5–2 m. Cross-pollinations were carried out among flowers isolated as described with the examination of self-fertility.

Results and discussion

In the fruit set, fruit development, extent of fruit drop and final size of fruits of apple a highly important role is played by the seed content of fruit primordia.

In some apple varieties even their own pollen induces fruit formation. In most

Table 1

Fruit set of flowers and seed content of fruits in apple varieties (Debrecen, 1988–1990)

Variety	Fruit set (%) full seed/fruit (n)		
	self-fertility	self-pollination	open pollination
Duncan Red Delicious	0.3 / 2.2	0.0 / -	10.2 / 5.8
Golden Delicious "Dánia"	2.0 / 6.0	4.8 / 116	20.1 / 9.1
Granny Smith	0.0 / -	0.0 / -	5.3 / 9.3
Gloster	0.0 / -	0.0 / 5.4	15.5 / 5.4
Idared	0.4 / 5.3	2.2 / 3.7	7.5 / 5.6
Maliga hibrid	0.4 / -	0.3 / -	10.1 / 5.6
Mutsu	2.0 / -	1.5 / -	9.8 / 3.8
Red Winesap	0.0 / -	0.0 / -	17.9 / 8.8
Red Jonagold	0.9 / -	5.3 / -	8.4 / -
Summerred	1.6 / -	0.1 / 0.0	11.1 / 7.1
Watson Jonathan	3.5 / -	4.0 / 2.3	10.8 / 5.7
Average	0.9 / 2.8	1.6 / 1.8	11.7 / 6.2

cases, however, the degree of self-fertility is very low, varies from year to year and is insufficient to achieve a satisfactory level of yield. We examined the effect of natural and artificial self-pollination on the fruit set and seed content, and found in both cases a low level of fruit set and seed content (Table 1). In an artificial way, plus with pollen applied to the stigma, the fruit set increased, but the average number of full seeds decreased. In some cases the fruits did not even contain seed. The maximum number of seed was 6.5 in the case of natural self-pollination and 5.5 with artificial self-pollination.

Free-standing flowers can be visited by the bees undisturbed, and the stigma receive pollen from different varieties. Thus, under favourable conditions, the fertility characteristic of the variety comes into full display and the characteristic seed content can develop. For the comparison of varieties and years the data of open pollination are considered the most suitable, though data obtained from cross-pollination can also be used, since we worked with a large number of variety combinations. Figure 1 shows the differences in seed content between the varieties on the basis of a three-year average. In the case of open pollination the number of seeds in the apples ranged from 5 to 7. Remarkably high values were observed in the fruits of *Golden Delicious "Dania"* (8.7 seed/fruit), *Granny Smith* (9.3 seed/fruit) and *Red Winesap* (triploid) (8.8 seed/fruit). The other two triploid varieties were characterized by a very low number of seed: *Mutsu* 3.8 and *Red Jonagold* 3.8.

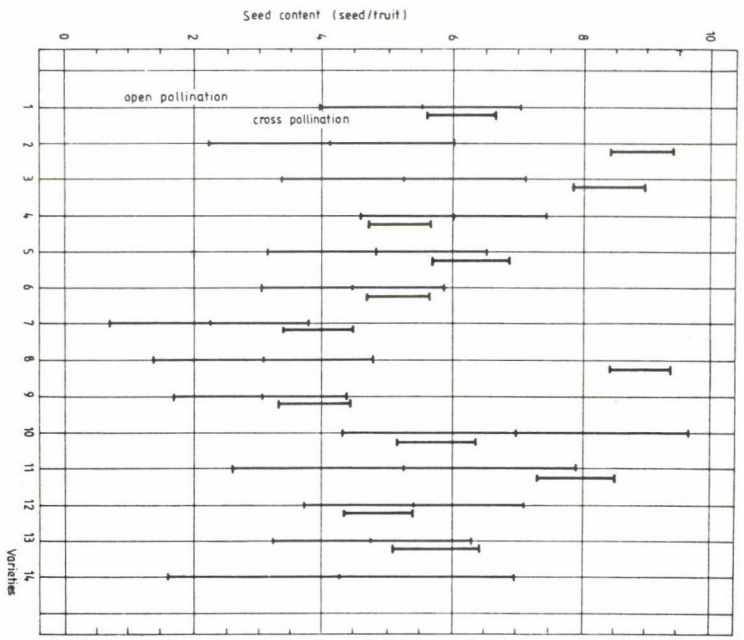

Fig. 1. Full seed content of fruits resulting from open and cross-pollination (Debrecen, 1988–1990)

The yearly variation of the seed content of fruit is of much lower extent than the fluctuation of fruit set. In 1988, following the frost damage of flowers, the number of seeds was found to be relatively high (6.6/fruit). In 1990, when beside a low density of flowers the proportion of fertile flowers was very high, the seed content was lower than average.

The seeds as centres of hormone production and nutrient accumulation have an important part in fruit set and development. A comparison of our self- and open pollination data also show that the extent of fruit set and the seed content of fruit are in correlation.

During the three years of our investigations, we carried out cross-pollination in 68 variety combinations. This number of replications was considered large enough to be taken for basis in calculatings correlation between the seed content of the fruits produced and the extent of fruit set.

The relationship between fruit set and seed content in the various cross-combinations is seen in Fig. 2. There is a very close positive correlation between the seed content and the extent of fruit set. The linear correlation is proved even at a level of p = 0.1%.

On the basis of our observations we have established that at a low seed content the extent of fruit set is low. A medium seed content (5–7 seed/fruit) is sufficient for the normal development of fruit, and in this case the fruit set data may show a very wide scatter.

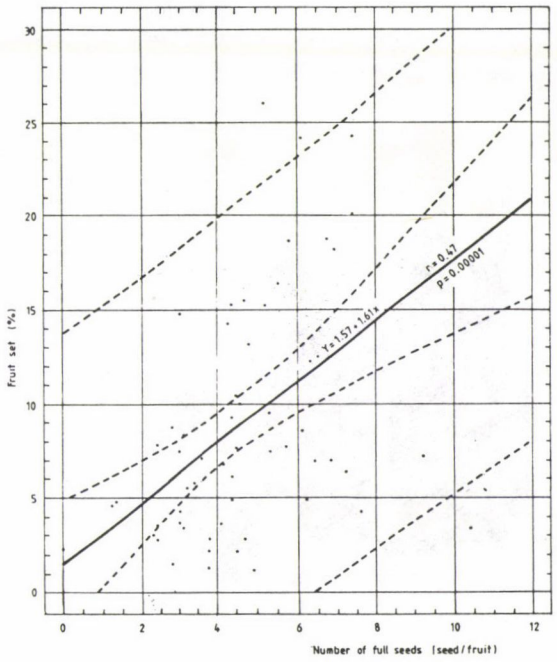

Fig. 2. Relationship between seed content and fruit set of fruits originating from crossing (Debrecen, 1988–1990)

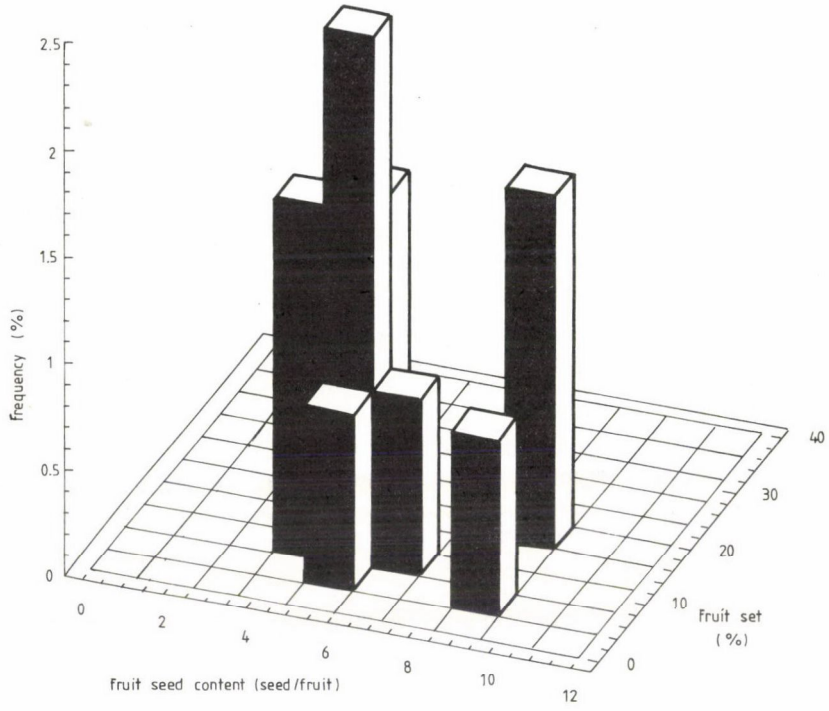

Fig. 3. Distribution of average full seed content and fruit set in apple varieties in the case of open pollination (Debrecen, 1988–1990)

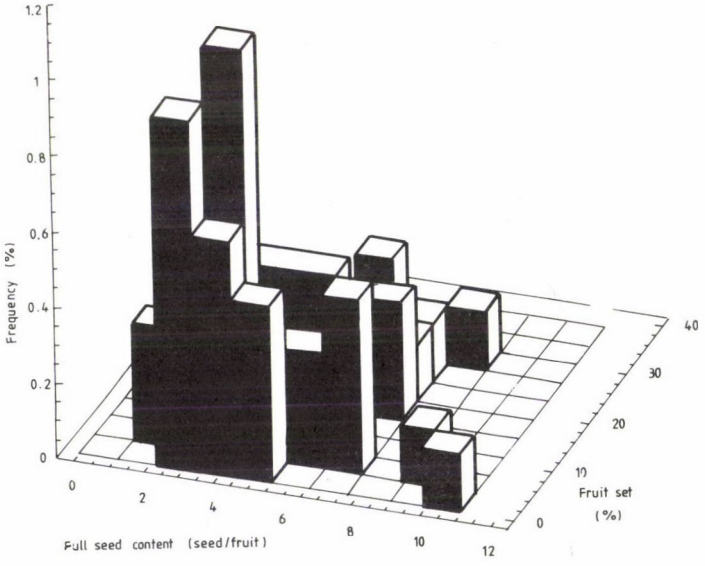

Fig. 4. Average full seed content of cross-combinations and frequency distribution of the extent of fruit set (Debrecen, 1988–1990)

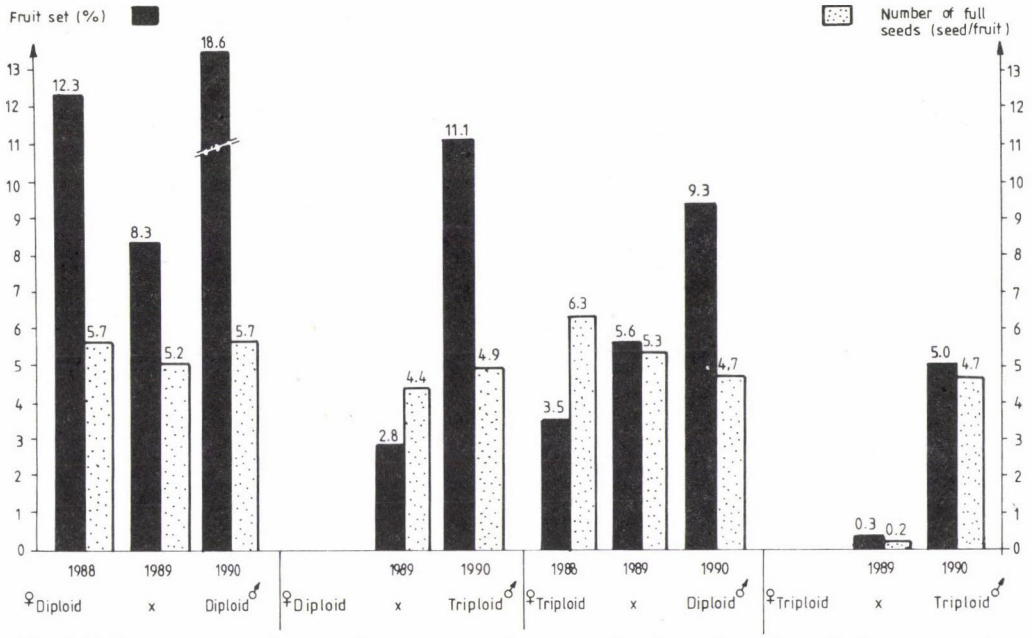

Fig. 5. Full seed content of apples from cross-combinations of various ploidy level (Debrecen, 1990)

In our opinion a sufficient seed content is an indispensable condition for the fruit primordia to remain in the tree. Thus, in the case of cross-pollination, those pollen donors are considered good whose pollen induces the development of many seeds in the fruit primordia of the mother variety.

Fruit set is, however, influenced not only by the number of seeds but by other factors too. That is why some observations seem to contradict the correlation mentioned above. When the environmental conditions of fruit set and development are favourable (or there are few flowers in the trees, as was the case in 1990), then a larger number of fruits containing fewer seeds also remain in the tree thus reducing the average number of full seeds. The distribution of the seed content of fruit and the extent of fruit set in the case of open pollination is shown in Fig. 3, while in the various cross-combinations in Fig. 4.

In the case of open pollination the fruit set in most varieties ranged between 10 and 20%, while the seed number in the larger part of the fruits was 4–5. In the course of cross-pollination those variety combinations were the most frequent where beside a 10–20 % fruit set the fruits contained 2–3 seeds.

The seed contents of fruits produced from the different cross-combinations are contained in Table 2. It can be seen that in most cases the seed content data change together with the fruit set percentages. The high fertility varieties have a higher seed content, and in fruits developed from flowers made fertile by good pollen donor varieties the seed content is also high. The largest number of seeds were found in the fruits of *Golden Delicious "Dánia"* and *Watson Jonathan*, and very few seeds were contained in the fruits of *Gloster* and of the triploid *Mutsu* and *Red Jonagold.*

As pollen donors the varieties *Gloster* and *Redspur Delicious* were outstand-

Table 2

Percentage of fruit set and number of full seeds in the various cross-combinations (Debrecen, 1988–1990)

Variety	1	2	3	4	5	6	7	8	9	10	11	12	Elstar	Malus sp.	Average	Self-fertility	Free-fertility
Duncan Red Delicious		15.5 4.7		15.2 5.2		13.2 4.8		4.8* 4.4							12.2 4.7	0.3 2.2	10.2 5.8
Golden Delicious "Dánia"	24.3 7.4			27.0* 8.2					3.4 3.1			20.1 7.4			19.7 6.5	2.0 6.0	20.0 9.1
Granny Smith				6.9 6.4			0.0 -	3.8 2.4		3.4 10.4			12.2* 4.3		5.3 4.7	0.0 -	5.3 9.3
Gloster	12.3 4.0	8.3 3.1	8.8* 2.8		14.2 4.3				7.8* 2.4			10.0 4.6	10.4 4.5		11.2 3.6	0.0 -	15.5 5.4
Idared				6.8 4.2		7.2 3.6	3.0* 2.3		7.7* 5.7		2.8 4.7	7.5 5.3	2.1 3.8		5.3 4.2	0.4 5.3	7.5 5.6
Maliga hibrid	4.9 6.2				8.6 6.1		8.6 4.4*	6.1 4.4*	1.3 3.8*		12.7 5.8*	7.0 6.8			7.0 5.3	0.4 5.3	10.1 5.6
Mutsu			10.5 3.8		14.8 3.0*	5.5 3.2*		4.8 1.3	0.0 -	7.1 3.6			7.6 4.5		6.1 2.7	2.0 -	9.8 3.8
Red Winesap	6.4 7.2		9.6 9.2	6.6 7.6		7.7 6.0	0.1 0.0		2.3 0.0						4.6 5.0	0.0 -	17.9 8.8
Red Jonagold	3.6 3.0	3.5 2.4		9.3 4.4		4.6 1.2*	1.5 2.8*	4.2 3.0*				1.5 3.0*	5.8 3.4	3.8 4.2	5.4 3.0	0.9 -	8.4 3.8
Redspur Delicious			26.1 5.2			12.5 6.0*	4.9 4.0*		1.2 4.9*						11.6 5.0	0.0 -	14.9 4.3
Summerred	9.5 5.5*				3.6 4.1								16.4 5.5	15.3 4.4	11.1 4.8	1.6 -	11.1 7.1
Watson Jonathan		12.2 6.3		20.2 6.1	18.8 6.7	12.5 6.5			2.2 4.5*				18.3 6.9	6.1	16.7 6.1	3.5 -	10.8 5.7
Average	10.9 5.5	9.8 4.1	13.7 5.2	13.7 6.0	12.0 4.8	9.0 4.8	3.0 2.2	4.5 3.1	3.2 3.0	5.2 7.0	7.7 5.2	10.4 5.4	10.1 4.7	9.5 4.3		0.9 2.8	11.7 6.2
L.S.D.$_{5\%}$ among the average fertility of varieties								4.4 - fruit set (%) 2.39 - seed content (n/fruit)									
Self-fertility average %	0.0 -	4.8 1.6	0.0 -	0.9 -	2.2 3.7	0.3 -	1.5 -	0.0 -	5.3 -	0.0 -	0.1 0.0	4.0 2.3	- -	- -		0.9	11.7

Notes: * = the examination was carried out in 1990 only

Table 3

Percentage fruit set and number of full seeds in the different periods of fruit drop in the case of open pollination (Debrecen, 1988–1990)

Variety	After clearing drop		After June drop		At the time of ripening	
	fruit set (%)	full seed (n)	fruit set (%)	full seed (n)	fruit set (%)	full seed (n)
Duncan Red Delicious	41.5	3.1	30.0	4.3	10.3	5.8
Golden Delicious "Dánia"	48.6	4.9	35.8	7.7	20.0	8.7
Granny Smith	20.3	6.2	6.9	6.9	5.3	9.3
Gloster	43.5	3.3	18.8	5.2	15.6	5.4
Idared	29.7	5.0	9.4	6.3	7.6	5.6
Maliga hybrid	17.6	2.3	10.9	4.2	10.1	5.6
Mutsu	41.1	3.2	31.3	2.3	9.1	3.8
Red Winesap	35.3	7.2	31.2	7.7	17.9	8.8
Red Jonagold	34.7	2.4	10.2	2.9	8.4	3.8
Redspur Delicious	48.1	4.5	36.5	4.6	15.0	5.1
Summerred	25.0	3.7	15.2	4.7	11.1	7.1
Watson Jonathan	39.6	5.1	22.5	5.4	10.8	5.8
Average	35.2	4.2	21.3	5.2	11.8	6.2

ing, because they produced the largest number of seed (6.0 and 7.0/fruit, respectively, on the average). *Seedless/parthenocarpic* fruits were found in those combinations where *Mutsu* and *Red Jonagold* were the pollen donors. The use of pollen from all the three triploid varieties resulted in the development of fruits with very low seed content.

The seed contents of fruits resulting from crossing varieties belonging to groups of the same and of different ploidy level, and the pertaining fruit set proportions are shown in Fig. 5. The diploid varieties when used as pollen donor resulted in the largest number of seed and the highest fruit set percentage, in the case of both the diploid and the triploid varieties. The unsuitability of the triploid varieties as pollen donor was proved by the fact that the fruit set average of such combinations was low and so was the seed content of the fruits.

The variation of the average seed number in the different periods of fruit drop was examined in the case of open pollination (Table 3). Evaluation of the data has led to the following conclusions. Towards the ripening stage the average seed number gradually increases, since fruit primordia containing fewer seeds drop in the first place.

The relationship between seed number and fruit set is supported by the data of Table 3. The differences in seed content characteristic of the variety can be seen already following the clearing drop (?). From clearing drop to ripening the seed content hardly changed in the varieties *Idared, Mutsu, Red Winesap, Redspur Delicious* and *Watson Jonathan*, while a very great extent of change was observed with the variety *Summerred.*

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AGRONOMIC TRAITS OF WHEAT LINES DEVELOPED BY THE DOUBLED HAPLOID, SINGLE SEED DESCENT AND PEDIGREE METHODS AFTER THREE CYCLES OF SELECTION

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Four winter wheat populations were advanced from F_3 to F_7 generation by the doubled haploid (DH), single seed descent (SSD) and pedigree (PD) breeding methods. The best selected lines from each method with the standard variety (Mv 15) were used to compare the relative efficiency of the methods for selection opportunities and maintaining genetic variation. On the average, DH lines were significantly shorter, more resistant to powdery mildew and earlier in days to heading than were SSD and PD lines and the standard variety. As for the yield components, none of the methods revealed superiority over the others, except that DHs showed higher grain weight/spike than did SSD and PD lines. On the other hand, PD lines had higher mean values and showed greater genetic variance than did DH and SSD lines in terms of quality characters (protein content and SDS-sedimentation volume). DHs had similar genetic variance to SSD lines for 7 out of 11 characters studied. These data suggest that the androgenetic doubled haploid method may be efficient not only in time saving, but also in selection toward certain characters such as plant height, disease resistance and earliness. However, pedigree methods seem to be more efficient for improving quality characters.

Keywords: wheat, doubled haploid, single seed descent, pedigree

Introduction

Hybridization, followed by evaluation of the segregating progenies, constitutes the basis of breeding procedures for many self-pollinated crops. In most cases it takes 4 to 6 years to reach the desired level of homozygosity after selection from the segregating population. This period offers a good opportunity for testing the adaptability of lines under different environmental conditions. By the time an advanced line becomes homozygous, it represents an adaptable genotype in the tested environments. The production of homozygous progenies through anther culture from hybrids in a single generation is a property that increases the efficiency of breeding by shortening the time between the original cross and the testing of homozygous progenies (De Buyser et al., 1987). However, the homozygous doubled haploids have to undergo similar selection procedures to test the adaptability of the same properties as advanced lines developed by classical methods. In most articles the agronomic performance of DH lines was compared with lines selected in the traditional way. It is always difficult to compare a population of DH lines with homozygous lines developed by classical methods in F_8 or F_{10}, since the plant breeder retains only the best lines (Henry and De Buyser, 1990). Extensive comparisons have been made on wheat (Winzeler et al., 1987), barley (Friedt et al., 1986) and triticale

(Charmet and Branlard, 1985) between DH lines and lines developed by either the SSD or the PD method. These authors found no significant difference in most agronomic characters, although no conscious selection had been applied in deriving doubled haploid progeny.

In order to get further information about the possibility of involving doubled haploids in wheat breeding, studies were carried out to compare doubled haploids with the respective single seed descent and pedigree lines after each had undergone three cycles of selection.

Materials and methods

Four winter wheat crosses (Mv15-85/Mv15, Mv39-85/Mv15, Mv40-85/Mv15 and Fatima/Mv15) were used in 1987 at Martonvásár to derive lines by the doubled haploid (DH), single seed descent (SSD) and pedigree (PD) breeding methods. From each cross, 50 to 70 spikes were selected in the 3rd generation. Two seeds from each spike were sown and from these plants the creation of DH, SSD and PD lines was started in parallel. One ear of each plant was used for haploid plant induction. The conditions of anther culture were the same as previously described (Abd El-Maksoud and Bedő, 1992). After vernalization, the haploid plants were treated with a 0.04% colchicine solution to achieve rediploidization. From the second ear of the same plant, 2 seeds were taken for the SSD method (as proposed by Goulden, 1939) and the remaining seeds were sown to develop the pedigree lines. Seed multiplication was carried out till the F_7 generation under the influence of selection on the basis of agronomic performance, and only the best lines from each method were involved in this study.

For comparing doubled haploid lines with the SSD and PD lines 23 DH, 28 SSD and 21 PD lines were sown together with the cultivar Martonvásári 15 (Mv15) in 1992 in a completely randomized block design, with 73 entries and four replications, and a plot size of 7.8 m^2.

Data were recorded on each plot for the following traits: 1. Plant height (PLH) in cm from the ground level to the tip of the spike. 2. Days to heading (DTH); number of days from 1st January till 50% of a plot having headed. 3. Powdery mildew infection (PMI) was recorded on a 0 to 9 scale (0= resistant, 9 = susceptible). 4. Spike length (SPL) in cm. 5. Number of spikelets/spike (SPS). 6. Number of grains/spike (GNS). 7. Weight of grains/spike (GWS) in g. 8. 1000 grain weight (TGW) in g. 9. Grain yield (GYP) in kg per plot. 10. Protein content (PRC) % using Trebor-80 apparatus. 11. SDS-sedimentation volume (SDS) in ml, based on the method of Axford et al. (1979). The values for traits 1, 4, 5, 6 and 7 were averaged from 15 randomly selected individual plants for each plot and measurements were made on the main tiller of each plant. All data were subjected to analysis of variance and the comparison of method means was performed according to Cochran and Cox (1957) and the standard error and ranges were also calculated. Estimates of genotypic variance were obtained for each breeding method from the separate analysis of variance based on the procedure outlined by Cockerham (1963). The analysis of variance, including Mv15, was carried out separately for each group of lines to determine the number of lines which were significantly higher (H) or lower (L) than the control variety (Martonvásári 15).

Results and discussion

In the 1st cycle 293 DH_0, 364 SSD (F_4) and 243 PD (F_4) lines were produced on average over the four populations. After 3 cycles of selection, 23 DH (7.8%), 28 SSD (7.7%) and 21 PD (8.6%) lines were left as the best ones derived from the populations. Regarding the individual crosses, the number of lines selected from the different methods varied with the cross as shown in Table 1. The proportion of lines remaining were 5.9% DH, 10% SSD and 7.9% PD from Mv15-85/Mv15; 15.4% DH, 7.3% SSD and 9.4% PD from Mv39-85/Mv15; 18.18% DH, 4.7% SSD and 8.2% PD

from Mv40-85/Mv15; and 8.3% DH, 7.8% SSD and 8.9% PD from Fatima/Mv15. The same general percentage of lines left from each method may be due to the fact that the selection pressure was of similar intensity.

Table 1

Number of lines from each method left after three cycles of selection

Crosses	DH		SSD		PD	
	DH_0	DH_3	F_4	F_7	F_4	F_7
Mv15-85/Mv15	186	11	120	12	63	5
Mv39-85/Mv15	13	2	82	6	64	6
Mv40-85/Mv15	22	4	85	4	49	4
Fatima/Mv15	72	6	77	6	67	6
Total	293	23	364	28	243	21

The comparison of overall means and ranges of all traits (Table 2) reveals that, except for 1000 grain weight, there were statistically significant differences between DHs and the lines derived by the SSD and PD methods. The DH lines were significantly shorter, appeared to be more resistant to powdery mildew and had higher grain weight/spike than were the SSD and PD lines. DH lines were earlier in heading time (about 2 days), and they had longer spikes than the PD lines, but their spikelet number/spike was lower. DHs were significantly better than the SSD lines in the number of grain/spike and protein content. The pedigree lines showed a slight improvement over the doubled haploids and/or SSD lines in terms of number of spikelets/spike and quality characters (protein content and SDS sedimentation volume). Comparing DH, SSD and PD lines to the standard variety (Mv15) they seem to be earlier, shorter in plant height and spike length and lower in terms of 1000 grain weight, protein content and SDS sedimentation volume. In addition, on average the DH lines were more resistant to powdery mildew than were the standard variety. No substantial difference was observed in other agronomic traits. The results agree with those published by Winzeler et al. (1987) in wheat and Friedt and Foroughi-Wehr (1983) in barley, showing that the DHs were shorter and more resistant to disease as compared to those from traditional breeding methods. These results confirmed the fact that the production of more disease resistant DH lines can be achieved via anther culture (Foroughi-Wehr and Friedt, 1984).

The low values of standard errors indicated that most of the lines of each population were close to the mean in most cases. The ranges of the three different populations showed a similar diversity for most of the characters. However, the PD lines gave a wide range of SDS sedimentation volumes, compared to the ranges of DHs and SSD lines, while the SSD lines showed a wide range in terms of 1000 grain weight.

Table 2

Means and ranges for agronomic characters of Mv15 variety and lines advanced by the doubled haploid (DH), single seed descent (SSD) and pedigree breeding methods (Martonvásár 1992)

		DH	SSD	PD	Mv15
PLH	Mean	86.56 ± 0.62a	88.74 ± 0.56b**	89.34 ± 0.52b**	94.01
	Range	74.68 – 97.75	70.90 – 95.65	79.28 – 98.25	
PMI	Mean	5.96 ± 0.16a*	6.42 ± 0.13b	6.43 ± 0.16b	6.25
	Range	3.00 – 8.25	2.50 – 8.00	4.50 – 8.25	
DTH	Mean	141.11 ± 0.30a	141.81 ± 0.27a	143.88 ± 0.21b**	144.50
	Range	137.00 – 147.00	137.25 – 147.75	140.25 – 147.00	
SPL	Mean	8.98 ± 0.09a*	8.96 ± 0.08a,b	8.72 ± 0.09b	9.23
	Range	7.88 – 11.00	7.50 – 10.60	7.05 – 10.36	
SPS	Mean	18.75 ± 0.10a	19.06 ± 0.11a	19.80 ± 0.13b*	19.65
	Range	17.55 – 21.14	16.60 – 20.65	17.00 – 21.07	
GNS	Mean	42.51 ± 0.48a**	40.49 ± 0.45b	41.26 0.45a,b	42.20
	Range	33.63 – 50.70	33.40 – 49.11	32.89 – 47.33	
GWS	Mean	1.65 ± 0.02a*	1.53 ± 0.02b	1.57 ± 0.02b	1.68
	Range	1.29 – 2.09	1.23 – 1.87	1.27 – 1.95	
TGW	Mean	37.21 ± 0.32a	36.51 ± 0.38a	36.30 ± 0.41a	38.02
	Range	31.53 – 42.62	29.21 – 45.09	30.24 – 42.20	
GYP	Mean	7.37 ± 0.08a,b	7.53 ± 0.06a*	7.29 ± 0.08b	7.85
	Range	6.45 – 8.73	6.39 – 8.46	5.67 – 8.46	
PRC	Mean	10.88 ± 0,15a	10.36 ± 0.14b*	10.94 ± 0.20a	11.90
	Range	8.73 – 12.33	8.83 – 12.35	8.82 – 14.00	
SDS	Mean	45.72 ± 0.62a	46.69 ± 0.58a,b	48.46 ± 0.90b**	50.38
	Range	35.50 – 58.25	39.25 – 60.00	33.25 – 60.25	

Note: a, b: Means followed by the same letter in the same row are not significantly different.
*,** significant at the 0.05 and 0.01 levels of probability, respectively.

The relative number of lines that were significantly higher (H) or lower (L) than the standard variety (Martonvásári 15) were calculated and are presented in Table 3. For plant height and powdery mildew resistance, the percentage of lines in the DH population being shorter and more resistant than the standard were higher (78% and 21.7%, respectively) compared to SSD lines (46% and 7%, respectively) and PD lines (57% and 4.7%, respectively). As regards to yield components, 17.4% of DH lines had higher GNS, 21.7% of them higher TGW and 8.7% higher GYP than the standard variety, while these percentages were 7.1%, 17.9% and 0.0%, respectively for SSD lines and 9.5%, 14.3% and 4.8%, respectively for PD lines. In terms of SPS and quality characters (PRC and SDS), a higher percentage of PD lines were significantly better than the standard variety, compared to the DH and SSD populations.

Highly significant genetic variation among the lines was detected in all traits studied, except for the protein content of SSD lines, which was significant only at the 0.05 level of probability (Table 4). Approximately 7 out of 11 characters showed similar genetic variances in both DH and SSD lines. Higher genetic variances were obtained in the PD population than in the DH and SSD populations for quality

characters (PRC and SDS). On the other hand, lower values of genetic variance were obtained for DH lines, compared to SSD and PD lines, for number of spikelets/spike and 1000 grain weight. When we compared the genetic variances maintained by DH to that of SSD (ratio of DH/SSD genetic variances), the differences were significant in the case of grain yield and protein content, while in a comparison of DH to PD (ratio of DH/PD genetic variances), it was significant for days to heading.

Table 3

The relative number of lines with significantly higher (H) or lower (L) values than the Mv15 variety

Characters	DH			SSD			PD		
	H	%	L	H	%	L	H	%	L
PLH	4.3		78.3	0.0		46.4	4.8		57.1
PMI	13.0		21.7	25.0		7.1	14.2		4.7
DTH	4.3		65.2	10.7		78.6	19.0		33.3
SPL	13.0		39.1	17.9		46.4	9.5		47.6
SPS	4.3		43.5	10.7		39.3	38.1		23.8
GNS	17.4		13.0	7.1		35.7	9.5		23.8
GWS	8.7		8.7	10.7		35.7	4.8		38.1
TGW	21.7		34.8	17.9		35.7	14.3		47.6
GYP	8.7		39.1	0.0		21.4	4.8		47.6
PRC	0.0		26.1	0.0		42.9	4.8		33.3
SDS	4.3		60.9	14.3		57.1	19.0		38.1

Table 4

Estimates of genetic variances for agronomic characters between lines within the groups of DH, SSD and PD lines

Characters	DH	SSD	PD	Genetic variance ratio DH/SSD	Genetic variance ratio DH/PD
PLH	31.93**	27.93**	18.19**	1.16	1.76
PMI	1.43**	1.37*	1.05**	1.04	1.36
DTH	8.44**	8.31**	3.41**	1.02	2.48*
SPL	0.55**	0.57**	0.62**	0.96	0.89
SPS	0.67**	1.05**	1.07**	0.64	0.63
GNS	13.46**	14.57**	12.69**	0.92	1.06
AWS	0.03**	0.03*	0.03**	1.00	1.00
TGW	7.69**	13.30**	12.75**	0.58	0.60
GYP	0.35**	0.17**	0.43**	2.06*	0.81
PRC	0.81**	0.35*	1.61**	2.31*	0.50
SDS	29.65**	29.91**	56.69**	0.99	0.52

*,** significant at the 0.05 and 0.01 levels of probability, respectively.

It should be mentioned that the populations used in this study inherited a semi-dwarf recessive gene (Rht8) and a 1B/1R translocation that controls quality characters from Mv 15 (one of the parents). DHs had a shorter plant height and a poorer

quality of characters (PC and SDS) than did PD lines and the standard variety. These results can be explained by the fact that the recessive characteristics and recombinants are fixed in the DH lines in one generation. In this way, the appearance of these recessive characteristics and recombinants was twice as high with the haploid breeding method as with diploid breeding methods (Hu Han, 1986).

Judging by the means, ranges, relative number of lines with higher or lower values than the standard and the magnitude of genetic variance, it can be concluded that androgenetic doubled haploids of wheat are identical to SSD lines in most agronomic characters and this agrees with the results published by Henry et al. (1988) and Choo et al. (1982). The DH method is efficient not only for saving time (De Buyser et al., 1987), but also gave a possibility of selection towards certain characters, such as shorter, earlier and more disease resistant plant types. The PD method was only more efficient for quality characters.

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GENETIC ANALYSIS OF DIALLEL CROSSES IN EGYPTIAN CLOVER *TRIFOLIUM ALEXANDRIUM* L.

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Five genotypes, including commercial cultivar Giza 1 of Egyptian clover, were crossed in a complete diallel. The results indicated that both additive and non-additive gene effects were involved in determining the performance of fresh and dry forage yield. The significant heterosis obtained over the better of mid-parental values for fresh and dry forage yields were correlated with positive significant s.c.a. effects of the respective hybrids. Five hybrids showed highly significant heterosis over the better parent for dry seasonal yield. Significant positive g.c.a. effects were shown by the parent Giza 1 "good general combiners" and could be exploited for breeding programs.

Keywords: *Trifolium alexandrium* L., diallel crosses, heterosis effect, seasonal yield

Introduction

Egyptian clover (*Trifolium alexandrinum* L.) is a major winter forage crop in Egypt. However, it has received little attention from both breeders and geneticists.

The study of combining ability is necessary in breeding programs to develop synthetic varieties, especially in forage crops. Limited studies of combining ability have been reported for Egyptian clover, but most studies in forage crops have been for alfalfa. Katta et al. (1980) reported that general and specific combining abilities were significant for green and dry yields at different cuts and annual yield in Egyptian clover under dense planting.

The main objectives of this study were to determine the relative importance of both general and specific combining ability and reciprocal effects, and to determine the magnitude of heterosis for forage yield in crosses of selected collections and Giza 1 of multi-cut Egyptian clover, after two generations of selfing.

Materials and methods

The present study was carried out during three successive seasons at the Experimental Farm of Assiut University, Egypt. Fifty-six multicut Egyptian clover accessions that belong to Miskawi type were evaluated and reported by Bakheit (1986). According to the results of that evaluation, four different genotypes from four different locations from Egypt were selected, one genotype from each of Sohag (P_1), Qena (P_2), El-Minia (P_3) and Assiut (P_4). The four chosen parents with the multicut variety Giza 1 (P_5) were used as parents in a complete full diallel crossing system, after two generations of selfing according to El-Gazzah and Chalabi (1981).

In the first season, the ten straight hybrids were made, each in a separate site. The crossing block of each hybrid included a large number of paternal plants and a few ones from a maternal parent near a honeybee-hive. Time and space isolation among the sites and the near Egyptian clover were considered (different sowing dates in different locations with

1 km between these locations). The crossed seeds were obtained from the maternal plants at each site. In the second season, the same procedure was followed to obtain the reciprocal hybrid seed.

In the third season, 600 seeds from each of the twenty-five entries (5 parents, 10 hybrids and 10 reciprocals) were evaluated in a randomized complete block design with three replications. Plot size was one m^2. All cultural practices were applied as recommended for Egyptian clover production. Four cuts were taken from each entry at 75, 110, 140 and 168 days after sowing. The characters studied were fresh forage yield (kg/m^2) and dry forage yield (g/m^2) for each cut and seasonal forage yield.

The data were subjected to the genetic analysis proposed by Griffing (1956) Method 3, model 1. Heterotic effects were computed as the percentage deviation of F_1 mean performance from its mid-parent and better parent.

Results and discussion

The analysis of variance for fresh and dry weights of different cuts and seasonal yields are presented in Table 1. There were significant differences among entries for both fresh and dry forage yields. Mean squares due to general combining ability "g.c.a." were significant for both traits, except for the fresh forage yield of the second cut. Likewise, specific combining ability "s.c.a." mean squares were significant for all cuts and annual fresh and dry forage yields. The results indicated that both additive and non-additive gene effects were involved in determining the performance of fresh and dry forage yields. These results are in general agreement with those reported by Katta et al. (1980).

Table 1

Analysis of variance for fresh and dry forage yield of different cuts in 5×5 diallel cross of Egyptian clover

Source of variation	Mean square				
	1st cut	2nd cut	3rd cut	4th cut	Seasonal
			Fresh forage yield		
Blocks	0.03	0.03	0.24*	0.09	1.55*
Entries	0.42**	0.20**	0.33**	0.28**	2.55**
g.c.a.	0.64**	0.07	0.57**	0.50**	2.96**
s.c.a.	0.60**	0.36**	0.13*	0.36**	4.54**
Recip. effects	0.14**	0.10	0.05	0.12*	0.39
Error	0.04	0.05	0.05	0.05	0.35
			Dry forage yield		
Blocks	3752.50	1452.00	5531.50**	3444.00	30920.00
Entries	5932.23**	4156.42**	7303.54**	14624.42**	6476.67**
g.c.a.	8210.00**	3703.50*	6467.00**	27017.00**	114952.00**
s.c.a.	7907.08**	6996.65**	13000.39**	15076.90**	97497.56**
Recip. effects	3046.17*	1497.42	1941.33	9214.68**	11962.42
Error	1476.58*	1105.96	1003.42	2541.83	11931.33

* and ** significant at 0.05 and 0.01 level of probability, respectively.

The reciprocal effects were only significant for first and fourth cuts in both fresh and dry forage yields (Table 1). The reciprocal effects in Egyptian clover were also reported by Katta et al. (1980).

Significant positive g.c.a. effects (g_i) were shown by the parents P_1 for high fresh and dry forage yields in the first and second cuts (Table 2), and by P_4 and P_5 in the other cuts and seasonal yields. Theoretically, an estimate of g.c.a. effect of a cultivar is not an absolute estimate. It depends upon the group of cultivars to which this particular cultivar was crossed in the diallel crossing system. If the cultivar is exactly average g.c.a., the exposed estimate (g_i) would be zero. Significant departures from zero, either positive or negative, would indicate that the cultivar is much better or much poorer than the average of the group involved with it in the diallel crossing system.

Table 2

GCA estimates for the two characters in a diallel cross of five parents of Egyptian clover.

No. of cuts	Parents					S.E.	
	1	2	3	4	5	g_i	g_i–g_j
				Fresh forage yield			
1st	0.142	–0.228	0.016	–0.040	0.109	0.032	0.051
2nd	0.072	–0.060	0.006	–0.013	–0.005	0.037	0.059
3rd	–0.099	–0.106	–0.003	0.074	0.134	0.036	0.057
4th	–0.117	–0.093	–0.070	0.127	0.153	0.036	0.056
Seasonal	–0.134	–0.405	–0.065	0.187	0.416	0.096	0.152
				Dry forage yield			
1st	16.580	–24.587	–8.687	7.847	8.847	6.28	9.92
2nd	13.967	–10.133	–5.500	–8.233	9.900	5.43	8.59
3rd	–14.833	–14.633	0.267	13.667	15.533	5.17	8.18
4th	–33.653	–16.053	–13.753	33.880	29.580	8.23	13.02
Seasonal	–25.347	–80.347	–17.847	49.954	73.587	17.84	28.20

S. E. = standard error of general combining ability for parents (g_i) and to compare any two parents (g_i-g_j).

Estimates of s.c.a. effects for all possible combinations are presented in Table 3. Four hybrids ($P_2 \times P_5$, $P_3 \times P_5$, $P_2 \times P_4$ and $P_1 \times P_2$) showed positive significant s.c.a. effects for both seasonal fresh and dry forage yields. In contrast to the g.c.a. effects, s.c.a. represented dominance and epistatic components of genetic variation which are non-fixable, and can be related to heterosis. Significant s.c.a. effects of a cultivar indicate that the cultivar behaved in that particular cross in a way not expected on the basis of its g.c.a. effect.

Table 3

Estimates of specific combining ability of the hybrids (above diagonal) and reciprocal (below diagonal) of seasonal fresh and dry forage yields

Parents	Parents 1	2	3	4	5
			Seasonal fresh forage yield		
1		0.205	0.509	0.184	–0.517
2	–0.215		–0.476	0.651	1.312
3	0.397	0.183		0.112	0.974
4	0.080	–0.052	–0.050		–0.224
5	–0.122	0.548	0.095	0.278	
S.E.	$S_{ii} = 0.271$		$S_{ij} = 0.198$	$S_{ij}–S_{KL} = 0.196$	
	$r_{ij} = 0.24$		$r_{ij} – r_{KL} = 0.339$		
			Seasonal dry forage yield		
1		–53.053	97.947	36.813	21.680
2	57.667		29.613	94.647	144.180
3	86.833	50.167		12.480	115.347
4	43.167	3.000	–18.333		–17.620
5	19.333	–16.500	45.833	40.000	
S.E.	$S_{ij} = 50.45$		$S_{ij} = 36.77$	$S_{ij}– S_{KL} = 36.41$	
	$r_{ij} = 44.59$		$r_{ij} – r_{KL} = 63.06$		

Standard error of sca of the parents (S_{ij}), hybrids (S_{ij}), reciprocals (r_{ij}), and to compare any two hybrids ($S_{ij}–S_{KL}$), reciprocals ($r_{ij}–r_{KL}$) have no common parent.

The superiority percentages of F_1 hybrids over the better and mid-parental values for fresh and dry forage yields are presented in Table 4. The significant heterosis obtained over the better or mid-parental values for fresh and dry yields were correlated with positive significant s.c.a. effects of the respective hybrids. The five hybrids $P_1 \times P_3$, $P_1 \times P_5$, $P_2 \times P_3$, $P_2 \times P_5$ and $P_3 \times P_5$ showed highly significant heterosis over the better parent for dry seasonal yield of 14.61, 12.70, 17.22, 17.30 and 19.60% respectively. Hence $P_2 \times P_5$ and $P_3 \times P_5$ hybrids could be considered as promising hybrids, since they outyielded the commercial cultivar Giza 1 by 17.30 and 19.60% respectively.

Table 4

Heterosis % over the better parent (above the diagonal) and mid-parent (below diagonal) for the fresh and dry forage yields of Egyptian clover

Parents	Parents 1	2	3	4	5
			Seasonal fresh forage yield		
1		2.821	8.597*	5.166	3.716
2	12.126**		2.871	6.817	18.328**
3	11.595**	9.356*		5.087	18.358**
4	6.510*	17.886**	9.380**		6.352
5	4.011	28.702**	21.289**	8.064*	
			Seasonal dry forage yield		
1		–0.327	14.611**	5.641	12.702*
2	8.066		17.217**	5.823	17.303**
3	12.380**	21.203**		4.560	19.598**
4	10.157*	19.209*	14.272**		8.499
5	14.167**	28.693**	27.168**	11.728**	

* and ** significant at 0.05 and 0.01 level of probability, respectively.

The production of F_1 hybrids appears to be economically feasible, and the heterotic effects of the promising hybrids $P_2 \times P_5$ and $P_3 \times P_5$ could be exploited for breeding programs.

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Reviews

CAUSES AND CONSEQUENCES OF SOIL ACIDIFICATION

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In the course of our investigations we found that the acidification of soils is caused by endogenous (soil and plant life) and exogenous (acid rains, fertilization) factors. In the soil the proton intake of endogenous origin is about twice that of exogenous origin. The amount of protons entering the soil through the "acid rains" is only a fraction (about 10%) of the exogenous protons produced by soil acidifying processes. Not only the fertilizers, but also the farmyard manure acidifies, according to the same mechanism.

The acidifying effect of the nitrogen fertilizers can be reduced by adding limestone or dolomite. The dolomite as an additive is better than the limestone, because the magnesium content of the dolomite increases the utilization of nitrogen and the composition value of the yield, and at the same time reduces the nitrate level. The increased magnesium content of the food and the lower nitrate level are particularly important because of their dietetic significance. Besides, the magnesium prevents the aluminium that dissolves during the soil acidification process from doing damage.

The acidifying effect of superphosphate, a phosphorus fertilizer, has been eliminated by a so-called liming technique, and this fertilizer is put into circulation under the name "limed" superphosphate.

The increased destruction of the Hungarian forests, primarily the sessile oak-forests, is a much debated question. In our opinion, the soil-type dependent and soil acidification caused release of aluminium, also the reduced uptake of water and nutrients (mainly magnesium) originating from its absorption and from the micorrhiza destruction, are responsible for the destruction of forests.

It has also been established that the main contaminants of the atmosphere come from heating (thermal plants, population), and traffic, and only in a negligible part from the chemical industry. In the forests of the Bükk Mountains neighbouring the industrial region of Borsod, a region loaded with the above air contaminants, leaf necrosis originating from atmospheric depositions has not even occurred with diseased trees.

Keywords: acid rains, aluminium, deficiency disease, endogenous and exogenous protonation, fertilization, magnesium, micorrhiza, nitrate, prevention, trachease

Introduction

Under the influence of natural factors and human interference, the acidification of our environment (soil, water) has been alarmingly increasing, especially for over recent years. Parallel with the acidification, the fertility of soils decreases, both in agricultural and forest areas. Acidification is no new problem; Kreybig already wrote in 1928 that in the Schomschich estate (Somogy county) where at the beginning of the century the land produced lucerne in abundance, owing to the acidification, lucerne was hardly produced. At the same time he gave the necessary instructions: liming had to be carried out, and as a result the area again became a good producer of lucerne. At that time (beginning of the century) "acid rains" due to excessive industrialization, and especially

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an overfertilization could not described. Consequently, they are not the only causes of soil acidification, but can and must only be taken into consideration as contributors of acidification. This statement is confirmed by the fertilization experiment conducted by Kreybig (1928) who found that both the farmyard manure and the fertilizers acidified, and the extent of acidification was a function of the type of soil. In originally acid soils the same rate of fertilization caused a higher degree of pH reduction. The acidifying effect of farmyard manure and fertilizer has the same mechanism. Namely, the farmyard manure and the fertilizer contain the same nitrogen active agent: nitrogen is present in them in the form of NH_3, NH_4^+ or NH_2^- . They equally introduce the ion in the soil; that is, they cause acidification either through nitrification, or the exchange of H-ions on the soil colloids, or through the ion-exchanged N-nutrient uptake of plants. Accordingly, a varying extent of acidifying effect decreesing the pH of soil must be reckoned with in the case of any kind (organic and mineral) of plant food. The organic fertilizers acidifying a lower degree than the mineral N-fertilizer with the same N content, because the humus brought about by the former has a compensating effect.

Causes of acidification

Consequently, the causes of acidification, the quantity of protons (H-ion) produced by the different causes and the amount of (dissolved) limestone required to bind them will be examined. The causes can be diccotomized as those of endogenous and those of exogenous origin (Sauerbeck, 1984; Kiss and Dombóvári, 1990), as shown in Table 1. It can be clearly seen in the Table that, through the respiration of the living organisms of soil and roots of plants, 10 kg/ha H-ion (proton) is released annually (Isermann, 1982). It may be mentioned here that the normal carbon dioxide content of air (0.03%), in case of balance, decreases the pH of water to 5.72, i.e. makes it mildly acidic. The average carbon dioxide content of the air of soil ranges from 0.3 to 1.0%, when the pH of the water in contact with it (soil solution) becomes 4.22–4.93 ('Sigmond, 1934). It is thus easy to understand why the carbon dioxide produced by the soil organisms acidifies, introducing some 10 kg H-ion/ha/year in the soil. In addition, some plants discharge 0.1–2.9 kg/ha/year H-ion because of a surplus uptake of cation which are preferred to anions. Owing to the ion exchange cation uptake ("root acids"), the roots of plants send 1.1–2.9 kg/ha/year of protons to the soil.

For the examination of the acidifying effect of fertilizers, we used the data of Murányi and Rédly (1987). With the atmospheric deposits the data given by Mészáros (1984) for the precipitation waters (1.9 $mg.L^{-1}$ S; 0.58 $mg.L^{-1}$ N and 573 mm annual precipitation) were taken into consideration. We left the quantity of proton produced in the course of nitrification of the nitrogen bound from the atmosphere out of consideration. The proton deposition thus calculated agrees closely with the data from Nürnberg (1983), Germany (0.6–1.3 kg/ha/year H-ion), where the rate of industrialization, thus the SO_2 and NO_x contamination of the atmosphere, is higher. It is worth mentioning that, while in 1902 the precipitation contained 0.2 $mg.L^{-1}$ N, in 1983 it even reached a value of 0.5–1.0 $mg.L^{-1}$ in Hungary (Mészáros, 1984). Finally, it must also be noted that not

all of the SO_2 and NO_x present in the air and entering the precipitation, respectively, reach the soil in the form of acid, a part of these may react with natural dust and alkaline substances already in the air. Therefore they do not act as acids. The extent of this neutralization shows a wide seasonal and local fluctuation (Hekes, 1983).

Table 1

H-ions of endogenous and exogenous origin in soils and their estimated values (Sauerbeck, 1984; Kiss and Dombovári, 1990)

Source	H-ion		Dissolved
	kg/ha/year	%	$CaCO_3$ kg/ha/year
Endogenous			
From root and soil respiration	10.0	57.5–39.7	514
From surplus cation uptake	0.1–5.0	0.6–19.7	5.1–257
Through root acids			
Total endogenous:	11.2–17.9	64.4–71.0	576–920
Exogenous			
From atmospheric deposits	0.58–1.0	3.3–4.0	30–31
From N-fertilizers (118 kg N/ha/year)	5.10–5.8	29.4–23.0	262–298
From superphosphate (77 kg P_2O_5/ha/year)	0.5	2.9–2.0	25.7
Total exogenous:	6.18–7.30	35.6–29.0	318–375
Endogenous + exogenous:	17.4–25.2	100	854–1295

When comparing the endogenous effect with the exogenous, we see that the greater part is of endogenous and the lesser of exogenous (atmospheric deposition + fertilizers) origin.

On the basis of the above and of the data of Table 1 we must agree with Isermann (1982), who states that the atmospheric deposition on the agricultural areas (arables) plays only a subordinate role in the H-ion balance; since it makes only 3–5% of the total proton production, and even of the exogenous protons it represents a mere 10–15%. We do not want to be little the importance of the "acid rains", but its exaggeration is not desirable either.

We note here that many are of the opinion that the chemical industry is mainly responsible for the contamination of air. We prove the error of this statement with the data of Table 2, on the basis of a publication by Vas (1990). Accordingly, the main contaminators are the power stations and other heating facilities, while the chemical industry is only responsible in a negligible extent for the discharge of SO_2 and NO_x though local effects can occasionally be noted.

Table 2

Places of formation quantities SO_2 and NO_x emissions in Hungary (Vas 1990)

Place	1980	1985	1990	1995	2000
			1000t/year		
SO_2 emission					
Power plants	709–806	662–746	647–764	882–996	1293
Other charging	668–770	650–749	651–748	669–786	793
Traffic	28–60	28–60	28–60	28–60	60
Chemical industry	18	18	18	18	18
Total Hungarian	1500	1400	1400	1700	1950
Import	1600	1600	1600	1600	1600
Export	2400	2400	2400	2400	2400
NO_x emission					
From fuel	204–245	195–235	193–236	216–236	291
Traffic	38–115	112–140	137–163	140–164	163
Chemical industry	16	16	13	10	10
Total Hungarian	330	360	380	400	430

Acidification of soils

The decrease in the pH value of soils greatly depends on their carbonate (lime) content greatly. Soils in which the free carbonate content ($CaCO_3$, $MgCO_3$) binds the H-ions maintain their original pH for a long time (as long as the carbonates are present), and the pH becomes lower only after the carbonate capacity has been exhausted (Filep, 1988). Accordingly, under the influence of the endogenous and exogenous H-ions, the carbonate content of the soils steadily decreases. Owing to the decreasing lime content of the soils, and the introduced protons, the percentage proportion of the low pH content (acid) soils increases (Podmaniczky, 1988).

According to a survey of MÉM-NAK (Baranyai, 1987), between 1980 and 1986, the pH of soils linearly decreased. The area of acid meadow soils increased almost twofold every 3 years. It is worth determining when the acidification of soils still calcareous will probably begin. A soil containing 0.2% $CaCO_3$ has in its upper 30 cm layer 9000 kg/ha $CaCO_3$. According to the data of Table 1 894–1295 kg/ha $CaCO_3$ can be expected to dissolve annually; that is, the calcium–carbonate content of this soil disappears in 10 year, and the buffering effect stops. Accordingly, in about 10 years a rapid lowering of the soil pH ensues (Kiss and Dombovári, 1990).

Table 3

Percentage of injured roots of Geum urbanum L. seedlings as a function of Ca and Al-concentration (Runge, 1984)

Ca ppm	Aluminium, ppm				
	0	2	4	8	20
0	48.9	95.8	98.9	100.0	100.0
10	13.6	72.5	94.7	93.7	100.0
30	5.5	24.4	41.8	86.0	97.9
60	5.2	19.8	32.3	70.9	97.9
90	1.0	6.5	26.0	66.0	95.0

Soil acidification and field production

Simultaneously with the acidification of soil, the nutrient conversion and, consequently, the volume of yield decrease. The soil pH dependence of various crops

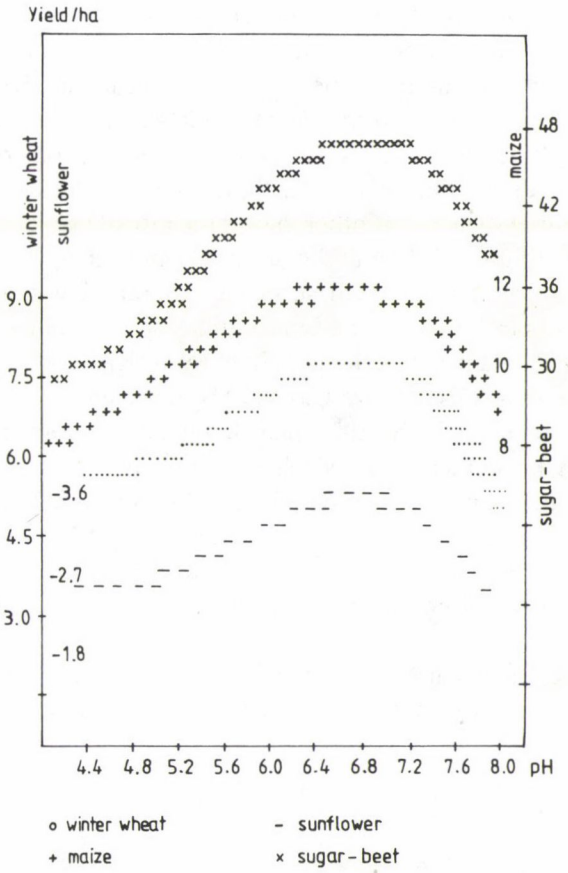

Fig. 1. Effect of changes in pH on the yield of some crops (Baranyai, 1987)

(wheat, sunflower, sugar-beet, maize) is shown in Fig. 1. (Baranyai, 1987). The figure clearly shows the influence of the soil pH on the volume of yield.

Saigusa (1980) examined the effect of soil pH on the length of roots in the case of burdock, barley and maize plants, and found the following correlations:

$y = 5.04x - 19.22$ $r = 0.857$ burdock
$y = 9.41x - 35.78$ $r = 0.872$ barley
$y = 6.51x - 22.24$ $r = 0.720$ maize

where y represents the length of root and x the pH. These equations also show the pH sensitivity of the plants. It is well known that plants with better root system produce larger yields.

According to Runge (1984) the decisive factor is the damaging effect of toxic substances, e.g. aluminium released with the decreasing pH, rather than the pH of soil. The symptom of the damage done by the aluminium is the coralloid growth of roots. This comes about by the necrosis of the apival meristem, from which relatively short-sector root branches appear, whose apices later also necrotize. The demage done by the aluminium may be equally reduced by the Ca and Mg ions. The effect of a change of ratio of aluminium and calcium ions in the soil solution is shown in Table 3 through the root damage of *Geum urbanum* L. It can be seen from the date of the table that the calcium ions have an inhibitory effect on the toxicity of aluminium. Unfortunately, the soil solutions "rich" in aluminium happen to be poor in calcium. Besides, the shortening of roots caused by the aluminium, hinders the uptake of various nutritive elements. The magnesium and calcium contents, in particular, show a considerable reduction (Kiss, 1983). Another cause of this decrease in the nutrient uptake is that the rate of aluminium adsorption is higher than that of other ions. If the rate of uptake of potassium is taken for 1, then that of calcium is 1.49 and the rate of aluminium uptake is 2.30 (Ratner, 1963). According to our examinations, if sufficient calcium and magnesium are present (at concentration higher by an order of magnitude than the concentration of aluminium), they can hinder the damaging effect of aluminium (Kiss, 1983). As already mentioned, it is exactly the acid soils that are Ca- and Mg-deficient.

In acid soils not only the aluminium but the cadmium too does damage (the latter is introduced in the soil with the phosphorus fertilizers). Damages by cadmium can also be prevented with magnesium salts (Kiss and Dombovári, 1990).

The pH-reducing effect of the different composition but identical level NPK fertilization varies with the soil type. These data are given in Table 4 after Murányi and Rédly (1987). As seen from the data of the table the acidity of soils grew by 0.3–0.4 pH in 10 years.

It must be noted that the risk of pH-reduction depends on the initial pH of the soil. Thus, in the case of a soil of 7 pH a 0.5 reduction of pH has almost no damaging effect,

while in a soil of 5 pH the same reduction causes considerable deterioration, which may even be disastrous for some crops.

Table 4

Changes in the pH of various soils in response to fertilizers of identical level but different composition of NPK (Murányi and Rédly, 1987)

Fertilizer dose kg/ha/10 years	On sandy parent material	With clay illuviation	Chernozem
		brown forest soil	
Control	4.7	4.98	5.73
1180 N (NH_4NO_3) 780 P_2O_5 (Superphosphate) 1040 K_2O (KCl)	4.29	4.75	5.50
1180 N (Carbamide) 780 P_2O_5 (Superphosphate) 1040 K_2O (KCl)	4.27	4.75	5.49
1180 N ($NH_4H_2PO_4$) 780 P_2O_5 (Superphosphate) 1040 K_2O (KCl)	4.27	4.69	5.42
Δ pH	–0.43	–0.32	–0.33

Of the fertilizers, those containing nitrogen as active agent carry most H-ions into the soil (Table 1). Therefore the Borsod Chemical Works has prepared a fertilizer containing carbamide as an active agent: the KARDONIT, and the ammonium nitrate-type AGRONIT, which contain a dolomite additive (Kiss, 1983). The dolomite additive binds the arising H-ions; whereby a fertilizer without acidifying effect is available. The examination of N-fertilizers of various type and dose proved that the fertilizers containing dolomite or limestone as additive had no acidifying effect, and spared the environment (Kiss and Kadlicskó, 1984). The N-fertilizers containing magnesium (dolomite) increased the yield better than those of the same N-level without magnesium (e.g. N-fertilizers with limestone as additive). Not only the yield of the crops increased in response to magnesium addition, but so did their magnesium-, protein and sugar content, while the nitrate content decreased (Kiss, 1990). Thus, the dolomite additive while inhibiting the soil acidification better then the limestone, also has a more favourable nutrition-physiological effect.

Among the phosphorus fertilizers the "limed" superphosphate, a product of the Tisza Chemical Works in Szolnok, also belongs to the non-acidifying fertilizers.

Environment acidification and the forests

Besides the agricultural areas, the soils of forests are also considerably acidified. A remarkable destruction of forests, primarily of sessile oak-woods, began in Hungary in 1978, in the Zemplén Mountains. The epidemic spread westward over to the Zala hill-country, which it reached in 1983 (Járó, 1990). The causes of forest destruction are being

studied all over Europe, and according to the general opinion it is due to the acidification of the environment (acid rains, soil, water) (Murach, 1983; Rehfuess, 1983; Huttermann, 1985; Jakucs, 1990, 1990a). Others (Járó, 1990; Führer, 1990; Szendrei-Kòren, 1990) insist upon soil acidification not being the cause if the increasing destruction of forests. The explanation given by Beetz (1984) of the apparent conflict is that healthy and diseased trees exits side by side. Neither the forest nor anything in its natural vicinity will be uniformly ill or attacked by disease. On the basis of 22 forest sectors and 41 soil samples, Szendrei-Koren (1990) indicates that the pH value of the soils does not reflect the health condition of the trees.

She attempts to prove her statement by the example of two forests of nearly the same age growing on soils with different pH (5.7 and 6.7) and titrable acid content ($y_1 = 4$ and 13, where y_1 is 0.1 N NaOH ml spent on neutralizing 100 g soil). According to her examinations, the destruction of forest was less severe on the lower pH soil. Unfortunately, she does not give either the type of soil or the soluble aluminium content, although these data might serve as an explanation.

As opposed to Szendrei and Jakucs (1990), having analysed the soils under the healthy and diseased trees of the industrial region as well as under the control trees, found differences in the pH and some soluble nutritive elements of these soils. The results of the analyses show that, in the acid soils under the diseased trees, the amount of soluble aluminium increased. We attach great importance to this and consider it to be one of the causes of forest destruction.

For the rapid destruction of sessile oak-woods, some authors (Jakucs, 1990, 1990a; Führer, 1990; Berki, 1990; Kovács, 1990) think the restricted uptake of nutrients is responsible. The decrease in the Ca and Mg contents of the soils of some forests in 20 years, with an almost identical and unchanged pH, as well as the great differences in forest destruction (Table 5, Kovács, 1990) suggest a nutrient deficiency as the cause. According to the leaf analysis data by Jakucs (1990) a decrease in the major nutritive elements (except N) was characteristic of the diseased trees while the Al content showed a considerable increase. Similar results were obtained by Rehfuess (1983), who found a 40–50% reduction of calcium and a 60–70% one of magnesium. In contrast to this decrease, the aluminium content increased by 10–40% in the case of diseased trees. According to Kovács (1990) the diseased trees of the forests suffer from magnesium deficiency, which agrees with Rehfuess' results.

Simultaneously with the acidification of soil, the number and activity of micorrhizal fungi living in symbiosis with the thin roots of sessile oaks decrease (Jakucs, 1990a). This symbiosis is similar to that with Rhizobium bacterium characteristic of the papilionaceous crops (root nodule bacteria), and it occurs through the so-called micorrhiza infection. The infectivity of the micorrhizae also depends on the calcium and aluminium ion concentration of the culture fluid (soil solution), in which the calcium ions promote the required infection. The Ca concentration of 2 mequv.L^{-1} resulted in a four-fivefold increase in the degree of infection (settling) compared to the medium containing 1 mequv.L^{-1} Ca (Hepper and Oshea, 1984). The aluminium, on the other

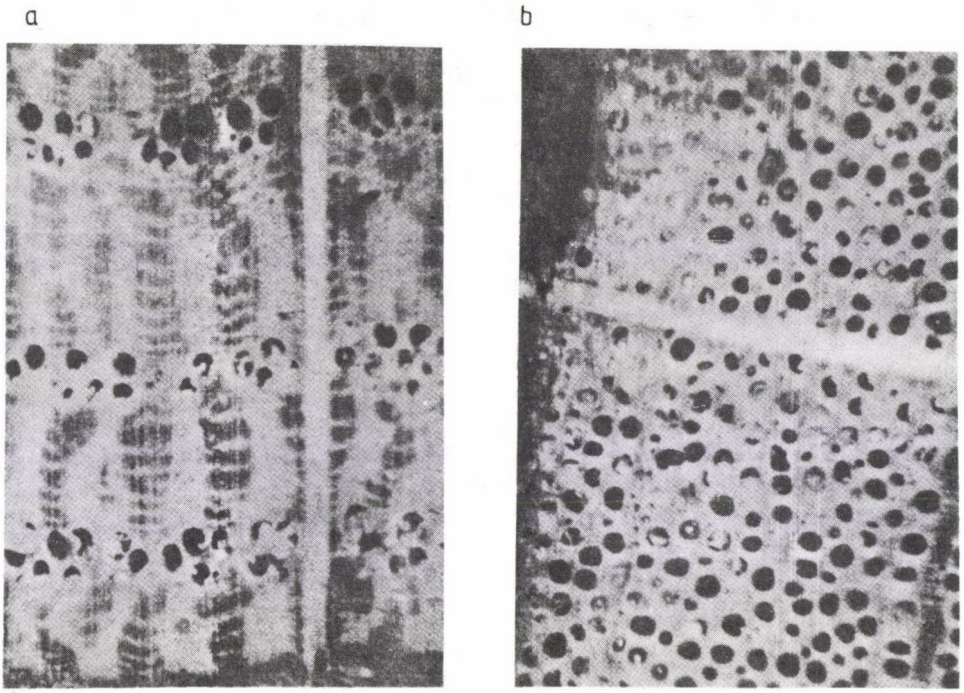

Fig. 2. Blocking off of tracheae in trunk sections from healthy (a) and diseased (b) trees (the black spots are the tracheae blocked off) (Jakucs, 1990)

hand, hinders the settling and symbiosis of micorrhizae, exactly as in the case of Rhizobia (Manzi and Cartwright, 1984; Kiss, 1989).

The micorrhizal fungi play an important role in the water and nutrient uptake of trees; that is, they increase the water and nutrient absorption capacity of the roots threefourfold (Papp and Papp, 1984). The absence or low number of micorrhizae may be one of the causes of the reduced water and nutrient uptake, especially in a warm, dry period. Besides, the transportability of nutrients in the trunk is also inhibited, owing to a negative pressure produced by the transpiration so that (if there is an insufficient supply of solution through the roots) the water columns may break in the tracheae of the trunk and embolia occurs. The tree protects itself against this by blocking the emboliae with various resin-like substances (Thyllises), whereby the possibility of restoring the transportation also ceases. At the time of the abscission of leaves in autumn, a similar phenomenon is in the process of lignification. However, when this process takes place in summer or in spring, when the transpiration and assimilation activity of the leaves would be the highest, it leads to the deficiency disease and destruction of trees. Fig. 2 illustrates dissections made by Jakucs (1990) of tracheae of healthy (a) and diseased (b) trees. The same authors examined the proportion of blocked tracheae during summer, and found it to be 2–6% in healthy and 30–40% in diseased trees. The blocking off of tracheae further reduces the amount of water and nutrients transported, therefore the width of the annual ring of the trees will be limited, and the yield of the forest lower.

Table 5

Changes in the pH, Ca and Ng content (mequv/100g) of soil from 1966 to 1986 on various forest areas, and extent of forest destruction in % (Kovács, 1990)

Place and trees of forest, soil type, depth of layer (cm)		pH		Ca		Mg		Forest destruction%
		1966	1986	1966	1986	1966	1986	
Mátrafüred 1.								
mixed forest, clayey mull-ranker	5–20	5.4	4.7	32.9	25.5	7.3	3.9	sporadic
	60–80	6.3	5.8	36.3	40.6	8.6.	4.4	
Mátrafüred 2.								
sessile oak-wood	0–3	6.4	4.8	53.5	18.0	10.2	3.7	
brown forest soil with clay	5–30	4.3	4.4	28.5	24.8	3.5	2.7	10–20
illuviation,	66–80	5.1	5.9	28.1	47.0	10.9	5.0	
Mátraháza								
mixed forest, brown forest	0–10	5.4	4.9	18.9	9.6	3.8	1.1	20–40
soil with clay illuviation	50–70	5.2	5.2	12.8	10.8	3.5	1.1	

Parent rock: pyroxeneandezite; exposure: southern slope in all three places
Mixed forest: Turkey oak-oak, *(Querco petraeae-cerris subcarpaticum)* maple and cornel *(Acer tataricum,* and *Cronus)*

In Fig. 3 a detail of the trunk cross-section of each of a 45 years old healthy (a) and a 50-year-old diseased (b) pine-tree is shown after Grill's (1984) photo. It is clearly seen that, in the case of the diseased tree, the cells and thereby the annual rings are thin, therefore the output is reduced.

According to Jakucs (1990) necrosis due to atmospheric deposits (SO_2, NO_x) was not found in the forests of Bükk even on the leaves of exposed and diseased trees of the industrial region, and not even at the end of vegetation. In oak-woods Berki (1990) found leaves that turned yellow early but did not necrotize; they showed an intervenial discolouring characteristic of magnesium deficiency. This can be caused partly by a reduced magnesium uptake and partly by the joint action of atmospheric SO_2 and NO_x. Beetz (1984) states that the "simple" (one-component) acid rain does not cause chlorophyll decomposition, because its pH is not below 3.5. On the other hand, if SO_2 and NO_x are present at the same time, then the adduct of these two compounds dissolves the magnesium of the chlorophyll, whereby the leaf loses not only its green colour but also its activity. Since this acid injury extends beyond intervenial area and covers the entire surface of the leaf, the loss of chlorophyll found by Berki to be restricted to the intervenial area must have been caused by nutrient deficiency.

In addition, the increasing aluminium concentration in the leaf also reduces the

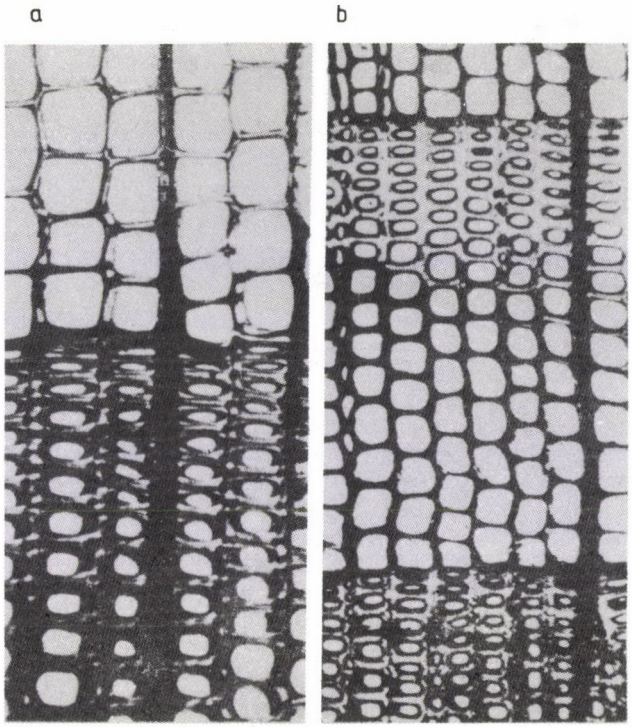

Fig. 3. Details of trunk cross-section from healthy (a) 45-year-old and diseased (b) 50-year-old pine trees (175x magnified) (Grill, 1989)

amount of chlorophyll and inhibits the photosynthesis, while increasing the photorespiration (Sarkunan, 1984). The value of 0.13–0.15 mmol/kg is considered to be the critical concentration of aluminium (depending on the sensitivity of the plant) and it reduces the chlorophyll content and photosynthesis "only" by 10% (Ohki, 1986). This is no enzyme inhibition, it is due to the injury of the chloroplast structure, as proved by Hampp (1976) with an electron microscope photo. The aluminium causes the disintegration of this structure partly by increasing the rigidity of the membrane, which occurs even at 25 μM Al concentration (Deleers, 1986).

We note here that the disintegration of the chloroplast even occurs in the absence of aluminium, at a reduced concentration of magnesium, as pointed out by Kiss and Mustárdi (1985) again by electron microscope examination. So the damages done to the chloroplast structure by the increasing aluminium and decreasing magnesium concentration accumulate and lead to the reduction of the photosynthetic products required for the plant life, that is to the decrease of output or even destruction of the tree.

We think it is important to show that the various environmental factors (acid rain, acidic gases, nutrient deficiency), while equally causing damage to or even destruction of the leaves of trees, have different action mechanisms. In Fig. 4 the different processes of leaf tissue damaging caused by acid rain (4a), acidic gases (4b) and nutrient. deficiency (4c) are seen (Fink, 1989). The acid rain first attacks the epidermis, then the

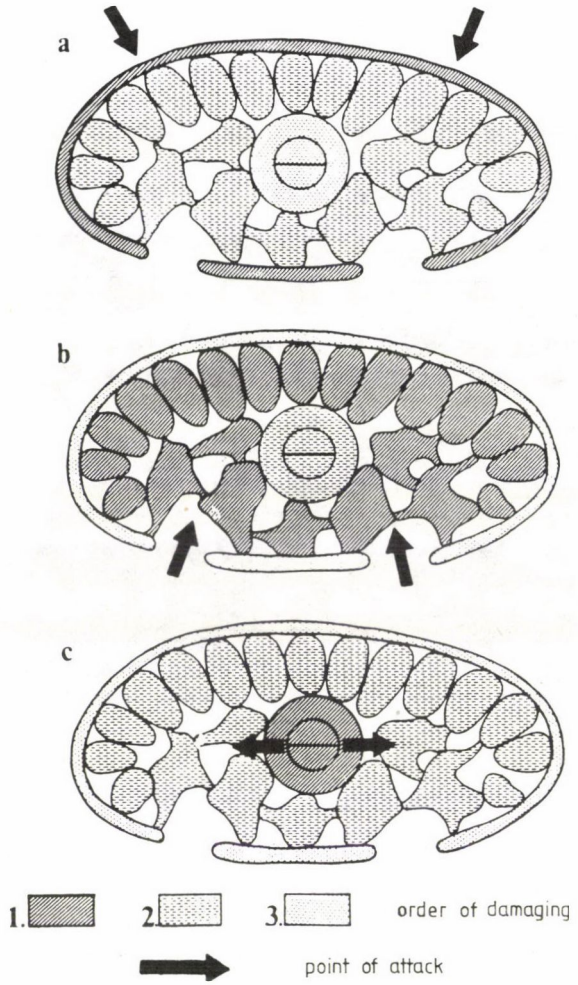

Fig. 4. Dynamics of the damaging action of acid rain, acidic gases and nutrient deficiency on leaves (Fink, 1989)

mesophyllic cells, and only ultimately the vascular bundles. In the case of nutrient deficiency the order of succession is inverted. The acidic gases entering the leaf tissue through the stomata first destroy the mesophyllic cells, then the vascular bundles, and only ultimately damage the epidermis. This observation may help in the subsequent determination of the damaging agent.

As shown by the above, a reduced nutrient content (in soil and plant alike), i.e. deficiency disease was one of the causes of the extensive forest destruction in the recent period, as supported by the forest fertilization experiments carried out by Berki (1990) and Heis and Koberg (1984). Latter authors conducted NPK, and Mg and Ca supplemented fertilization experiments in 30 fertilized and 36 untreated (control) plots in Scotch fir and spruce forests in 3 districts. They found that under the influence of fertilization the number and output of healthy trees was 1.4-fold with spruce and 1.2-fold

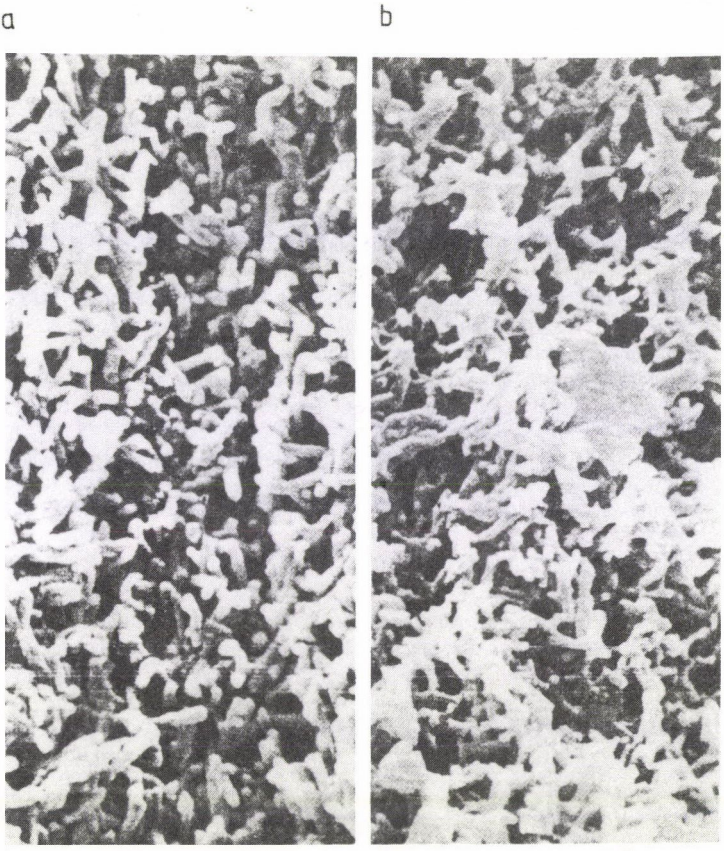

Fig. 5. Microphoto of the waxen coating of healthy (a) pine needles and of those damaged by atmospheric deposition (b) (6000x magnified) (Grill, 1984)

with Scotch fir-compared to the untreated plots. Accordingly, the harmful effect on forests of soil acidification and a subsequent nutrient reduction as well as of acid rains can be lessened with NPK+Mg+Ca fertilization.

The other form of damage caused by the acidic deposits is the destruction of the waxen coating of leaves (pine needles). The microphotographs taken by Grill (1984) in Fig. 5 show the waxen coating of healthy pine needles (a) and of those damaged by atmospheric deposition (b). Owing to the damage done to the waxen coating, the evaporation of the leaves increases, the loss of water will be great, which combined with the reduced water and nutrient uptake caused by the blocking of tracheae and absence of micorrhiza may result in a "sudden" death of trees.

In summation, as for the effect of acidification on agriculture and sylviculture, the damage seems to occur mostly (or almost exclusively) through the soil. The low nutrient and symbiotic microorganism (micorrhiza) content of the acid soils, further, the increased concentration of soluble aluminium in the soil solution, are the causes of a decrease in the production of plants and of the increased destruction of forests.

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BIOTECHNOLOGY IN *POPULUS* SPECIES: AN OVERVIEW

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Populus species have become one of the most intensively cultivated and investigated forest trees. The increasing number of articles published in the international papers on *Populus* seems to substantiate this claim. This review tries to provide a short guide on the *in vitro* methods applied for and also on the results achieved on poplar species.

Keywords: *Populus, in vitro* micropropagation, tissue culture, haploid induction, protoplast isolation, genetic transformation

Introduction

In vitro techniques of forest species have developed dramatically over ten years. Among the woody plants, various species of the genus *Populus* have been studied extensively for growth and differentiation in relation to rapid clonal propagation; particularly, propagation of difficult-to-root aspen (*Populus tremula*). At the level of micropropagation, two methods (axillary budding and adventitious budding) are now commonly used. Callusing and neoformation of buds, because of genetic instability are rarely applied, though these methods have been used on aspen (Boulay, 1987). The commercially applied micropropagation method for aspen was reported by Ahuja (1983 and 1984). Nevertheless, commercial micropropagation of poplar species and also other woody species remains limited. Limitation is based on the need to rejuvenate elite trees selected at a mature stage.

The main methods of plant biotechnology, such as genetic transformation, somaclonal cell selection, somatic cell hybridization and haploid production have also been successfully utilized for the genus *Populus*, but required reliable and efficient adventitious shoot regeneration procedures. Compared to other woody plants, some *Populus* species and clones have a relatively high capacity to form adventitious buds on excised leaf, stem and root segments, and stem internodes or callus derived from these organs while some do so with difficulty when cultured *in vitro* (Lee-Stadelmann et al., 1989).

The failure of cells and tissues to regenerate shoots is the major limitation preventing the application of plant biotechnology to forest tree species (Coleman and Ernst, 1989). The majority of the success in the cell culture of poplar has also occurred with species or hybrids incorporating species of the Leuce section (aspen

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and white polars). The successful cell and tissue culture species of the Algeiros group (the cottonwoods) has been limited. *Populus deltoides* is an economically important species of the Algeiros group of poplar and is extensively cultured. Successful *in vitro* shoot regeneration of *Populus deltoides* has been reported from explants of immature embryos (Coleman and Ernst, 1989) and anther culture (Uddin et al., 1988). Non-embryonic tissues and interspecific hybrids of *Populus deltoides* have been recalcitrant to attempts of *in vitro* shoot regeneration and shoot culture (Sellmer et al., 1989). Douglas (1984) reported the *in vitro* production of buds and shoots from internodal stem explants of *Populus deltoies*, but the procedure was very laborious and involved multiple transfers.

1. *In vitro* micropropagation

In the most cases the methods used included adventitious and/or axillary budding. Callusing and neoformation of buds is rarely used (Boulay, 1987).

1a) Adventitious budding

This method has been often used for propagation of *Populus* species, particularly for the multiplication of seedlings. Adventitious shoots have been regenerated from stem internodes of *Populus hybrid* TT32 (Douglas, 1985). For this purpose, internodal explants of 2 and 6 mm long were cultured on modified Murashige and Skoog (1962) medium supplemented with zeatin, indoleacetic acid (IAA) and abscisic acid (ABA). After five to six weeks, on the explants of 6 mm length, adventitious buds were formed on the basal medium. Explants of 2 mm length were non-morphogenic when cultured on a basal medium, but produced buds when cultured on a medium supplemented with zeatin (0.1 mg/l). In the absence of sucrose, explants also remained green but failed to produce either buds or shoots; a significant increase in morphogenic response was obtained by increasing sucrose concentration from 1% to 2%. The morphogenic response of 6 mm explants were potentiated by the concentration of zeatin and fivefold increase was obtained with a concentration of 5.0 mg/l. IAA present at high concentration (5.0 mg/l) inhibited formation of adventitious buds in 6 mm explants.

Adventitious shoots have also been regenerated from calli originating from leaf disks of *Populus deltoides* (Coleman and Ernst, 1989). Adventitious shoot formations from stem internodes of *Populus deltoides* was published by Coleman and Ernst over the years 1989 and 1990. In 1989, adventitious shoots regenerated from internodal explants of three genotypes of *Populus deltoides* on a modified version of woody plant medium (Lloyd and McCown, 1980) designated WNA supplemented with cytokinins (6-benzyladenine, 2-isopentyladenine and zeatin). For each of the 3 genotypes, the greatest number of shoots were consistently regenerated on a medium (WNA) supplemented with zeatin [the mean number of

shoot regenerated from 1.43 (4.0 mg/l zeatin) to 2.41 (0.5 mg/l zeatin) shoots per explants]. Internodal explants cultured on a WNA medium supplemented with BA browned and rapidly became necrotic. The mean overall shoot regeneration on a WNA medium, supplemented with BA, ranged from 0.04 to 0.89 shoots per explant. The mean number of shoots regenerated on a 2iP supplemented WNA medium ranged from 0.02 to 0.36 shoots per explant.

In 1990, shoot cultures were established from adventitious shoots regenerated from stem internodes by Coleman and Ernst (1990). The stable shoot cultures were then used as explants to investigate the effects of zeatin concentration and genotype on axillary shoot production and growth. The mean number of axillary shoots produced was significantly different among the zeatin treatments. A zeatin concentration of 1.0 mg/l stimulated the greatest number of axillary shoots. The greatest growth per axillary shoot occurred when stem explants were cultured on 0.25 mg/l zeatin. These results suggest that optimization of *Populus deltoides* axillary shoot cultures may require high zeatin concentration for maximum proliferation and reduced zeatin concentration for maximum individual stem growth.

Formation of adventitious buds from stem internodes of *Populus* species was studied by Douglas (1984). In this method stem internodes of the following poplar species formed adventitious buds when cultured *in vitro* on a basal medium in the absences of exogenous growth regulators; *Populus nigra, P. deltoides, P. tacamahace, P. trichocarpa, P. alba, P. wilsoni* and *P. hybrid* TT32. The number of buds formed per internodal explant varied with each genotype.

The formation of adventitious buds *in vitro* on a micro-cross-section of hybrid poplar leaf midveins was also reported by Lee-Stadelmann et al. (1989). After preparing a particular explant of leaves (Micro Cross Section MCS) in the size of 100, 200, and 300 μm by a lancer vibratome, cultures were kept on a woody plant medium (WPM) supplemented with 2% (W/V) sucrose, 0.2 mg/l benzyladenine (BA) and varying concentration of naphthalene acetic acid (NAA), and solidified with 0.7% (W/V) agar. In the presence of NAA, explants of different sizes showed equal bud forming capacity and produced adventitious buds (average: four shoots/MCS explant). The maximum shoot number was obtained with the addition of 0.01–0.02 mg/l NAA to the basal medium which contained 0.2 mg/l BA. The indirect method of adventitious budding has a great potential for multiplication but several problems remain. First, the problem of obtaining true-to-type genetic copies which is the main purpose of clonal forestry. Second, the variation in the growth behaviors of resulting plants remains uncertain. Heterogeneity between the plantlets coming from adventitious budding was also reported on other forest species such as *Pinus taeda* (Leach 1979). He reported that in the field trials, the growth of *in vitro* plantlets is more heterogeneous than that of a population of seedlings. The results of Patel and Berlyn (1982) have also shown a genetic instability among the buds obtained using this method on *Pinus coulteri*. Evidence supports the view that direct adventitious budding can be useful tool for multiplication of poplar species such as other tree species.

Nevertheless, in the case of adventitious budding which is not direct and

passes through a callusing stage, it cannot be a reliable technique for micropropagation because of genetic abnormalities. There are some reports in which plantlets have been regenerated from direct adventitious budding for multiplication of seedlings without showing any significant genetic abnormalities.

1b) Axillary budding

This method is used particularly in angiosperms and some commercial application. The methods of *in vitro* multiplication of poplar and aspen has also gone through some development. Mass propagation by this method is still limited to young selected trees. By using this method even mature trees can be propagated but with a great decrease in rate.

Nevertheless, the multiplication of mature selected trees using axillary buds has succeeded in aspen (Ahuja, 1984). Boulay (1987) suggested that the pretreatment of the selected mature tree of these species is not necessary and in most cases successive transfer on multiplication medium (i.e., cytokinin) improved the rooting ability in the *in vitro* produced shoots. Boulay (1983) noted that explants taken from suckers, or sprouts on stems, or grafting adult scion onto a juvenile seedling, and even regrafting, improves the initial reaction of the explants and accelerates the multiplication. Engelmann (1982), working with two clones of *Cunninghamia lanceolata*, one juvenile (one-year-old) and one mature, found that juvenile type explants produced two kinds of plantlets; one looks like a normal seedling but the second is heavily branched and lacks a leader branch. All copies of the older clone are plagiotropic. For this clone, the rooting percentage improved during successive *in vitro* transfers but none of the applied treatments to the mature tree were able to overcome plagiotropism. Nevertheless, some propagules of the mature tree displayed morphological typically juvenile characteristics, which means that this problem can be overcome.

Ahuja (1984), took meristematic explants from buds (dormant, axillary or apical) and cultured on modified woody plant medium [designated aspen culture medium (ACM)], supplemented with low levels of cytokinin and auxin. Following shoot differentiation/proliferation on the bud explants of the responsive aspen clones, the micro shoots were rooted in a soil-free potting mixture. A few thousand plantlets from a large number of mature selected aspen clones have regenerated by this relatively simple two-step method. Axillary budding methods for *in vitro* propagation of poplar on the base of juvenile and rejuvenated explants have been investigated by Chun et al. (1986). They studied influences of medium consistency and shoot density on *in vitro* shoot proliferation of *Populus hybrid (P. alba* × *P. grandidentata)*. In this procedure, shoot clusters originating from axillary shoot explants cultured in Gresshoff and Doy (1972) basal medium without hormones were cut into 5 to 6 segments, and transferred to a proliferation medium, composed of Murashige and Skoog basal medium supplemented with 0.2 mg/l BAP. One of the two kinds of proliferation media was prepared with agar (agar-solidified medium) and the other without agar (liquid medium); and for each medium, 3 sizes of vessels

were tested. The mean number of shoots regenerated on a liquid medium was also significantly more than that of the agar-solidified medium. The total mean number of shoots per explant produced on liquid and agar-solidified media was 2.5 and 0.9 respectively, after 2 weeks of culture. There were no significant differences in total mean number of shoots among the vessel sizes. The sizes of culture vessels did not influence the shoot proliferation of poplar.

Seumer et al. (1989) cultured shoot tips of six *Populus* clones and obtained stable shoot cultures from all clones except *Populus deltoides* x *Populus nigra* "Eugenei". The poplar clones that formed a stable shoot culture were placed as two-node explants on either a Murashige and Skoog medium or woody plant medium containing benzyladenine to determine the rate of shoot multiplication. All five poplar clones showed rapid shoot multiplication when cultured in the presence of 0.4–1.0 μM benzyladenine on a Murashige and Skoog medium. *Populus tremula* "Erecta" produced a greater number of healthy shoots when grown on a woody plant medium.

2. Morphogenesis

2a) Organogenesis

Indirect methods of adventitious shoot induction have a great potential for multiplication in micropropagation, but are still unreliable because of some problems; first, the problem of obtaining true-to-type genetic copies, and second, the variation in the growth behaviors of resulting plants. *In vitro* regeneration of plants from callus culture is often an essential final step for somatic hybridization, genetic transformation of plants by foreign DNA, and can be used to generate somaclonal variation for selection of desirable traits (Son and Hall, 1990; Lester and Berbee, 1977; Ostry and Skilling, 1988). There are, however, relatively few reports on multiple shoot differentiation from callus cultures of the *Populus* genus (Noh and Minocha, 1986; Park and Son, 1988). Coleman and Ernst (1990) determined the shoot regeneration competence and callus induction of 15 genotypes of *Populus deltoides* by transferring internodal stem explants onto a callus-inducing medium (CIM, a modification of the WNA medium) supplemented with 0.5 mg/l zeatin or 2,4 -D. Internodal stem explants initially cultured on CIM were transferred to shoot inducing medium (SIM) supplemented with 0.5 mg/l zeatin. The number of adventitious shoots regenerated per explant and the number of explants that regenerated shoots varied among the genotypes. When cultured on a callus inducing medium for 10 days before transfer to a shoot-inducing medium, all 15 genotypes failed to regenerate shoots. Son and Hall (1990) established callus cultures derived from leaf, stem internodes and root segments of *in vitro* shoot cultures of *Populus alba* × *Populus grandidentata* Michx, on a WPM medium, supplemented with 0.5 μM BA and 2.5 μM 2,4-D. Shoot regeneration from organogenic calli occurred on a

WPM medium supplemented with BA, 2iP, and zeatin. Shoot formation increased by subculturing the selected organogenic calli on a regeneration medium. The highest rate of multiple shoot formation was obtained by using 0.05 μM IBA in combination with 22.5 μM 2iP, 22.5 μM zeatin and 12.5 μM 2iP respectively.

Noh and Minocha (1986) induced callus from leaf segments of aspen (*P. tremuloides*) on a modified B5 (mB5) medium with 0.5 mg/l BA and 0.1 mg/l 2,4-D. For determining the efficiency of shoot regeneration, the resulting calli were either subcultured to a solidified woody plant medium (WPM) supplemented with 0.5 mg/l BA or sieved into a liquid mB5 medium for suspension culture. After growing callus clumps in a suspension culture, they were transferred onto a solidified WPM supplemented with 0.5 mg/l IBA. Nearly 100% of the clumps formed shoots on the WPM medium when subcultured directly from mB5 to WPM (average 6 per callus). When calli were transferred from suspension culture mB5 to a solidified WPM medium, an average of 6 shoots per callus were produced from 51% of the calli. Srivastava et al. (1991) multiplied Chinese poplar clones (*P. tomentosa* CARR) as callus cultures and regenerated them into plants. They concluded that the regeneration success is mainly genotype dependent. Organogenesis and regeneration of complete plantlets was possible only on a medium M2 (MS+0.1 NAA,+0.1 IBA+0.1 Z,+500 CH, all in mg/l).

2b) Somatic embryogenesis

This method can be very useful in propagating tree species for two main reasons: First, if one is able to obtain somatic embryogenesis from a mature tree, we can assume that true rejuvenation has been achieved. Also, most problems linked with the maturation and behavior of plantlets in the field will be overcome. The second point concerns the possibility of maintaining large quantities of somatic embryos in a small volume of liquid culture.

Working with one *Populus* hybrid (*P. alba* × *P. grandidentata*), Michler and Bauer (1991) obtained somatic embryogenesis from leaf tissue explants. A high frequency of somatic embryogenesis was induced on embryogenic callus originating from nonembryonic explants on a MS (Murashige and Skoog) medium, supplemented with 5 mg/l 2,4-D, 0.05 mg/l BA and 30 g/l sucrose. After 2, 4, 6 and 8 weeks of culture somatic embryogenesis and development of embryogenic callus occurred in the medium. For embryo production from cell suspension culture, both the auxin to cytokinin ratio (5 mg/l 2,4-D to 0.05 mg/l Zeatin), and sucrose concentration (30 g/l) was optimized. For stimulate germination, somatic embryo development was monitored microscopically weekly for 6 weeks and transferred to a MS medium supplemented with 5 mg/l IAA and 0.05 mg/l BA for one week and then transferred back to a MS medium without IAA. An important issue for this method to be used at a commercial level is to ensure that the variability of genetic copies is acceptable.

One possible solution is to freeze-preserve embryogenic cell lines. Dividing each cell line in two lots, one in a genebank and the other for regeneration (in micropropagation or improvent processes), clonal field tests can be conducted with

regenerated embryogenic plantlets. Once the results of field tests are known, after 6 to 10 years, multiplication of cell lines and production of plantlets from the best clone can be attempted. The limitations to using this technology for mass propagation are the inability to initiate embryogenic callus from non-embryogenic tissue, low frequency of embryo formation, low germination rate, inability to control aberrant morphology and difficulty in acclimatizing germinated plantlets to *ex vitro* environments.

3. Cellular and molecular methods in poplar improvement

Biotechnological strategies have great promise for the improvement of trees, but have not progressed much beyond various types of micropropagation (Sellmer et al.,1989). Some of the most advanced applications of biotechnology with woody plants have been done with *Populus* (Ho and Raj, 1985; Russell and McCrown, 1986; Fillatti et al., 1987; Ostry and Skilling, 1988; Michler and Bauer, 1988) which is emerging as a model system for forest tree improvement studies.

3a) Protoplast isolation and plant regeneration

The regeneration of plant from protoplast is central to the utilization of new developments in somatic cell genetics for plant breeding progress. The mesophyll protoplasts in *Populus* species have been isolated in *Populus alba* (Park et al., 1987; Satio, 1980), *P. alba* × *grandidentata* (Chun, 1985), *P. alba* × *grandulosa* (Park and Han, 1986), *P. tremuloides* and *P. tremula* (Ahuja, 1984). Park and Son (1987) investigated the composition of four types of enzyme solution for the isolation of protoplasts from callus and suspension cultured cells of *Populus alba*, derived from stem cambium tissues of an *in vitro* culture. *Populus alba* cultures were grown on a MS medium supplemented with 0.5 mg/l 2,4-D, 0.1 mg/l BAP, 30 g/l sucrose, and 7.5 g/l Difco bacto agar (Park and Han, 1986). Protoplasts were easily isolated from callus and suspension culture cells. They concluded that the ratio of amount of cells and enzyme solution also affected the protoplast yield and viability (250 mg fresh weight of cells and 5 ml of enzyme solution). The optimum protoplast yield in 0.5% cellulase, 0.5% macerozyme and 0.02% pectolyase from callus was 5.48×10^5 protoplasts per germ. 4.87×10^5 protoplasts per fresh weight with a viability of 93% was obtained in 2.0% cellulase, onozuka R-10, 0.5% dricellase and 0.1% pectolyase Y-23.

Plant regeneration from protoplasts of *Populus nigra* × *P. maximowiczii* leaf mesophyll was reported by Park and Son (1989). In this procedure, intact viable protoplasts were isolated from leaf mesophyll with a mean yield of 10.5×10^6 protoplasts per fresh weight by the enzyme digestion. The MS medium (minus NH_4NO_3) supplemented with 0.5 mg/l BAP and 2.0 mg/l 2,4-D showed the highest frequency of diving protoplast to form cell colonies by a liquid planting method. After six weeks of growth, colonies were transferred to a semi-solid 0.5% agar

medium and callus formation was observed. Protoplasts derived calli produced numerous shoots when transferred to a regeneration medium of MS supplemented with 1.5 mg/l zeatin. Leaf protoplasts were isolated from shoot cultures of two hybrid poplar clones by Russel and McCown (1988). The protoplasts originating from cells divided and formed calli. Shoots regenerated from these calli were maintained as shoot cultures. Plants developed from microcuttings were rooted *ex vitro* and grown in the greenhouse and field.

3b) Androgenesis

Anther culture for the development of haploids and dihaploids is especially useful if a purely homozygous forest tree is desired for breeding. In most trees, recurrent inbreeding to increase homozygosity is not fruitful due to the long generation cycle, high initial levels of heterozygosity (Hamric et al., 1979) and inbreeding depression caused by the expression of recessive deleterious/lethal genes (Koski, 1973). In dioecious species such as members of the *Salicaceae*, attaining homozygous lines via inbreeding is even more prolonged. The recognition of the value of homozygous lines, which are extremely difficult to obtain by inbreeding in a tree, stimulated research in anther cultures, especially in *Populus*, which are intensively bred and cultured worldwide (Stoehr and Zsuffa, 1990).

An anther culture of *Populus maximowiczii* was reported by Stoehr and Zsuffa (1990). They described a novel mode of haploid induction via embryogenic callus of *Populus maximowiczii* originating from microspores at the mononucleate stage and at the tetrad mononucleate and pollen stage, cultured on a MS medium with two levels each of 2,4-D and kinetin. Anthers in mononucleate, tetrad and pollen stages developed normal parenchymotous unorganized (non-embryogenic) calli. Some anthers also formed unusual calli. After 4 to 8 weeks anthers on MS medium with 0.5, 1.0 or 2.0 mg/l 2,4-D in combination with 0.1 mg/l kinetin developed calli. After the transfer of anthers with embryogenic calli to MS medium with a low hormone level, microspores started to divide and initiated independent meristematic nests, which developed into an embryoidal structure. The embryoids germinated precociously without developing cotyledons. After being transferred to a medium with a range of levels of BA, adventitious shoots developed. Adventitious shoots were rooted in a half-strength MS medium supplemented with 0.025 mg/l NAA.

3c) Plant transformation (genetic engineering)

One prerequisite for the application of recombinant DNA technology to forest tree species is the development of gene transfer systems (Fillatti et al., 1987). There are a number of characteristics that make *Populus* NC-5339 ideal for transformation studies. Firstly, *Populus* is an important forest tree species worldwide. A major factor limiting the establishment and management of short rotation *Populus* plantations is the lack of a broadspectrum herbicide which effectively controls weeds

(Akinymiju et al., 1982). Production of a *Populus* variety, resistant to such herbicides would thus be economically attractive. Introducing the chimeric gene for glyphosate tolerance (Comai et al., 1985) into *Populus* hybrids offers a unique opportunity for weed control. Secondly, *Populus* has been known to be a natural host for *Agrobacterium tumefaciens* for over ten years. Wild type strains such as strain 27 (Keen et al., 1970) and strain AT 181 (Sciaky et al., 1978) have been isolated from galls on *Populus* sp. more recently. *Populus* was reconfirmed as host for *A. tumefaciens* by demonstrating that T-DNA sequences were present in gall tissue (Parsons et al., 1986). Thirdly, *Populus* is a member of a genus of forest trees which are amenable to manipulation *in vitro*. Shoot cultures of *Populus* can be maintained *in vitro* and used for clonal propagation, thus providing a sterile source of explant material for bacterial co-cultivation.

Fillatti et al. (1987) induced a plant transformation and developed a regeneration system for transformed poplar hybrids. For this purpose leaf explants from stabilized shoot cultures of a *Populus* hybrid NC-5339 were co-cultivated with *Agrobacterium tumefaciens* on a tobacco nurse culture. Shoots did not develop when leaf explants were co-cultivated with the binary disarmed strain of *A. tumefaciens.* Transformed plants with and without the wild type T-DNA were obtained using an oncogenic binary strain of *A. tumefaciens.* Leple et al. (1992) produced transgenic poplars by co-cultivating leaf or stem explants of a hybrid poplar clone (*P. tremula* $\times$ *P. alba*) with an octopine or a nopaline disarmed *A. tumefaciens* modified strain. Transformed poplar shoots readily regenerated from the explants.

Summary

Due to its valuable economic characters, such as growth rate, short rotation cycle and wide application (e.g. energy plantations, packaging materials, building industry and the common application in agroforestry in marginal private lands, flood plains of rivers etc.), the poplar has become a main object of plant tissue culture. Tissue culture technology offers prospects for mass cloning of superior genotypes for reforestation programs, storage of tissue under low temperatures, isolation of new genotypes through protoplast fusion and gene transfer and utilization of genetic variability that has been detected in the protoclones or somaclones. As a summary, the results attained in micropropagation, morphogenesis (organogenesis and embryogenesis), cellular and molecular methods in poplar improvement are shown in Table 1.

Table 1

Achievements of tissue culture work on poplar

Species	Explant	Culture media+Supplements	Results	References
Populus deltoides (16 clones)	Internodal stem	WNA+BA+2iP+Zeatin	Adventitious shoot regeneration	Coleman and Ernst (1989)
Populus deltoides (15 genotypes)	Internodal stem	WNA+Zeatin+2,4-D (callus induction) WNA+Zeatin (shoot induction)	Shoot regeneration	Coleman and Ernst (1990)
Populus deltoides	Adventitious shoot segments	DKW+Zeatin	Axillary shoot proliferation	Coleman and Ernst (1990)
P. alba ×*P. grandidentata*	Root tip of *in vitro* plantlets originated from bud	WPM (bud culture) WPM+BA (shoot culture, proliferation and elongation) WPM+BA+Kinetin+2iP+Zeatin (root culture)	Multiple shoot regeneration from root culture	Son and Hall (1990)
P. alba ×*P. grandidentata*	Leaf disc, stem internodes, root segment	WPM+BA+2,4-D (callus induction) WPM+2iP (shoot induction)	Callus formation and plant regeneration	Son and Hall (1990)
P. alba ×*P. grandidentata*	Leaf disc (non-embryogenic tissue)	MS+2,4-D+BA	Somatic embryogenesis	Michler and Bauer (1991)
P. alba ×*P. grandidentata*	Axillary bud	Gresshoff and Doy medium (bud culture) MS+BAP (shoot tip culture)	*In vitro* shoot proliferation	Chun et al. (1986)
P. alba ×*P. grandidentata*	Nodal explant (shoot culture), leaf explant	WPM+BA Modified WPM+NAA+BA (semi-solid)	Plant regeneration from protoplast	Russel and McCown (1986)
P. alba ×*P. grandidentata*	Leaf explant co-cultivated with Agrobacterium tumefaciens	MS (shoot culture) MS+BA+Zeatin (regeneration)	Agrobacterium mediated transformed plants	Fillatti et al. (1987)
Populus clones (six)	Shoot tips	MS+BA, WPM+BA	Rapid shoot multiplication	Sellmer et al. (1989)

Table 1 (cont)

Species	Explant	Culture media+Supplements	Results	References
P. nigra × *P. maximowiczii*	Protoplast from leaf mesophyll	MS(minus NH_4NO_3)+BAP+2,4-D (callus induction) MS+Zeatin (shoot induction)	Plant regeneration from protoplasts	Park and Son (1989)
Populus maximowiczii	Anthers	MS+2,4-D+Kinetin (callus induction) MS+NAA+BA (embryogenic callus induction) MS+BA (shoot regeneration)	Haploid plant induction	Stoehr and Zsuffa (1990)
P. tremula × *P. alba*	Leaf and stem internodes, explants co-cultivated with *A. tumefaciens*	Modified MS	Expression of chimeric genes in poplar	Leple et al. (1992)
Aspen P. termula	Bud, stem, leaf and root segment	Modified WPM (ACM)+BAP+NAA+adenine sulfate	Rapid clonal propagation	Ahuja (1983)
Aspen P. termula	Apical meristem or axillary bud	ACM-1+BAP+adenine sulfate (bud break) ACM-2+NAA (shoot growth) ACM-3+IBA+NAA (root induction)	Micropropagation	Ahuja (1984)
Populus hybrid TT32	Internodal stem segment	Modified MS+zeatin+IAA+ABA	Adventitious shoot formation	Douglas (1984)
Populus tremuloides	Leaf segment	Modified B5+BA+2,4-D	Shoot regeneration from callus	Noh and Minocha (1986)
P. nigra var. betulifolia × *P. trichocarpa*	Leaf midveins (micro-cross-section)	WPM+NAA+BA	Adventitious shoot formation	Lee-Stadelmann (1989)
P. alba × *P. grandidenta* *P. nigra* × *P. trichocarpa* Aspen (*P. tremula*)	Leaf protoplast and shoot culture	Modified WPM+coconut water+casein	Plants regenerated from leaf protoplast	Russel and McCown (1988)
Populus alba	Stem cambium tissue	MS+2,4-D+BAP MS liquid+2,4-D+BAP	Protoplast isolated from callus and cell suspension culture	Park and Son (1987)

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THE POSSIBILITIES AND CHANCES OF A HUNGARIAN BIOETHANOL PROGRAM

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The use of ethanol gained by fermentation from agricultural products (bioethanol) as fuel for internal combusting engines plays an increasing part in the agribusiness of numerous developed and developing countries. Bioethanol-production gives a good possibility for stabilizing the agricultural market situation, while both decreasing the environmental pollution and increasing the self-sufficiency in energy resource. This article analyses the various logistical, energetic and organizational aspects of bioethanol production under Hungarian conditions.

Keywords: biotechnology, ethanol fermentation, logistic organization, energy, economic analysis

Introduction

Using agricultural products as raw material for fuel ethanol production has great traditions in Hungary. Between 1927 and 1942, an ethanol-petrol blend called Motalko was used as fuel in cars. In decades of cheap energy, the bioethanol-production had no economical importance as the centrally planned economy neglected the viewpoint of environmental protection, agriculture had no problems with the realization of products on the Soviet market, and there were firm ideological barriers concerning the use of products fit for human nutrition products as fuel in cars.

The situation dramatically changed due to the rapid collapse of COMECON and the so-called socialist system. The aim of this paper is to give an economic analysis of the possibilities to use agricultural products for bioethanol-production in Hungary. The Hungarian economy and the ecological potentiality of the state similar to the other Central and Eastern European countries. Thus, the results of the analyses can be adapted for other countries as well.

General conclusions of national bioethanol-programs

In recent decades, almost every developed and numerous developing countries have set up a national bioethanol-program, so it is possible to draw some general conclusions. The methods of programs, the raw materials, the technology, the economical and ecological environments differ, but the programs have some characteristic features and conclusions in common:

1. Active and effective governmental support is a necessary precondition for the success of bioethanol programs.
2. Exaggerated state support in raw material production may cause a distortion in the agriculture of the state.
3. Mono- or oligopolistic positions of bioethanol-producers may hinder competition and innovation, so there is a very great danger of maintaining unreal bioethanol price-levels.
4. Introductions of bioethanol-gasohol blends must be followed by an effective public-relations policy for the end-users, stressing that the ethanol-gasoline blend is not harmful for the engine.

Main factors, determining the economic eficiency of bioethanol-production

The economic efficiency of production depends upon various factors, the most important of which are:

a. cost of raw material production
b. cost of bioethanol production
c. price of petroleum, as the higher the cost of petroleum, the more important the use of renewable fuels

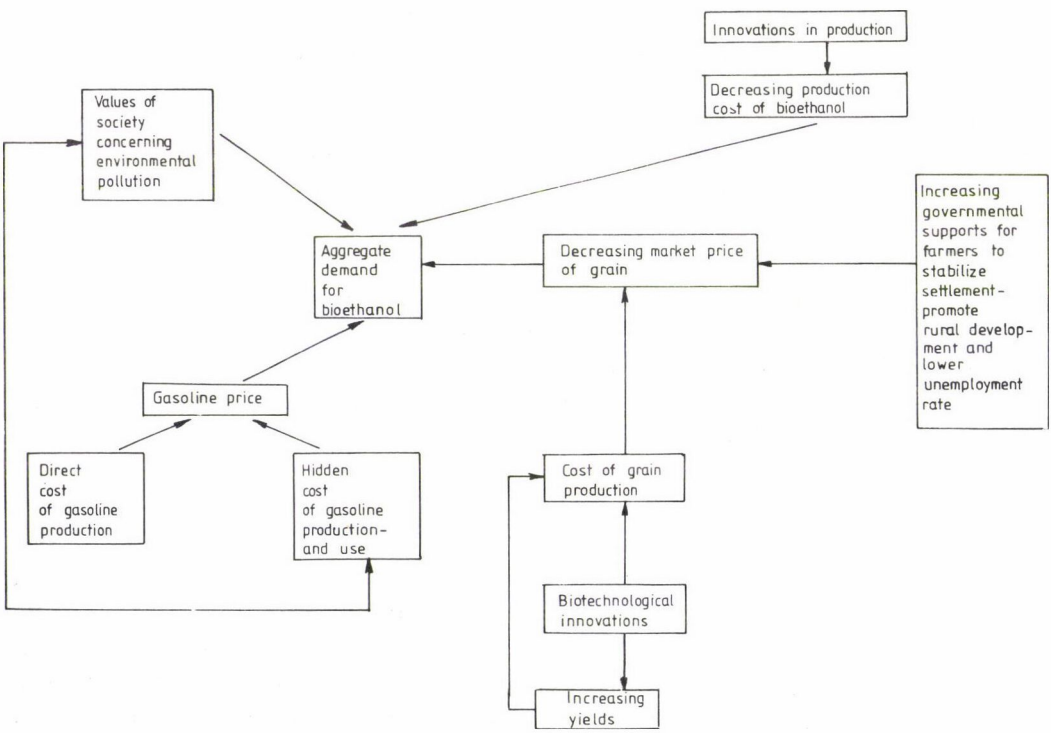

Fig. 1. Relationships of factors determining the demand for bioethanol

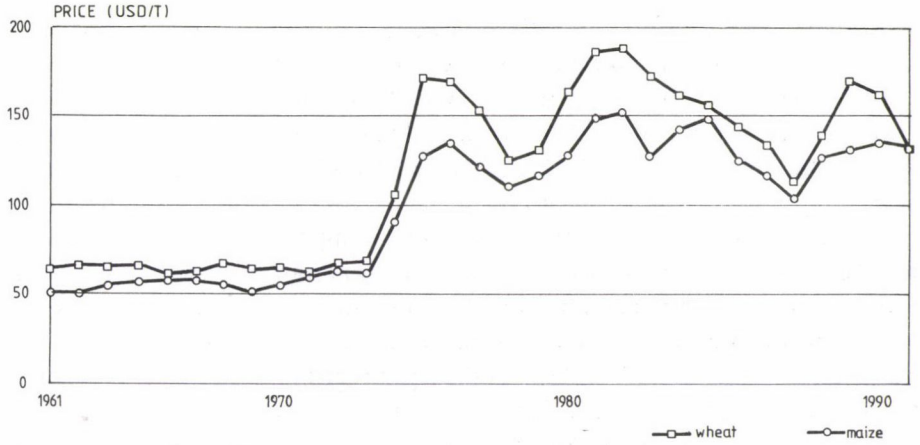

Fig. 2. World market price of wheat and maize (1961–1991)

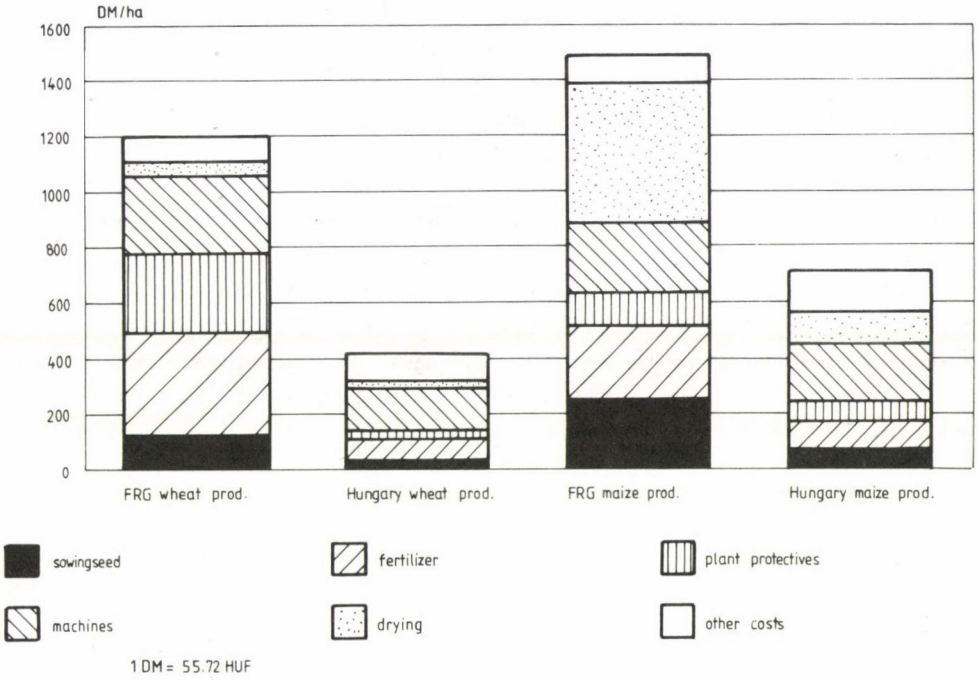

Fig. 3. The direct-cost structure of wheat and maize production in FRG and Hungary

d. values of society, expressed by the support of environmental protection techniques and materials.

The relations among the most important factors, determining the economic importance of bioethanol-production are shown in Fig. 1.

Let us consider the present-day and future position of the above factors from the viewpoint of economic efficiency of bioethanol production.

(a) It can be seen from Fig. 2 that the market of grains shows no stable upward tendency. In the world market there is a considerable surplus caused by the lack of effective demand. In Hungary, which is one of the largest and most effective (Fig. 3) grain producers and exporters per capita, this tendency is very unfavourable and underlines the importance of alternative uses of various agricultural products. The Hungarian agricultural production has been considerably increased during the last thirty years, but the variance between years was important, and much greater than in the Western-European states. This phenomenon can be explained by the great differences among weather conditions of the various years. Of course, the economic re-structuring of Hungarian agriculture caused a considerable decrease in production during the last two years, but the trend of production increase is very linkely to continue. This assumption is justifiable when we consider the trend of increasing the self-sufficiency ratio of EC-member states. For example, in FRG the self-sufficiency ratio in regard to cereals in 1969 was 85%, in 1980 105%, in 1989 106%. This tendency expresses that even sufficiently regulated states could not slow down the increase of agricultural production, although various economic measures have been taken to achieve this.

(b) Oil prices in the world market can be characterized by swift changes. Considering the long-range tendencies of oil prices it can be stated that there is a continuous upward tendency there (Fig. 4) due to increasing production costs, but we cannot expect drastic price increasing, as the oil price has a determining effect on the development of the world economy. Thus, after the oil crisis in 1973, great efforts were made to stabilize markets. The stabilizing mechanisms worked very effectively during the political crises of recent years (e.g. the Gulf war), so the oil price can be forecasted by a slowly but evenly increasing upward tendency. Of course new oil fields or other energy resources may decrease the oil price, but on a short or middle run this is improbable.

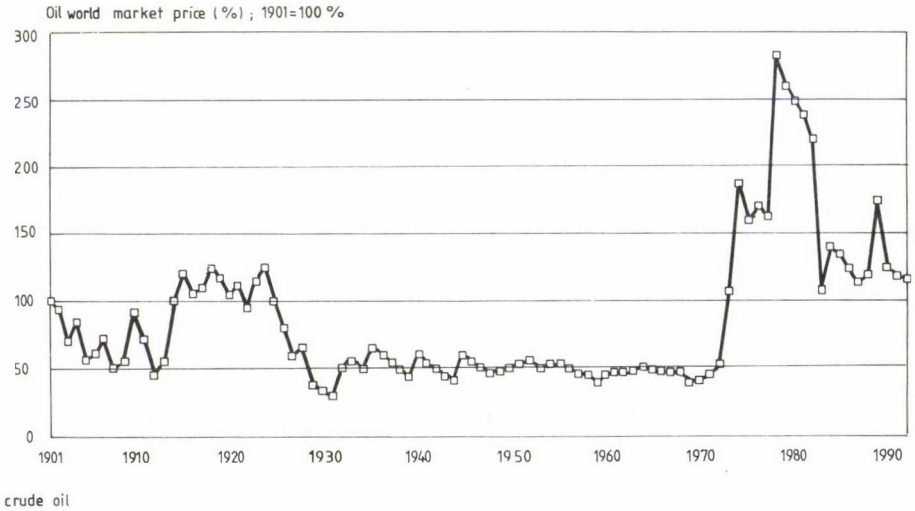

Fig. 4. Changes of oil world market real price (1901–1992)

(c) Costs, cost-structures and the energy balance of bioethanol production vary in by countries, depending upon the raw material, technology, and the scale of production (Table 1). The relative cost of production is higher than the cost of gasoline, so – in the case of even retail price – the governmental revenue is lower. Yet this phenomenon does not reflect the real economic aspects of the question, because the gasoline price does not contain the so-called hidden costs (Hubbard, 1991) of gasoline production and use. (The cost which is not expressed in the product price, e.g. the cost of military support of oil fields and transport lines, the cost of environmental protection measures, costs for the decrease of environment pollution caused by the use of lead-based octane enhancers, etc.)

(d) The octane-number is traditionally enhanced by adding lead-containing agents. The adverse effects of lead are well known, so every country promotes the reduction of environmental pollution, but the intensity of measures for environmental protection is naturally determined by the system of values and the economical potential of a certain state. The use of ethanol instead of lead as an octane-enhancer is a very effective way of environmental protection. As is well known, there are many other ways of octane-number enhancement (using other chemicals), but every alternative has its technical and/or economical disadvantage. As a summary of this chapter, it can be stated that practically all the factors that determine the economic position of bioethanol production are incentives for the development of the Hungarian bioalcohol production.

Logistical concept of a bioethanol-producing system

The technology of bioethanol production is completely analysed in the literature, but the logistical aspects of bioethanol production are less known. Most of the authors suggest large-scale plants in order to reduce the fixed costs of production. However, we suggest a two-stage system, which could produce bioethanol in symbiosis with the agricultural production and environment. In the first phase raw alcohol production takes place, realized in small-scale plants, the capacity of which is no more than 100 hl alcohol a day. This system allows the reduction of transport costs and the by-products can be used as feedstuffs for animal breeding or for sustaining fermentation in the biogas plants of an animal-breeding plant (in Hungary the continuous work of biogas plants without using external energy resources – heating, using other substrats – is not yet achieved). Energy produced by biogas plants could be used for heating rectificators. So the process of bioethanol production would be an environmental-friendly one.

Table 1

The cost structure of bioethanol production according to various resources

GUIDOBONI G. I. (1984):		the cost-profit structure of bioethanol production raw material: 72% other materials: 6% wages: 3% general costs: 2% insurance: 1% amortization: 3% R+D: 4% profit margin: 9%
MOURRIS B.(1984)		cost structure of ethanol-production
	from sugar cane	from sugar beet
raw material	5400000	6393600
depreciation	1700000	1700000
fuel mat.		1157000
wage	486000	972000
maintenance	200000	200000
chemicals	100000	100000
total cost	7886000	10522000
cost of ethanol production		0.187 GBP/1 0.236 GBP/1
DELLVEG, H., LUCA, S. F. (1988):		the cost structure of bioethanol production raw material: 67% amortization: 10.6% energy: 11.4% wages: 12%
KEIM, C. R., VENKATASUBRAMANIAN, K. (1990):		the cost structure of production (USD/l ethanol) net maize cost: 0.133 energy: 0.026–0.053 enzymes: 0.01 yeast: 0.01 water: 0.01 wages: 0.005-0.53 amortization: 5–10% of the plant cost maintenance: 3–4% of the plant cost insurance: 1–3% of the plant cost
BRYAM, M. (1991):		The share of raw materials in the cost structure of bioethanol production: 65% the production cost in 1980:0.409 USD/l in 1990:0.23 USD/l as an average in the USA, but in large plants: 0.193 USD/t
BRIDGWATER, A. V., DOUBLE J.M. (1991):		production cost of fuel alcohol from wheat, produced by fermentation: 180 GBF/t (19.8 GBF/GJ)

Pure alcohol production would take place in the second phase of the system. This stage is a more capital-intensive one. As raw alcohol requires transport, the final dehydration would take place in a central plant, with a capacity sufficient for dehydration of 100 hl raw alcohol. The bioalcohol could be transported to petrolstations from these plants where a 1:9 bioethanol-gasohol blend could be produced.

So one module of the system would consist of ten small-scale raw alcohol producing plants and one plant, where the dehydration takes place. This structure allows

- minimizing the total cost of raw material and by-product transportation
- minimizing energy costs
- a building-block system of project development as a function of the financial conditions.

Energy-balance of bioethanol-production

The energy balance of bioethanol production considerably differs according to various raw materials and technologies (Table 2).

Table 2

The energetic aspects of bioethanol production according to various resources

LIPINSKY, E. S. (1978):	the net energy gain of bioethanol production from various raw materials (in % of input energy): manioka 25% sugar cane 36% sorghum 7%
BERNHARDT, W., MENRAD, H. (1980):	manioka: 5.5 kg/l ethanol sugar cane: 14.3 kg/l sorghum: 12.6 kg/l potato: 9 kg/l corn: 2.9 kg/l sugar beet: 11.8 kg/l the energy structure of bioethanol production from sugar beet: seed: 1.4 % fuel oil of machines: 13.2 % labour: 0.8 % fertilizers: 8 % machine work: 4.3 % industrial processing: 72.3 %
HUNGARIAN RESEARCH INST. OF DESTILLERY IND. (1980)	5.2 kg steam/l ethanol 2.5 kg maize/l ethanol
WOHLMEYER, H. F. J. (1981):	energy input/output ratio by ethanol production from various products (data in GJ/ha) wheat: 23/63 potato: 31/91 maize: 29/63 sugar beet: 32/145

GIBBONS, W. R., WESTBY, C. A. (1984):	ethanol production of various plants (l/ha): sugar beet: 6600 sweet sorghum: 5400 sugar cane: 3700 Jerusalem artichokes: 3300 corn: 1700–2000 fodder beet: 5900
OMETTO, J. G. S. et al. (1985):	the structure of energy input in Brazilian bioethanol production: fuel for trucks: 22 % fuel for tractors: 21.2 % fertilizers and pesticides: 34.9 % seeds: 4.3 % industrial equipment: 9.9 % labour: 5.5 % external energy: 2.2 % input/output ratio (production from sugar cane): 1:9.02 input/output ratio (production from corn): 1:0.625
TECHNIP Co. (1985):	46.4 kg ethanol/100 kg monosacharid molasses: 9.5 kg/l ethanol canel: 13 kg/l ethanol cassava: 5.2 kg/l ethanol corn: 2.5 kg/l ethanol
PÁNDI F. (1987):	energy balance of bioethanol production (output/input ratio): sugar beet: 3,50 sorghum: 2.8 corn: 1.1–1.3
MOLLE J.-F.(1988):	energy balance of ethanol production from sugar (MJ/ha) seed: 335 fertilizer: 17350 machine: 11500 ethanol: 6350 energy balance of ethanol production from wheat (MJ/ha) seed: 125 fertilizer: 15000 machine: 8150 ethanol: 12000 energy yield of various plants for bioethanol production (kWh/m^2) potato: 1.88 sugar beet: 2.71 corn: 1.18 rape: 1.26
KEIM, K., VENKATASUBRAMANIAN K. (1989)	energy use in bioethanol production from corn by conventional technology: 10–13 MJ/l bioethanol with energy-saving technology: 5–7 MJ/l

Energetic aspects of production were determined upon the empirical facts of some great Hungarian grain-producing farms and on the base of our calculations. The structure of costs is shown in Fig. 5. It can be seen that, due to favourable agroecological conditions of the state, the net energy gain is comparatively high.

The average Hungarian gasoline consumption is 1.7×10^7 t a year. Assuming that 10% of the gasoline would be replaced by ethanol, the energy gain would be

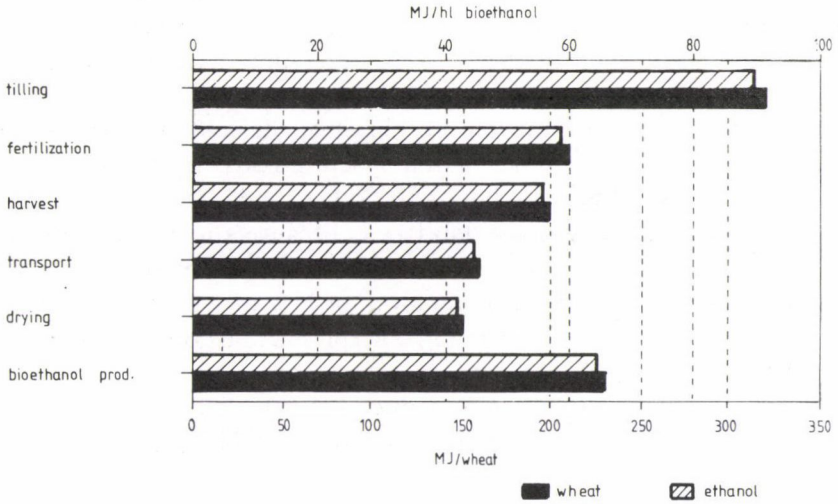

Fig. 5. Energy demand of bioethanol production

5.3×10^{12} J, thus approximately 1700 kt gasoline could be saved. In this way the export-import balance of the state could be improved.

Cost-benefit analysis and work organization of a bioethanol-producing plant

The structure of costs in Hungary according to our estimations are shown in Fig. 6 It can be seen that the costs mainly depend on raw materials. In Hungary the cheapest raw material is wheat. The appropriate use of raw materials is very important for the optimum utilization of the plant. In Hungary, autumn barley is suggested as the first raw material of production in June; then from the end of June to the harvest of maize it should be wheat (stored in aerobic conditions); and then

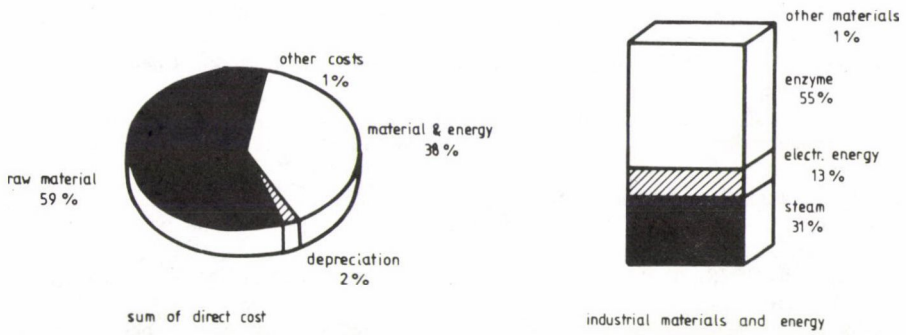

Fig. 6. Direct cost structure of bioethanol production in Hungary

the raw and stored maize are the most economical raw material. The suggested organization allows continuous work in the plant from June to February, without storage during more than half of the work. The optimum ratio of various raw materials can be determined by using linear programming.

The cost of bioethanol-production is higher than the cost of normal gasoline. A 10% subtitution of gasoline by bioalcohol may cause a $50 * 10^6$ ECU decrease for the Hungarian governmental budget, but on the other hand

– Hungarian corn markets could be stabilized, because the whole capacity of suggested bioethanol–producing systems needs 600 kt maize or 628 kt wheat, representing 8% of Hungarian maize production, or 11% of wheat production. The industrial use of such a quantity of Hungarian corn production could result in a stable corn market in Hungarian agribusiness

– lead pollution of environment could be reduced by 60–80%

– the external balance of the Hungarian national economy could be improved by 3–5%.

Summary

We have analysed the economical aspects of a Hungarian national bioethanol program. It was determined that using agricultural products for the production of fuel materials makes the reduction of environmental pollution and energy demands possible. It calls for an optimum structure organization of bioethanol plants. The introduction of bioethanol as an octane enhancer could result in a cleaner environment and in an increasing energy self-sufficiency as well.

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JENŐ SZUNDY, A GREAT PERSONALITY OF HORTICULTURE IN HUNGARY

Life and activity

Jenő Szundy was born on 27 March 1883 in Makó, Csanád county, to an intellectual family. His father, Károly Szundy was a secondary school teacher of mathematics and geometry. His mother, Kornélia Borcsányi raised six sons and a daughter. From his father he inherited a keen insight, from his mother a sense of recognizing the errors of the world. She wanted her son to develop a strong character.

Szundy completed his elementary and secondary school studies in his native town. In 1898 he was employed as a horticultural trainee on Count Nádasdy's estate where he spent two years. In September 1900 he registered at the Royal Hungarian Horticultural High School in Budapest and graduated on 29 June 1903. After a one-year military service, he entered government service in the autumn of 1904 as a teacher at the State Gardener School, first at Nagybocskó, then in Lőcse. In the spring of 1908 he was charged with the management of the State Nursery of

Akadémiai Kiadó, Budapest

Trencsén; then from the autumn of 1911 was a teacher at the Agricultural School at Szilágysomlyó. In 1912 he had study tours for two months in Germany, France and England with a state subsidy, and acquired a comprehensive knowledge of the most developed horticultural sciences of then. Having returned home he was appointed head of the horticultural department at the State Agricultural School of Pápa for a one-year period. However, even before the term expired he was charged with the management of the State Nursery of Munkács. In the framework of a grafting campaign, he promoted the modernizatian of fruit growing through the change of varieties. In 1914, the year when World War I broke out, he occupied a post in Szabadka, as a teacher of horticulture at the agricultural school of Palics. During the war he did horticultural work instead of combat service. In order to ensure the seedling supply, he established some 100 vegetable gardens in 72 settlements on the area of his division. He was discharged as a lieutenant in 1918 and continued his pedagogical activity at Palics, teaching in Serbian language. For he refused to Serb subject he was sent off. He went home his native town - in the age of 37 - he was discharged. From 1920 to 1927 he was a leading member of the Agricultural Society of Csanád county; he was engaged in itinerant agriculture and organized agricultural exhibitions. Agriculture, as a condition of efficient fruit growing was greatly promoted by him. There is no fruit yield without the presence of agriculture this was his conviction. On 16 January 1927, he founded the County Orchard Association of Csanád-Arad-Torontál, and was its general manager director for 23 years. The organization functioned with 4700 members. The main tasks were to give expert advice, carry out plant protection activity, prepare a fruit register, discuss the experiences, organize exhibitions and shows. Jenő Szundy established 400 new orchards.

The orchards and gardens were replanted with fruit species and varieties suited to the region. In 1937 Szundy established a large nursery on a 2.57 hectares area in order to supply the county with excellent quality grafst, and established stock plantations and demonstration gardens too. He held educational lectures to teach the members of the Association the most up-to-date technology of fruit growing and methods of vineyard plantation, and did his best to have the theoretical knowledge carried into practice. Not only as a teacher of horticulture, but also in his capacity as a manager, he made every effort to deepen the professional knowledge of vini- and viticulturists, apiarists, flower and vegetable growers and other horticulturists. As a patron of bird protection he enriched the science of ornithology by organizing programmes and issuing educational publications. He had a collection of 837 various nesting boxes, feeding tables and other devices to demonstrate his lectures. In admonitions sent to competent persons in the Ministry of Agriculture, and in warnings addressed to the regional superintendents of schools, Szundy constantly repeated: We will have larger yields and help ourselves by protecting the birds, as birds kill millions of insects. He published professional guide-books, scientific papers, books, and he was for 12 years editor of fruit-growing calendars. The number of his publications in about 1100. He occupied leading posts in the National Council

of Fruit Growers, the National Society of Fruit Growers (as its manager he had professional authority all over the country), the National Pomological Committee, and the National Committee on Judging New Plants. He was an exemplary master of his era, county and people.

Szundy realized that, in the absence of skilful tending after World War II, the Hungarian orchards ran wild, while the fruit-trees were attacked by numerous pests and pathogens. The 4500 waggons of apple and pear of 1938 fell to 200-250 waggons by 1946. On 20 December 1946, Szundy was commissioned by the National Hungarian Association of Vine- and Fruit Growers to take an active part in the Hungarian horticulture. He then travelled, argued, and organized, working 14-15 hours a day. Politics, however, interfered. In the new system, old values got lost and earlier merits became sins. Szundy's nursery and experimental area at Medgyesháza were taken over by the state without compensation. The Pomological Association of Csanád-Arad-Torontál county was dissolved on 3 April 1949, on the departmental order No. IV/30.134/4/1949 signed by the Home Secretary, its assets confiscated by the sub-prefect of Makó on the decision. No. 18533/1949. The estated value of the associaton was 103.219 Forint according to a registration on 15 June, 1949. From 1949 Szundy found it difficult to make both ends meet, and there was hardly anyone to help him. Tenants moved in to his home, so for more than 15 years he could not receive his sisters and brothers who lived in distant places of the country. In 1950 he was employed as a physical worker in the Horticultural Enterprise of Makó. From 1951 to 1954 he trained small commodity producers in tending fruit-trees. In the meantime he wrote the history of fruit growing in Csanád-Arad-Torontál counties and submitted it in 1955 to the Section of Agricultural Sciences of the Hungarian Academy of Sciences. This work was accepted by Prof. Dr. Rezső Manninger and it gained a monetary reward. His studies that remained as manuscripts are: "Fruit-tree and the child" and "Fruit-growing pioneers in the Csanád lowlands". On 15 June 1963, Szundy founded the Horticultural Cooperative of Makó and established a propagation material plant for the propagation of perennial drought-resistant flowers to be planted in 234 streets of the town.

Szundy intended to replace the old own-rooted vine-grape varieties by new varieties and gradually eliminate prohibited vine-grapes harmful for consumption. The initial basis of the propagation work was a stock of 341 rooted vine grafts given by the Research Institute of Vine- and Viticulture. By 1968 2050 grafts suitable for plantation were available for the producers of the county. Szundy also started the establishment of an ornamental nursery. As a first step he produced some 10,000 shrub-roses and nearly 1000 ornamental shrubs for public places and parks. As a recognition of his activity in the field of horticultural production and research, the University of Horticulture and Food Industry gave him a golden diploma on 25 February 1961, a diamond diploma on 17 May 1965, and an iron diploma on 27 May 1968. Honour was never conferred upon him. He worked much in his life, serving for more than 70 years the development of horti- and viticulture in Hungary, but moral and material compensation was never given him.

His disease turned for the worse after 20 May 1974. On 27 June 1974 Prof. Dr. Endre Probocskai, educational Sub-rector, Head of Department, visited Szundy in his home on behalf of Prof. Dr. András Somos, Rector of the University of Horticulture and Food Industry, to wish him recovery and hand over a 2000 Ft special grant of the University to him. Szundy died on 4 July 1974 at 3 a.m. in Makó at the age of 91. Two days later, he was buried in the Calvinist old downtown cemetery, in the so-called parcel of the poor, beside his wife and mother-in-law. At the zenith of his career Szundy professed that. One has to love his vocation, without it humanity could not develop forward. Szundy wrote his name in the agricultural history of Hungary and his example will never be forgotten.

B. Halász

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Book reviews

ANDREW HIATT (Ed.): *Transgenic Plants: Fundamentals and Applications*
Marcel Dekker, Inc. New York, Basel, Hong Kong (1993) 340 pp. 45 figures and 17 tables
ISBN: 0-8247-8766-8

Transgenic plants are those that have had inserted into their genome the DNA that has been manipulated in the laboratory by recombinant DNA methods. These methods have been developed during the past 20 years, and by the end of 1992 there have been over 200 field experiments with transgenic plants worldwide.

This book tries to summarize the recent results in plant genetic engineering from specific areas of practical significance (e.g. transgenic pathogen protection) to the new initiation, utilizing plants as bioreactors. This volume contains 16 articles and reviews, written by 39 contributors in 5 chapters comprising 340 pages, dealing with detailed problems of plant transformation, new vector strategies, expression of foreign genes, antisense and catalytic RNAs.

The main weakness of this book is that, although on the one hand there are some well-known names (e.g. J. Schell, I. Potrykus, Montague), on the other hand some interesting applications (e.g. herbicide resistance) are missing. Nevertheless remainders are discussed in full depth. Consequently it has a strong set of values for those who study plant biotechnology as well as those who work in plant molecular biology.

The content of chapter I, "Transgenic Manipulation of Metabolism" explains the manipulation of hormones, seed storage proteins, intercellular transport and altered fatty acid metabolism in transgenic plants.

The second chapter "Viral Pathogen Resistance" focuses on coat protein- and nonstructural Viral gene sequence-mediated resistance only.

Few methods are discussed in chapter III. "Techniques for Transformation of Photosynthetic Organisms" among others are microprojectile bombardment and viral-based vectors.

In chapter IV, "Molecular Farming", production of pharmaceuticals, antibodies, human proteins, and bacterial enzymes are the new specific areas that hold great promise. The modification of crops for specific industrial purposes may lead to new processes and products ranging from the improved use of plants in the food and feed industry to new production methods of pharmaceuticals, specifically chemicals and bulk industrial products. The first steps in studying the potential of crop modification for industrial purposes are discussed in this chapter.

Finally in chapter V, emerging techniques "Ribozymes and Antisense RNA" are dealt with. The success of antisense strategies for modifying gene expression and creating single-gene mutations in transgenic plants are demonstrated. Antisense genes have been used to modify plant development (ripening), flower color, photosynthesis, etc. The use of ribozymes to inhibit fungal or bacterial infection of plants requires much more basic knowledge.

The chapters are well written and logically organized. There are not only fact described, but the contradictory results are also critically discussed in all chapters. I recommend this book not only for the specialist working in the field of plant transformation but also for the sciencist involved in plant biotechnology and plant molecular biology, and all other areas of plant science.

L. E. HESZKY

I. KÁDÁR: *Principles and methods in plant nutrition*
Akaprint, Budapest, 1992. 398 pp.
ISBN 963 400 874 7. Hardbound. (In Hungarian)

There are a great number of monographs written in foreign languages and dealing with one or another field of plant nutrition. These works, beyond the fact that they do not give an overall view of the discussed subject, are not prepared for the Hungarian reader. For the adaptation of results and experiences obtained under other circumstances, active research performed in Hungary is needed.

While presenting the basic principles and methods related to plant nutrition, the author refers to the major questions which appear to have motivated researchers in the past. The scientist who concentrates exclusively

upon contemporary research, while neglecting the historical roots of his subject, does so at the risk of "discovering the wheel". More importantly, there is no better way for the reader to develop ability of judgement and criticism, while learning the subject matter, than by studying the experiments and interpretations of our scientific predecessors. Hungarian literature in plant nutrition and soil science is extraordinarily rich, and the author has relied upon this national treasure.

In addition to the historical approach, he also strives to reveal a broader background of the phenomena. The plant is an organism similar to man, since both alike need air, water, sunlight, etc. Plant and man are also connected by the food chain. Man consumes plant food and after his death, when he becomes "dust and ashes", he serves as food for the plants. All living organisms, of which man is only one, are related to and dependent on each other. Our environment is one and the same: the air we breathe, the water we drink and the land and sea on or within which we live.

The book is intended for researchers, teachers, university students and anybody with an interest in agriculture, plant nutrition, environmental management. It summarizes the new results obtained in plant nutrition in Hungary and abroad. Comprising almost 400 pages, the book contains nearly 180 tables and 11 figures. An English summary and the inscriptions of the tables and figures in English are attached.

The 10 chapters include the history of agriculture and fertility of soils; principles and methods of estimating nutrient balances; principles and methods in soil testing, plant analyses, field-, pot- and solution culture experimentation environmental issues in plant nutrition; plant nutrition in alternative agriculture; and fertilizer recommendation for farmers.

The review of the problems of environmental protection within the frames of plant nutrition may command the strongest interest, because this chapter deals with the sources and consequences of environmental pollution, the toxicity and the limit concentrations of the polluting sources.

The relationship between man and his environment in earlier days is discussed as well.

The role and the cycle of nutrient elements and of heavy metals causing pollution in the environment are also represented in this part. Relying on his own research work and his experimental data, the author casts light on the processes of heavy metal enrichment in the environment caused by traffic, habitations, industry and fertilizer use.

Brief sections of sample collection, the preparation of samples and interpretation of material obtained are also added.

B. LÁSZTITY

STEVEN FOSTER (Ed.): *Botanikal booklet series*
American Botanical Council, Austin, 1992

The American Botanical Council has most recently announced the publication of the Botanical Booklet Series authored by the renowned botanist Steven Foster. Each booklet of the series comprises 8 pages and provides comprehensive, at the same time concise and accurate, information on the following major medicinal species:

Latin name	Common name	Volume No.
Echinacea	Purple coneflowers	301
Eleutherococcus senticosus	Siberian Ginseng	302
Panax ginseng	Asian Ginseng	303
Gingko biloba	Gingko	304
Silybum marianum	Milk Thistle	305
Mentha x piperita	Peppermint	306
Matricaria recutita et Chamaemelum nobile	Chamomile	307
Panax quinquefolius	American Ginseng	308
Hydrastis canadensis	Goldenseal	309
Tanacetum parthenium	Feverfew	310
Allium sativum	Garlic	311
Valeriana officinalis	Valerian	312

In my view it is the consumer that can profit most from the booklets. However, by providing the most relevant botanical descriptions, history of use both in the past and present, including also information on the active substances as well as clinical/therapeutic uses of selected species, the booklets could become an ideal resource for pharmacists, physicians, nurses, nutritionists, teachers, etc. as well.

Consequently, it is to be hoped that both the prolific botanist author and the American Botanical Council will carry on with this excellent publication series, since the affordably priced booklets not only disseminate reliable information for a wide choice of expertise, but also educate the public toward a better understanding and more reasonable/rational use of both traditional and currently popular phytopharmaca.

Á. MÁTHÉ

PÁL KOZMA: *Vine and vine growing*
Akadémiai Kiadó, Budapest,
1991, 339 pp. and 1993, 403 pp.

Habent sua fata libelli - books have their own fate, as the old saying goes. However, in the case of monographic works by an author with a renowned

career, the works have evolution and sometimes even "reincarnation".

This holds true for the academician Pál Kozma's great two-volume synthesis "Vine and vine growing" which can be safely described as unique among the scientific yet practical manuals that summarise the material of knowledge of a branch of agriculture. Synthesis means, in this case, not only an updated literary review, but also the rearrangement of a new body of knowledge based on the author's own investigations and experiences. In the world literature of his profession, only the manuals of the French Branas (1974) and the Italian Fregoni (1985) are similar works, but what Kozma offers is different and exceeds theirs.

The evolvement of the work can be traced back in the Author's preface. Its "precursor" was published by the Mezőgazdasági Kiadó under the title "Vine growing": Vol. I. in 1964, then in a second edition in 1967; Vol. II. in 1966. Vol. I. was published in the Japanese language too:"Budo szaibano kiszoririon" (Fundamentals of vine growing), 1970.

During the quarter century since it was published, the knowledge of natural sciences has considerably increased, and the aspects of social sciences have changed in many respects. Therefore, the former book has become - in the Author's words - "partly or totally out-of-date". Thus, it was necessary to rewrite and republish it, which was made possible by the support of the Section of Agricultural Sciences of the Hungarian Academy of Science, and with the contribution of the Akadémiai Kiadó.

However, the Author not only updated his book but also made it easier to understand and more readable. He strictly stuck to discussing only the material of ampelology in detail, merely referring to interdisciplinary relations, while making use of their achievements. Kozma was right in neglecting a detailed description of the vine-grape varieties - since up-to-date ampelographic works have been published by Csepregi and Zilai. Nevertheless, of the varieties and their use he expresses his opinion.

As to its literary form, the book is the full monograph of a profession /or production branch with emphasis laid on the biological and ecological aspects. It encompasses the entire area of the profession and is both of scholarly character and practical; finally, it is interesting, comprehensive and colourful.

Amateur-, home-garden- and hobby vine growers are numerous and may represent the largest segment of the potential book market. One willingly reads books even of higher than the necessary level on subjects (e.g. astronomy, history etc.) of personal interest. Kozma's book can also fulfil such demands.

Furthermore, this book with its content, pictures and fine layout makes a suitable present (which in the market economy must not be underestimated).

Yet, in the first place, it was written for research workers, instructors, university students, horticultural engineers and future farmers, satisfying their highest demands. All this will be clear when we look at the structure and content of the book.

As to its structure, it is a "twin-book" rather than a two-volume work, not only because of the two years between their appearance (1991 and 1993) but also for the separation of the subjects and even of the subjects indexes. This has the advantage that either volume can stand by itself, while the difficulty of looking up something is its disadvantage. (The index of the second volume should refer to pages in the first volume with differently printed numbers.)

Volume I with the sub-title "Historical, biological and ecological bases of vine growing" treats the subject on 339 pages (42,5 printed sheets) with 229 pictures in a readable style.

The first three chapters contain the history of vine, covering 100 million years up to the present day; the Author discusses the different periods according to their importance, and more recent ones at increasing lengths.

A brief introduction deals with the utilisation of the fruit of vine, referring here to viniculture (it is, though, outside the scope of the monograph; most people hearing the word vine think of wine, champagne, brandy).

After a thorough botanical description of the origin of vine (pp. 15-19) the Author gives a culture historical characterisation of the development of vine growing and its global expansion, illustrated with excellent pictures (pp. 20-35). After a rapid progress over the different periods, the section ends with detailed world statistics of viti- and horticulture in the near past.

The development and present situation of vine growing in Hungary is discussed in a similar way (pp.36-55). The description of the period from the mediaeval initials to the phyloxera plague reveals the connection between the history of vine and that of the Hungarian people; and the twentieth century history of viticulture is by itself an excellent analysis. In out age the "present" situation becomes anachronistic in a few years and can better be said "near past". Here let me make a remark: a book of this type must not contain anything that falls out-of-date in - say - five years. And the necessary "lasting validity" is particularly rare in the socio-economic sphere.

The biology of vine growing (pp. 56-202) reminds one of a text-book of high professional level. The excellent (and original) photos even make the taxonomy readable. The same can be said of the morphological and anatomical characterisations of the vine plant. The flower morphology is uniquely

excellent. Then, a detailed physiological and plant nutrition description follows. Original is the treatment of the cycle of the vine plant, of the annual biological cycles, the shoot growth, and finally of the reproduction: flowering, fruit production and ripening.

In describing the ecological factors, the Author was led by his long since expressed opinion and his investigations rather than by the fashion of "emphasising the environment" (pp. 203-261). Since viticulture has spread all over the world, its production geography is also very interesting. The effects of light, temperature, moisture, etc., are always shown as reflected by the vine plant. The exploration of the mincroclimate of the vine plant within the stand is unexampled and may arouse the interest of related professions too. The section on soil science deals with one of the most important factors of the growing site.

In describing the vine growing regions of Hungary (pp. 245-261) the wine districts are discussed not only on the basis of statutory provisions, but the varieties grown in each of them are also characterised.

Volume II. sub-titled "Propagation and production technology of vine" is a detailed guide-book of practical ampelology.

After a brief introduction in which the content of the second volume is brought into connection with that of the first one, the vine pro–pagation is dealt with (pp.11-80), from the traditional methods of propagation to micro propagation. The description is so clear that it may even be suitable for medium level teaching.

The reason why an instruction in vine planting (pp. 81-156) is so important is that some plant the vine before learning the profession. It is good that the choice of variety is mentioned again, and the raising of young plantations is also dealt with here, together with a detailed description of the supporting systems.

After these two relatively short chapters, the technology of vine growing seems to be oversized (pp. 157-342), not so much because of the length of the chapters as owing to the disproportion between the different sections: For example, phytotechnics running from p. 157 to p. 235 could have been separate chapter and the section on technology could be called in itself a book. It is difficult to write criticism of this book as it contains what all text-books written on the subject should and do contain. It covers plant protection, nutrition and soil cultivation (and even machines). Of the irrigation of vine, so much has not been read so far. On the other hand, of the harvest and the now fashionable postharvest technologies, I should have liked to read more. The root-stock cane cultivation would have been out-of -place here; its proper place would have been in the chapter on vine propagation. Finally, very peculiar is the way the Author discusses the productivity of the plantation, its inner structural balance, the changes in the ecological conditions and the transformation of the plantations.

The bibliography deserves special atten–tion. It covers the full world literature of this branch, and with its help the reader acquires orientation for further investigations. In the text there is hardly any reference, but each chapter gives an excellent "literary compass" to reader who, on the other hand, when searching for a particular author may have considerable difficulties. With this solution the Author followed the international practice, so work needs no excuse.

Vine growing is part of our human culture, not merely a branch of agriculture: It plays a role in our way of living, it forms our habits, literature and history. And this book serves this culture. Scientific approach and practical popularisation go hand in hand in it, scientist and farmer equally find in it the knowledge they need.

P. TOMCSÁNYI

REVIEWERS OF MANUSCRIPTS, VOLUME 42
1993

Every scientific contribution in Acta Agronomica Hungarica is reviewed by two scientifically qualified persons. The Editorial Board is pleased to publish the following list of reviewers for the manuscripts of the manuscripts of the 1993 issues, who by their unselfish contribution have significantly contributed to ensure the scientific standars of Acta Agronomica Hungarica.

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