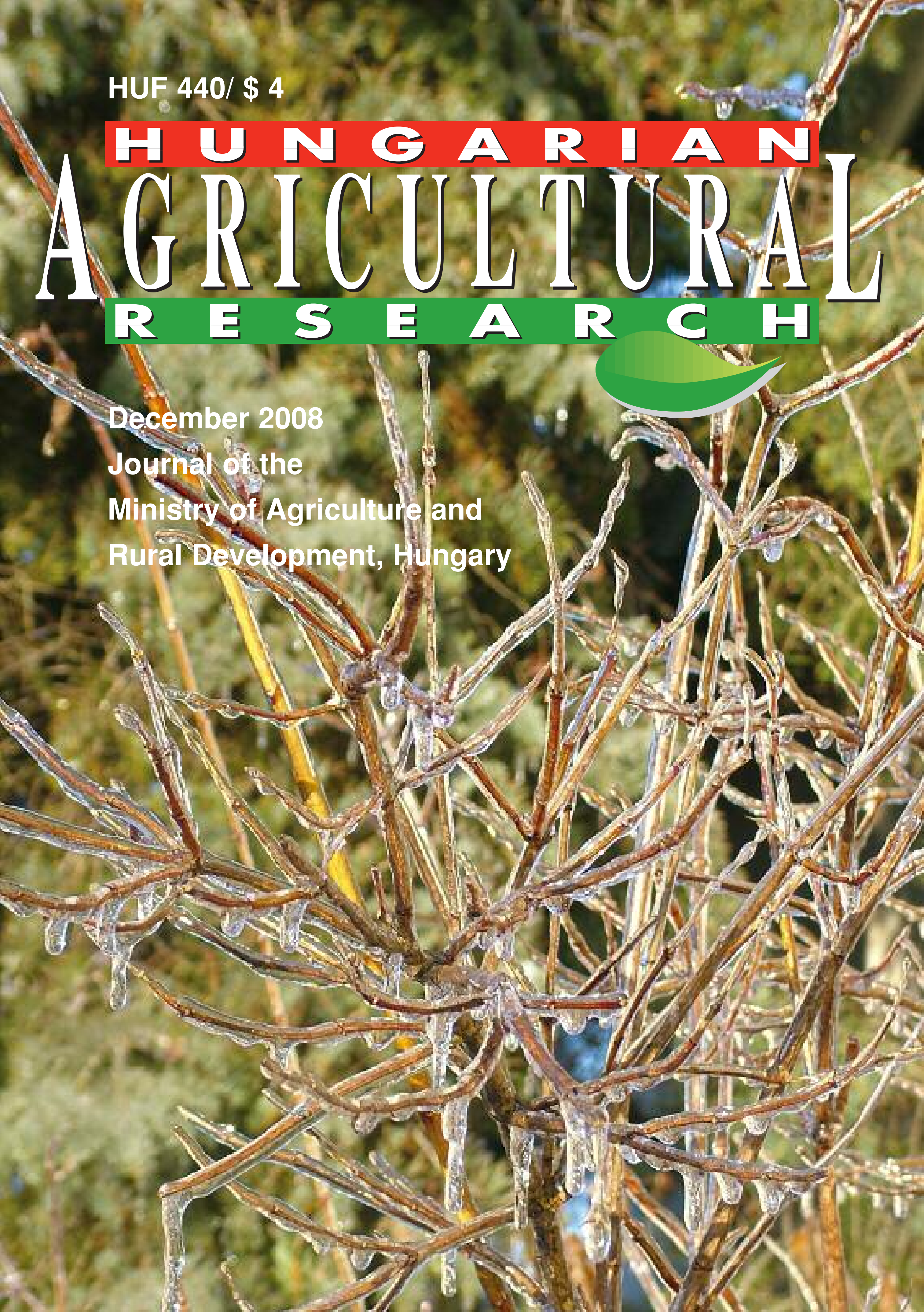
HUF 440/ $ 4

HUNGARIAN AGRICULTURAL RESEARCH

December 2008

Journal of the Ministry of Agriculture and Rural Development, Hungary



Report of the 29th Session of the Codex Committee on Methods of Analysis and Sampling

Péter Biacs and Mária Váradi

The Codex Committee on Methods of Analysis and Sampling held its Twenty-ninth Session in Budapest, Hungary, from 10 to 14 March 2008, by courtesy of the Government of Hungary. The Session was chaired by Professor Péter Biacs, Professor at the Corvinus University of Budapest. Professor Pál Molnár, Department of Food Science of the University of Szeged, acted as the Vice-Chairperson. The Session was attended by 159 delegates and observers representing 59 Member Countries, one Member Organisation (EC) and 8 international organisations.

The Committee endorsed or updated several methods of analysis in Codex standards. These are the following: a number of standards for milk and milk products, standards for infant formula and formulas for special medical purposes intended for infants, standards for some contaminants (mercury, arsenic, cadmium, lead), as well some standards for Asia and Near-East.

The Committee had a brief discussion on the approach to be taken with regard to health and safety concerns of methods. The Committee agreed that if there was a choice between different methods, that the safer option should be given preference. The Committee further noted that concerns of health and safety should be addressed through good laboratory practice and that standards development bodies should continue to take into account these factors when developing new methods.

The Committee discussed some guidelines as follows: Proposed Draft Guidelines on Analytical Terminology; Draft Guidelines for Evaluating Acceptable Methods of Analysis; Draft Guidelines for Settling Disputes on Analytical (Test) Results.

The Committee agreed to consider further at its next session the development of guidelines for establishing methods criteria for identification of relevant analytical methods; guidance on sampling uncertainty; the question concerning conformity assessment in the presence of significant measurement error; and methods of analysis for dioxins and dioxin-like PCBs.

The Committee agreed to undertake new work on Guidelines for Criteria for Methods for the Detection and Identification of Foods Derived from Biotechnology and on the revision of the Guidelines on Measurement Uncertainty.

The Committee was informed that the 30th Session of the Committee would be held in Budapest in March 2009.

The Committee expressed its warm thanks and appreciation to Professor Péter Biacs and Professor Pál Molnár, on the occasion of their last session as Chair and Vice-Chair respectively, as the excellent chairmanship of the Committee throughout the years had contributed significantly to the progress made by the Committee on many complex issues of great importance for the work of Codex in the area of analysis and sampling.

Winter
(Photo by Erika Bakk)

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**Vol. 17, No. 4.
December 2008
Journal of the
Ministry of Agriculture and
Rural Development, Hungary**

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Published by AGROINFORM Publishing
H-1149 BUDAPEST, Angol u. 34. Hungary
www.agroinform.com, studio@agroinform.com

Editorial Office
Department of Mechanics and Engineering Design
Szent István University, Gödöllő
H-2103 GÖDÖLLŐ, Hungary

Subscription request should be placed with the Publisher (see above)
Subscription are HUF 1200 (only in Hungary) or
$16 yearly plus $5 (p & p) outside Hungary
HU ISSN 1216-4526

László Heszky[1]

Challenges of plant breeding early in 21th century

At the beginning of the 21st century, agriculture faces many challenges. I intend to briefly summarize these questions and problems with special respect to the plant breeding. In addition, I try to briefly summarize the main approaches in breeding what we have to take into consideration in the near future in order to meet the challenges. The main facts and issues, what we have to face are:

Growing human population

Most of the problems we face in the 21st century relate, directly or indirectly, to the growing human population. World population stands at 6.3 billion, and expected to stabilize at 10–12 billion during the next 50–70 years. This bear doubling of world population will require more than doubling of world food production. The main problem from breeding respect is that the population is growing faster than increases in food productivity. Grain production is 0.5%/year, which is below the rate of population growth *(Fig. 1)*. The need to improve crop productivity, to reduce the use of harmful agrochemicals and to produce nutritious and healthful food is greater today than ever before.

Sustainability *(Protection of Environment and Biodiversity)*

The international agricultural community faces this challenge at a time when population is growing faster than increases food productivity, when there is less per capita arable land available for food production (0.44ha/1961, 0.15ha/2050), and when more than 42% of crop productivity is loss owing to various biotic and abiotic factors *(Fig. 2)*. To conserve our natural resources, biodiversity needs the decreasing of the use of agro-chemicals which cause soil and water pollution.

During the 21st century, human society faces a new relationship with the natural world. The new relationship is captured by „sustainability", a concept implies meeting current human needs while preserving the environment and natural resources needed by future generations. To meet this demand, it may be the organic breeding. The 1st meeting of the Organic Breeding Working Group of EUCARPIA was organized in Wageningen in October 2007, entitled „Plant Breeding for Sustainable Low input Agriculture".

Globalization

There is a world wide competition among the largest Multinational (as global) and National (as local) Companies, for the global and regional sowing seed market. Consequently, a conflict is being generated between multinational and local Seed Companies, and between the multinational and national breeders.

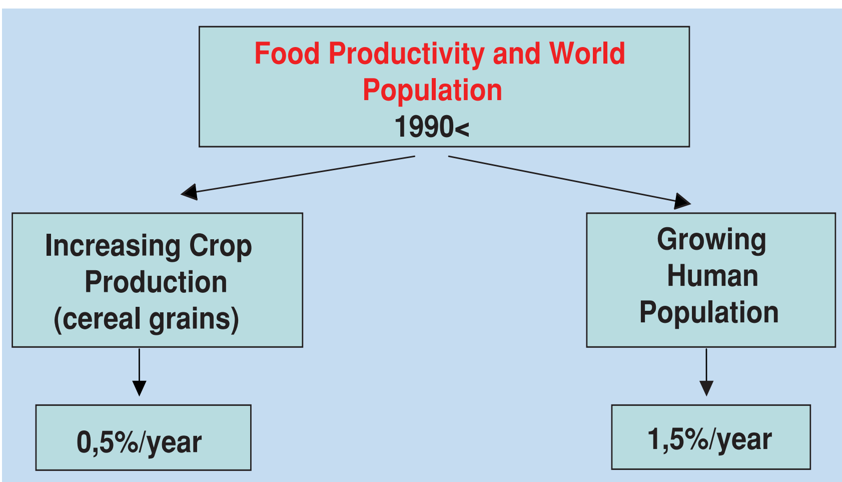

Fig. 1. *Population is growing faster than increases in food productivity.*

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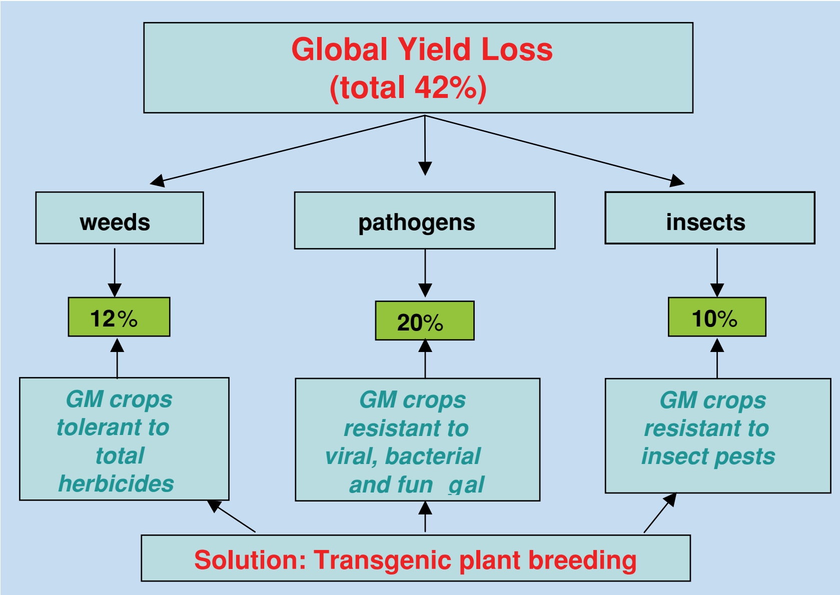


Fig. 2. *Global yield loss and the solution*

background of plant response and transfer new genes of interest into the best conventional varieties.

Conventional or organic breeding?

Organic sector of European agriculture is rapidly growing. Organic crops and varieties are required transgenic free, implicitly organic farming sector needs special varieties. Therefore a new section the Organic Breeding Working Group has been set up during the last Eucarpia Congress in Vienna 2004.

Global warming

Global climate change may lead to changing the local environmental conditions also in Europe. The consequence includes the change in quality and quantity of biotic and abiotic stress factors. Many examples of invasive pests and diseases, that caused significant economic disruption to agriculture, for instance the current spread of the American corn borer in maize *(Fig. 3),* and new bacterial and fugal diseases in sunflower threaten the production. Frequency of dry seasons and years included drought has been increased. It needs new and adaptable varieties. Special aim is to breed varieties capable retaining high yields even under abiotic stress conditions as well. In order to meet this demand, it is necessary to identify the genetic

Fig. 3. *Insect resistant GM corn (MONSANTO)*

Breeding for food or biofuel use?

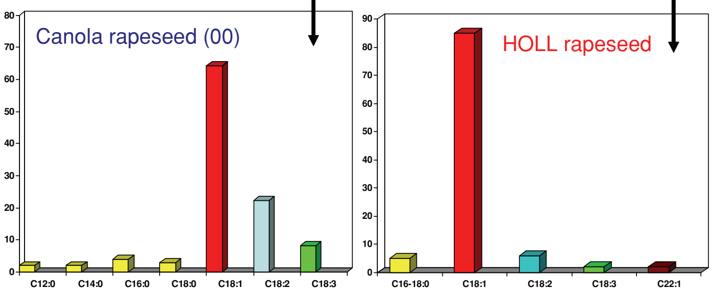
Due to the increasingly unsustainable consumption of energy resources, agriculture will once again be called upon to significantly contribute to civilization's energy needs. One of the questions that Europe must address is what role our agriculture will play in producing the new energy crops? Europe has deficit of diesel oil and petrol (gasoline). In the next years biodiesel production should definitely expand both in EU and in worldwide. In EU there have already been significant programmes to produce biodiesel; and bioethanol of plant origin, then used as a component of car fuel.

The new challenge of oil and starch crops' breeding is to improve new varieties for both food and biofuel industry *(Table 1).*

The main task of oil plant breeders are the optimization of the fatty acid composition for both food and biodiesel use. Both conventional breeding and biotechnology have a role to play in producing desirable oils in sunflower and canola. The biosynthetic pathways, that allow plant cells to produce the

***Table 1.** Biofortification of rapeseed for food and biodiesel use*

Fatty acids	Fatty acid composition of conventional 00 rapeseed oil	Biofortified composition (HOLL) for food and biodiesel use
Saturated fatty acids	7%	< 7%
Oleic acid	61%	75% <
Linolic acid	21%	–
Linolenic acid	11%	< 3,5%



individual fatty acids, of which oils are composed, are well understood. This knowledge allows breeders to modify parts of these pathways to control characteristics such as the level of saturation of the oil. High level of mono-unsaturated fatty acid (oleic acid) can be produced by the improvement of high oleic acid varieties (HO). These goals can be achieved both in canola and sunflower as well by conventional and mutation- or transgenic breeding. In the longer term, cost effective sources of the nutritionally important poly – unsaturated omega-3 fatty acids may also be available by breeding.

Main starch crops in Europe to be used for bioethanol production are in the first place corn (maize), moreover potato and sugar beet. The main breeding aim recently is to increase the starch content in the yield. Another breeding goal is to modify the composition of starch, namely the amilose and amilopectin ratio in order to improve the starch and ethanol conversion by improving the efficiency of ethanol fermentation.

Conventional or transgenic breeding?

Most of the issues mentioned earlier, beg for biotechnical solution in plant breeding. Biotechnology based plant breeding, offers appealing opportunities to develop new varieties for energy use in oilseed rape, sunflower, corn, potato also in Hungary.

As regards protein plants that are soybean, pea, alfalfa the main tasks are to increase the limiting amino acid content, and to eliminate the allergen proteins.

The pipeline for future applications of biotechnology for agricultural crops is clouded by number of factors (consumers' acceptance, intellectual property protection, patented technologies and genes, small crops, gene flow, etc).

Crop-to-crop transgene movement is much more likely then crop-to-wild gene flow. Crop-to-weed gene flow has created the appearance of new or more difficult weeds. When gene flow problems are realized, they can sometimes be costly. The issue of „coexistence" of genetically engineered crops with organic farming may be most

***Fig. 4.** IN VITRO BREEDING: Using in vitro haploid method in wheat breeding (Pauk et al. Cereal Research Non-Profit Company, Szeged, Hungary)*

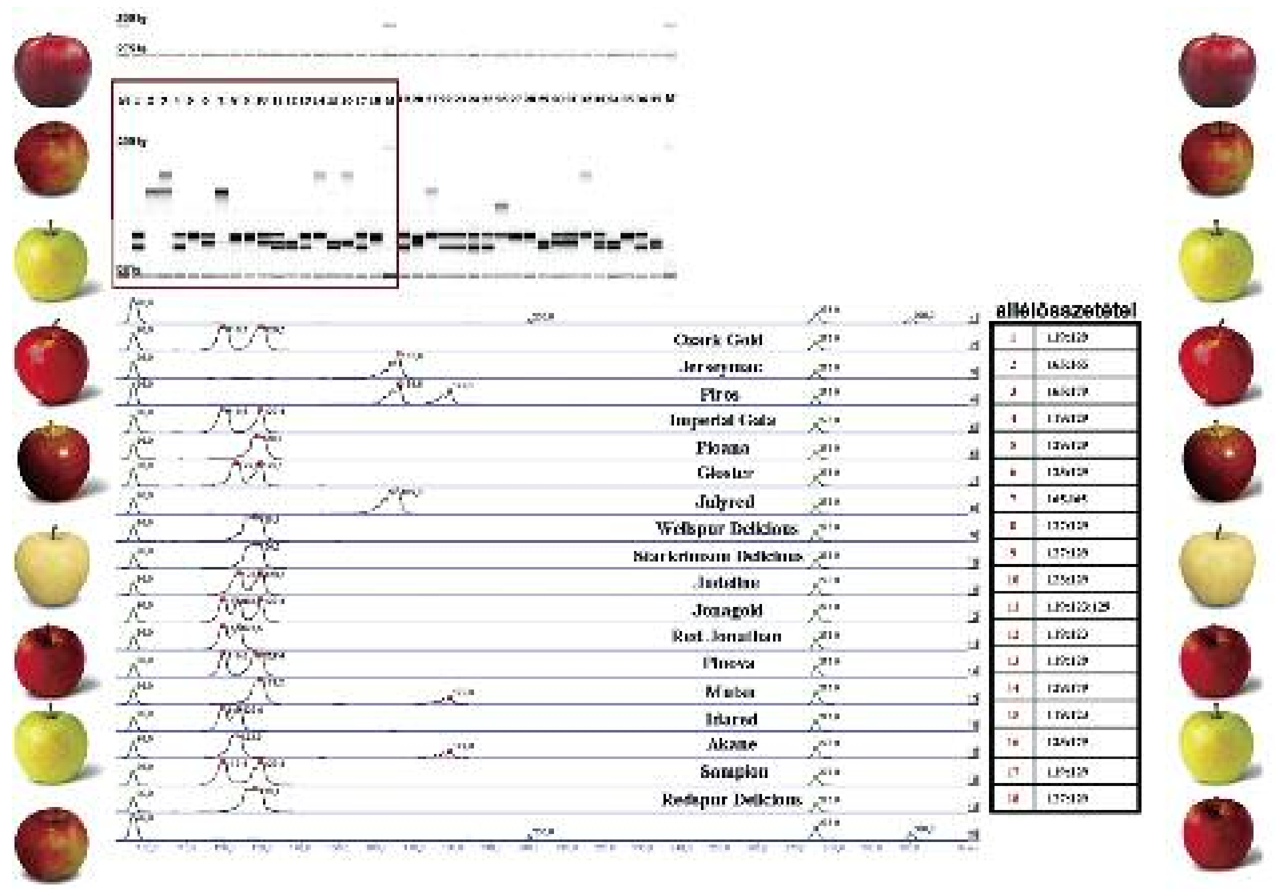


***Fig. 5.** Molecular fingerprinting of apple cultivars (Galli Zs, Halász G., Kiss E., Heszky L., HortScience, 1974–1977)*

important. „all is permitted” or „nothing is permitted” prevents rational social progress, on this issue in Europe and in Hungary too.

Plant breeding contribution to meet the challenges

There were the challenges; now let us see the new approaches in breeding methods, what we have to take into consideration in order to meet the challenges.

Considering the magnitude of the challenges we face, we should in general use all new knowledge and tools, that can contribute to overcome the previous mentioned problems and to maintain our long-term productivity and sustainability. The successful production and application of new knowledge is necessary to overcome the previous mentioned problems.

Fundamental research in plant biology including molecular genetics, molecular biology, genomics, proteomics, bio-informatics, and molecular physiology is necessary to increase our knowledge with improving crop plants. The new interdisciplinary fields, as **in vitro breeding, molecular breeding and transgenic breeding** will help our capability to prevent the bad consequences or to overcome difficulties discussed earlier.

New approaches of In Vitro breeding:

- Induction of haploid and DH (doubled haploid) forms, and improving DH varieties (cereals, hybrid canola, corn and sunflower etc.). *Fig. 4.*
- In vitro selection of herbicide tolerant mutant and breeding herbicide tolerant varieties and hybrids (e.g. SUMO, IMI hybrids in corn and sunflower)
- Production of new synthetic *Brassica napus* forms by sexual crossing and protoplast fusion of the two parental species (*B. oleracea* and *B. capestris*)
- Improvement of automized technologies for vegetative micropropagation and application of artificial (synthetic, somatic) seed production.

New approaches of molecular breeding:

- Identification of the sterile cytoplasm by molecular markers in food and industrial crops (oilseed rape, sunflower, wheat, corn etc).
- Pedigree analysis of cultivars and hybrids by DNA hybridisation and PCR based methods *(Fig. 5).*

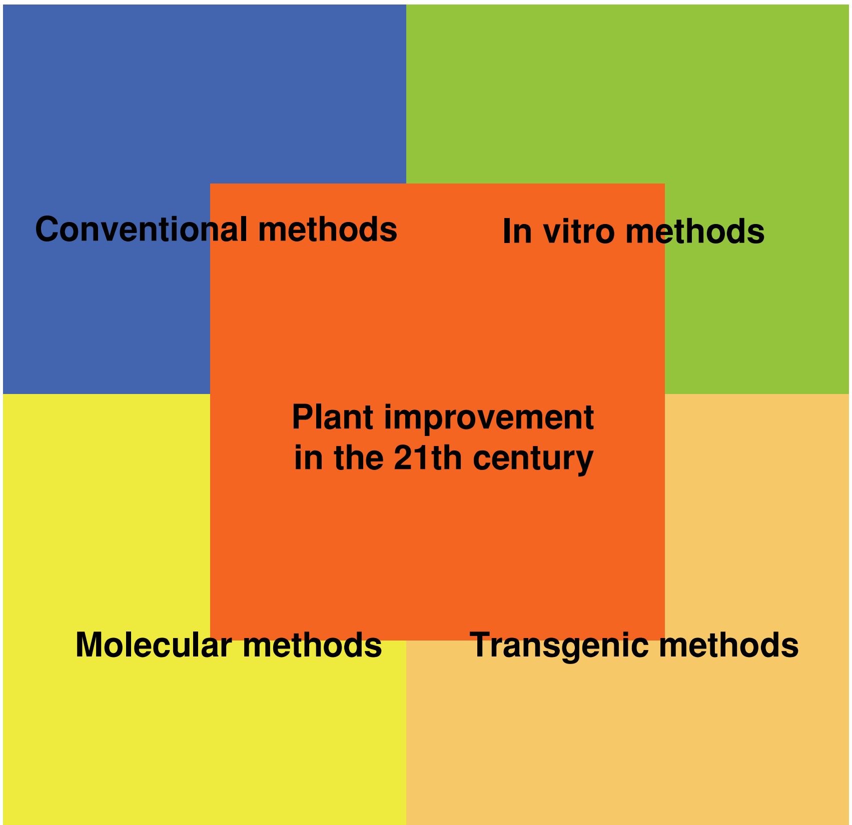


Fig. 6. *Combining the different techniques and approaches to meet challenges of 21st century*

- Application of Molecular fingerprint approach for patenting and protection of varieties and hybrids in various species
- Utilization of MAS (marker assisted selection) for improving biotic and abiotic stress tolerance of varieties and hybrids etc.

New approaches of transgenic breeding (gene technology)

- Elimination of allergen proteins for food consumption by using sense and antisense genes and RNA interference (soybean, wheat, etc.).
- Increase of oleic acid content and decrease of linolenic acid level in sunflower and canola oil for biodiesel and food industry purposes.
- Increasing of protein content, and improving protein quality in staple crops (corn, legumes, grasses etc.).
- Production of total herbicide tolerant GM varieties and hybrids
- Hybrid breeding by using transgenic male sterile parental lines. (canola, wheat, rice etc.)
- Isolation, identification, functional analysis of targeted genes confer resistance against biotic stress such as pathogens and insects etc., or involved in tolerance of abiotic stress like the drought, total herbicides etc.

Conclusion

It is clear that the challenges we face in the 21st century are greater than those we faced in the last century. Agriculture is called upon to produce more food and feed, more fuel and more industrial raw materials, I hope, modern plant breeding used in vitro, molecular and transgenic approaches is a magic bullet that will solve all of above mentioned problems and will play a key role in achieving those goals *(Fig. 6)*.

There are lots of tools for breeders to improve varieties of cereals, protein and oil crops. I hope plant breeding can contribute to sustainable agriculture and to the improvement of food in quantity and quality as well as security and safety. Looking to the future, it is one of the keys to combine the biotechnological and molecular techniques with conventional plant breeding procedures.

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Viktória Szente[1]

Consumer trends in organic foods in Hungary

In Hungary controlled ecological farming is carried out on 130 000 hectares (according to 2005 data). As for production structure, the dominance of crop production (arable production) can be observed. Ecological animal production has a slight role and a lot of people are unsuccessfully seeking organic fruits and vegetables. Ecological farming has come to a standstill during the past years. Fortunately the interest of consumers and the more vivid trade activity kicked it into motion.

Total turnover of ecological products is about 10.5–14 billion HUF in Hungary. It is less than 1% of total food sales. Consumption level of organic foods is even lower, about 0.5% of the total.

Despite the unfavourable market tendencies and ever sharp competition ecological products of Hungary are sold mostly abroad. Dominance of raw material production seems to continue. Producers, processing plants and certain commercial companies are endangered not only by narrowing export possibilities but also by the widening import channels. Beside export-focused production retail shops hardly offer a wide choice of Hungarian products, consumers can buy imported and in some cases inferior quality goods. The market has simultaneous supply and demand characteristics: sometimes products are marketed that no one buys and sometimes the required products have narrow choice or insufficient quantity.

Consumer preferences of organic food

In order to understand the consumer behavior, the factors influencing food choice need to be reviewed. Hundreds of research results verify that besides price, food choice is strongly influenced by the healthy, chemical and additive free nature of products. According to the Eastern-Central European life style research by GFK Hungaria, approximately 65% of Hungarians consider organic foods of the ideal aliment of the future (*www.hvg.hu, 2005*). A survey conducted by Szente (*2005*) shows that the wholesomeness of foods is an important factor for 83.7% of the people purchasing organic foods, which definitely reflects the more conscious consumer behavior of this group. Among people not purchasing organic foods this ratio is 64.5%, which shows their strong willingness as well.

In the last years, the consumption of organic foods only moderately increased, and it can be surely stated that these foods will never become mass products in Hungary. This trend, among others, is due to the following factors:

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- The increasing demand is primarily directed towards basic foods (milk and dairy products, bakery products, vegetables and fruits). This is reflected by the broad assortment of these products in stores, including domestic and import foods as well. The turnover of processed, ready-to-cook products is low; they are present on the shelves as assortment broadening only.
- There is a limiting factor present when buying vegetables and fruits, the belief that 'what looks good cannot be healthy'. Many people do not trust organic foods even if they can see the control certificate or the organic label.
- Price sensitivity is still present among people purchasing organic foods, the cheaper products are demanded. The influence of price on the decisions can particularly be perceived in the case of basic foods.
- During shopping, people visit stores where they usually shop, and other goods can be purchased, too. Therefore, a re-arrangement of the retail structure can be perceived, in which the winners will be the hyper- and supermarkets, and within a couple of years, following the trends in Western Europe, discount stores. Besides their easy availability, their main attractive factors are convenience and lower prices of basic organic foods.

The change of the retail and distribution structure is more and more strongly reflected in the consumer behavior Due to the various services offered by different types of stores, this new structure is capable of segregating different consumer groups, thus fulfilling individual needs. In the following chapter the change of the Hungarian retail structure is presented.

Purchasing place of organic food

In countries like Hungary, retail structure is constant motion in present times. The re-arrangement of export and import markets and the progress of EU accession induced perceivable changes in the food consumption behavior.

At the beginning, organic products were available usually at the producers and in unprocessed form only. By the middle of the 90's, the majority of the sales was settled by organic markets and health food stores and reform stores. By the millennium, the network of specialist stores became stronger, organic foods became available at several places in the rural towns, too. By this time, processed organic products appeared in hyper- and supermarkets. At first, the presence of organic foods was almost imperceivable in these stores, but since then, the volume has been increasing, and they win over more and more customers from health food and reform stores.

In the past years, more and more positive changes could be perceived regarding the alternative distribution channels in Hungary. Local delivery, which used to be almost unknown, moved out of a standstill, and 100% organic food

packages are available in more and more cities. Besides seasonal vegetables and fruits, traders offer processed foods such as milk and dairy products, meat products, vegetable pastes and tropical fruits as well. The number and assortment of health food and reform shops is growing, too. The most successful distribution channel, however, is the organic market in Budapest. The consumers, impressed by the imposing environment and the opportunity of direct purchase from producers, pay the higher prices of organic products almost unnoticed.

The increase of the small-scale food processors is considered to be a significant change. They market more and more differentiated, often old, "forgotten" products, primarily through alternative distribution channels. The increase of demand, however, has brought up several weaknesses of specialist shops and alternative channels against hyper- and supermarkets. These obstacles are the following:

- Small floor-space
- Lack or limited number of technical appliances
- Simultaneous selling of fresh vegetables, fruits, milk, dairy products, meat, and bakery products require huge investments
- Often under-educated shop assistants.

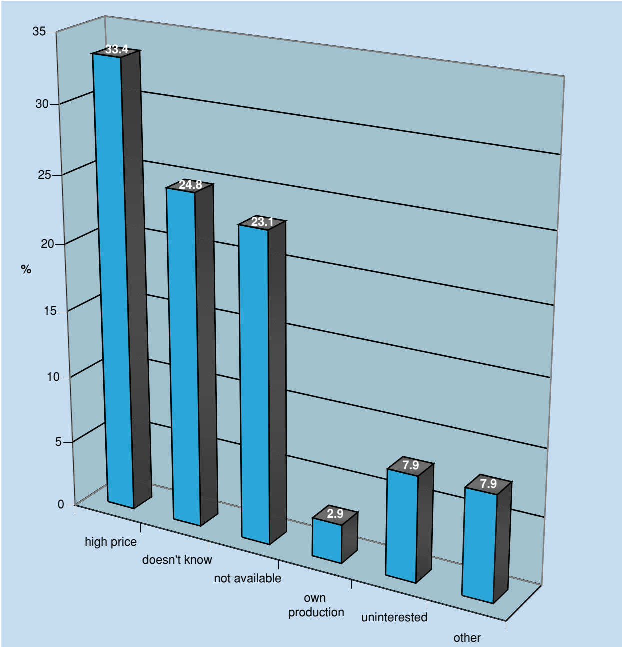
Besides the above listed obstacles, the organic sector needs to face another challenge from June 2005: according to the EEC Regulation No. 2092/91, all retailers of organic foods are required to undergo a control process. The regulation incorporates several anomalies.

Anomalies of retail regulation

Just as producers and processors, retailers of organic food are required to comply with the regulations covering topics of storage, separation from conventional products, waste management and packaging materials in the first line.

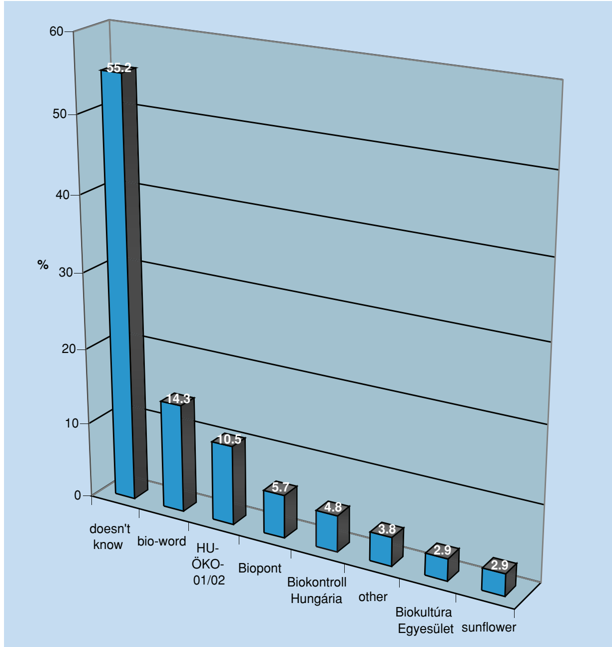
Earlier, the retailers selling organic foods were not required to be inspected, but starting 1 July 2005, health food and reform shops, hyper- and supermarkets, and all other food retailers and wholesalers are subjects to the above mentioned regulation and the Council Regulation (EC) No. 392/2004 as well (*Roszik, 2006*). All sellers have to have an inspection made, which enables the retailer to use the label 'controlled organic shop'. The purpose of the regulation was to increase consumer trust, since several intended and unintended trespasses occurred at the stage when the consumer meets the product. However, the efficiency of the new regulation is strongly questionable. The major dilemmas are the following:

- Retailers consider the control as unnecessary, time-consuming and costly. (Besides the control fee, retailers need to comply with the regulations on separated storage, waste management, packaging, etc., what is difficult or even impossible to accomplish for smaller shops).
- They pass along these higher costs to the consumers, who consider organic products too expensive even today (*Figure 1*), they mentioned high prices as the major counter-argument against the purchase of organic foods.
- The label 'controlled organic shop' cannot improve the consumers' trust, due to the fact that they are not familiar with the regulations and they do not even know the controlled product labels, either. This is presented in *Figure 2,* showing that only 10.5% of customers of health food- and reform shops could



Source: Szente el al., 2006

Fig. 1: *Counter-arguments against buying organic food (n=197)*



Source: Szente, 2005

Fig. 2: *Knowledge of organic product symbols (n=105)*

correctly define the letter-number combination destined to mark organic products.

It would be more reasonable, if the denotations bio (biological), eco (ecological) or organic were solely allowed for products and retailers complying with the regulations, the inspections, however, should not be restricted to subjects signed in into the control system. Through strict control, the use of these denotations could be banned for several producers and retailers gaining market advantages by abusing the consumers' good faith.

Possible alternatives in the future

After these thoughts a question may emerge: what will the future bring on the market of organic foods? The progress may have two outcomes:

1. The organic will slowly disappear from the shelves, and their place will be taken by other products of trust or functional foods with health benefits.
2. In the more favourable case, the market will grow. In the near future, the surplus of supply will remain, and due to the strong activity of retailers, the interest of consumers in organic foods will increase.

The only way to sustain or increase the consumption level is to intensify marketing activity, with special interest on common marketing. Among the elements of STP marketing, positioning should receive special attention, since research results show that their wholesomeness in the most important factor in choosing organic products. Therefore, producers and retailers should position their products by accentuating their scientifically proved nutrition benefits. Using target group oriented, individual marketing, there is a chance to maintain or improve the market positions. The current regulation structure should be thought over in the fields of trade, control and subsidies. The purpose of the regulation should be the easier accomplishment and the encouragement of market process. There is a chance to maintain the organic food market only if it is driven by the consumers' willingness to buy organic products.

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István Szabó[1], Lajos Fogarasi[2]

Energy-Purpose Utilization of Miscanthus in Hungary

Abstract

In Hungary, the ratio of 'green' electric power will be as high as 12 to 15% following 2010. In the EU, the share of biomass is 50% of the total renewable energy sources. Taking the natural resources (limited wind and hydro power potential) into consideration, this ratio is about 80% in Hungary and the cheapest process of the energetic utilization of biomass is direct firing in boilers. Practically, the plant Miscanthus can be recommended to farmers and suppliers of the greater 'green-energy' integrations: all the farms have got the necessary basic tillage equipment for plantation, the nutrient demand of Miscanthus is not significant and there are no known pests or pathogens of Miscanthus in Hungary. The critical operations in the growing technology are the planting process and the harvest. The improvement of their technology and machinery is the most important task in the R+D project of the Institute of Mechanics and Machinery (Faculty of Mechanical Engineering, St. István University, Hungary).

Introduction

After the Kyoto Protocol (the international Framework Convention on Climate Change), Europe attempted to decrease the carbon-dioxide emission, and, at the same time, make herself independent from the dwindling energy carriers (natural oil and gas) imported mainly from beyond her borders. The European Union and the member countries have committed themselves to the use of renewable energy; the proposed object is to reach a 12% proportion (about the double of the present EU average ratio) of renewables in the total energy balance, and 22% in the total electric-power production up to 2010.

In Hungary, the ratio of 'green' electric power was already 4.5% in 2005; however, the expectable requirement will be 12 to 15% following 2010.

Renewables and Hungarian agriculture

In the EU, the **share of biomass** is 50% of all renewable energy sources. Taking natural resources (the limited wind and hydro power potential) into consideration, this ratio is about **80% in Hungary**.

The energetic utilization of biomass can be performed by *firing* in boilers (to generate heat and electric energy) and in the forms of *bio-engine-fuels* or *biogas*. The **cheapest** process of the utilization of biomass as energy is the technology of **direct heat production** (direct burning).

At present the actual amount of the firing-purpose materials gained from forestry cultures is enough to fulfil the public demands, and to supply with fuel the smaller power plants converted to biomass-firing; however, **the larger amount of biomass** required by future development **can be provided only from new resources** – either by gathering agricultural by-products and waste or growing **energy-purpose plants or plantations**.

After implementing the conditions needed in 2010, due to the expected increased demand for these plants or products in the neighbouring EU-member countries, the production of energy plants may exceed domestic sales as well. By exploiting the excellent land bearing capacities, the energy plants produced in Hungary may be potential exports.

Miscanthus

The appropriate materials of biological origin for production of heat are the forest and woodworking products and waste, the wood energy plantations

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(acacia, poplar and willow), natural reed, grown energy reed (e.g. Miscanthus Sinensys Giganteus) domesticated by plant-breeding, various herb-form energy plantations (energy grass); the field-land by-products (straw, maize- and sunflower-stalk), the horticultural wastes and other by-products and wastes. Cereals can also be used as energy crops; however, by the European mentality, it is a crime to burn bread-crops.

Due to the high oxygen content of biomass fuels, their calorific power (12 to 17 MJ/kg) is similar to that of the low-grade coals *(Table 1)*. At the same time, the SO_2 content in the flue gas is low (maximum 60 ppm). *Bense* (2008) measured essentially the same calorific data with Miscanthus chops.

However, the biomass-to-coal ratio is 1:2 to 1:4 accordingly the extent of the storage, preparation plant and transport or conveying machinery has to be increased. Biomass, because of its fibrous structure, cannot be milled by conventional coaling mill-separator devices. Accordingly, it is necessary to improve the technology of the selected thermal powers plants.

Practically, the plant Miscanthus can be recommended to farmers and suppliers of the greater 'green-energy' integ-rations. Its calorific value – considering the plantation life and the specific yield – is well acceptable. Miscanthus Sinensys Giganteus is a perennial endemic plant in East-Asia but it can be successfully grown in the most regions of Europe. The applied technical machinery is similar to that of corn-production or rather – the Sudan-grass culture. A plantation may subsist even for 20 years and during this period the soil does not have to be cultivated at all. The soil state is conserved by the regeneration of rootage and the soil protecting effect of the fall of leaves. The treading soil-damage can be prevented by the harvest in winter on the frozen ground.

All the farms have the necessary tillage equipment for plantation (subsoiler, plough, disc-harrow, field and inter-row cultivators, seed-bed preparing combination, ring- or clod-breaker rollers etc.). The nutrient demand of Miscanthus is not significant. Spreading waste-water or other organic sludge matters (e.g. slurry) can be substituted for artificial fertilizers since Miscanthus is not used for food or foodstuff.

There are no known pests or pathogens of Miscanthus in Hungary; spraying may be necessary against diphyllous weeds but only in the first two years. The established plantation keeps the weeds down.

In Hungary, now the main industrial project is to establish the primary production chains for Miscanthus, and to solve the actual mechanization problems.

Preparatory works of Miscanthus plantation

Miscanthus Sinensys Giganteus is planted in the spring. The life of the perennial plantation may be 20 years or longer as well and during this period the soil cannot be cultivated at all. Accordingly, before planting, the perfect cultivation of stubble completed with sub-soiling and deep-ploughing tillage, herbicide

Table 1

Composition of some biomass fuels

(measured by CHN analysis; ash 1.0 to 1.7 %)

(% m/m)	Poplar	Acacia	**Miscanthus**	Hemp cavings
C	48.9	48.0	46.6	47.1
H	6.4	6.8	6.8	6.0
N	1.6	1.0	0.2	1.2
O	43.2	47.2	46.5	45.7
Combustion heat	17,650 kJ/kg	19,830 kJ/kg	**17,810** kJ/kg	19,340 kJ/kg

(Kotsis-Marosvölgyi, 2006)

application and basic fertilization (with artificial and farmyard manure) is necessary.

After planting in the first two years, for weed killing, inter-row cultivators can be used; spraying may be necessary against the diphyllous weeds. The soil state will be conserved by the autonomous life action of the plants.

The critical operations in the growing technology are the planting process and the harvest.

Planting

The Miscanthus can be planted by transplanting seedlings (plants) or rhizomes. The planting depth is about 10 cm with rhizomes and the stand density is 1 to 4 plants per square metre with both methods. When growing the plant directly for sale as an energy crop, the stand density is 1 to 2 plants per square metre; the actual row space is 0.75 to 1 m and the space between plants in a row is 0.5 to 1 m.

Theoretically, any transplanter is suitable for planting Miscanthus seedlings however the supply of plants is problematical. With free-root seedlings, the length of 25 to 30 cm is advantageous or, with plants in soil blocks, the block size of 4.5 × 4.5 cm is suitable in terms of mechanization.

Figure 1: *Manually fed rhizome planter (Petényi Ltd. Co. Hungary)*

The planting of rhizomes can be carried out by potato planters. Unfortunately, the widely used **scoop planters are not suitable** due to the tangled, meshing root-cuttings. However, the auto-manual (semi-automatic manually fed) revolver-type designs can be successfully used. (*Bense, 2008*)

Temporarily, manually fed simple transplanter attachments equipped with spacing indicator *(Figure 1)* are used. Their area-performance is extremely low due to the low (inching) travel speed and the limited working width and they require a considerable amount of manual labour.

Industrial farms prefer the full-automatic designs. The *Hvidsted Energy Forest Co.* developed a special Miscanthus-rhizome planter *(Figure 2)* in Denmark. This 'bulk-flow' or spreader-type

Figure 2: *Rhizome planter NORDIC (Hvidsted Energy Forest)*

machine plants a fifty-fifty soil-rhizome mixture. A scraper conveyor delivers the planting matter along the plateau of 5-metric-tonne capacity to the front of the machine (actually – a manure spreader) where a horizontal master drum and the vertical beater (shredder) rotors throw the rhizomes into the collecting vibrated cones. The row space is 75 cm and the planting depth can be adjusted between 2 and 20 cm. The plant spacing depends on the ratio of scraper-convener velocity to the travel speed. The rows are covered by a rubber press-roller loaded by 150 kg in order to a good soil-rhizome contact.

The area-performance of the type NORDIC is high, and requires only a little manual work. However, the **distribution of plant number is unequal** due to the spreader mechanism. The working principle of the special Miscanthus planter of the company BICAL is similar to that of the NORDIC.

According to the modelling calculations (*Bense, Fogarasi, 2008*) on the pattern technology, the mechanical work demand to establish plantation is about (probably less than) 10% of the total technological cost of the plantation however the quality of planting and the cultivation in the first two or three years basically determine the success of the investment.

Harvest of the Miscanthus

Theoretically, the harvest of Miscanthus is mechanical; the roughage-harvester machine line can be used in herb-like energy plantations as well. The Miscanthus can be gathered by the usual (self-propelled) forage chopper-harvesters; however, the dry-matter mass per one cubic metre of chopped energy reed is only 80 kg! In addition, it is expedient to store the cut energy reed in a closed bunker protecting the material from precipitation or the effect of wind. Storage has to be ventilated for fear that the bulk may heat.

The Miscanthus harvest can be carried out by the machinery line of hay making as well; however, now several new technical problems arise: the period of harvest (late winter or early in spring), the windrowers cannot be used because of the stiff and hard canes. The double-knife mowers are not suitable.

Figure 3: *Flow of the cut crop through the special rotary mower (Kemper)*

The row-independent rotary mowers with counter-blades *(Figure 3)* are capable of laying down the cut stand in a narrow windrow required by the pick-ups only if the high canes are tilted forward by an auxiliary frame before (and during) cutting. The guide frame is not an accessory of the mower; it is a custom-built piece, adapted to the actual mower type.

Both the rectangular and the roll balers are suitable for gathering Miscanthus. By materials-handling aspects, the conventional **power stations prefer the rectangular (square) big bales of higher density** but the small municipal incinerator plants are capable of utilizing small bales as well. The storing and transporting aspects include the density and the space filling efficiency of the bales – the rectangular big bales exceed the round bales from both points of view. In the case of the moisture content of 20 to 25%, the bale density is 120 to 150 kg/m^3 with round bales, and about 170 to 220 kg/m^3 with rectangular bales. The **higher densities can be achieved only by slicer-balers**. In addition, **in Hungary, the harvesting moisture content of the Miscanthus is 7 to 10%**, with much worse compressibility.

According to the tests carried out by the CLAAS Company between 1990 and 1995, the conventional pick-up device of the baler does not work properly due to the properties of the plant (length and springiness); the picking-up is problematical (either much material is missed or the device mixes earth in the bales).

Figure 4: *Self-propelled baler (Deutz-Fahr)*

The perfect solution would be a direct baler. The firm Deutz-Fahr fabricated the self-propelled baler type Power Press 120H in series *(Figure 4)*.

The Austrian firm HUMER designed a tractor-implement combination. The main parts of the machine combination are a row-independent mower unit mounted on the front hitch of a 150 kW agricultural tractor, an under-mounted belt conveyor and a high-density baler type Krone HDP Big Pack (with the performance 0.7 to 0.8 ha/hour or 15 to 16 500 kg bales per hour).

Conclusions

The technological machine fleet of the plants commonly grown in Hungary is suitable for the mechanization of the production of Miscanthus Sinensys Giganteus. **The only problems are the planting process and harvesting.** In the conditions of Hungary, the design of a rhizome planter similar to the manually fed, revolver-type, mechanically spacing potato planting machines which requires less concentration of the operators; they can feed more cells at the same time and it facilitates to avoid the deviation (lag) from the planting rhythm.

Harvesting can be carried out by the common machines of forage harvest. However, storage difficulties will arise when using chopper-harvesters and contrary, the baling process will be accompanied with pollution – mixing of soil (snow) in the bales. A single-pass, self-propelled harvester-baler would be the best solution but such machines are not manufactured at present. Custom-made machines might be ordered; however, it has to be reasoned even if the production volume is quite large.

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Genetic Transformation: tool for genetically improving grapevines

Grape is one of the oldest cultivated plants, as well as a worldwide commodity. Vineyards have recently continued expanding due to the demand for wine and development of wine production and fruit processing. Grapes top the world's fruit crops. On the other hand, in the coming decades plant scientists need to face more and more urgent enviromental challenges. The impacts of climate change limit the capacity of agricultural production. Genetically engineered crops can survive unfavourable or extreme circumstances to flourish as researchers work hard on creating the varieties which belong to quicker and wider adabtability in order to cope with these circumstances. GM plants can play a critical role in the sustainability and ensruing yields worldwide. Furthermore, they can contribute to the protection of the enviroment in that less chemicals will be used owing to disease and insect resistant varieties. Researchers are struggling to combat different diseases of grapes such as downy and powdery mildew, *Botrytis, Phylloxera*, crown gall caused by *Agrobacterium,* as well as adverse climatic and soil conditions. Therefore, grapevine growers maintaining the competitiveness of the sector will require a deeper knowledge on the genetic background of grapevine. Breeding of grape is aimed at protecion against biotic stress; infection from pathogens and abiotic stress such as drought, low and high temperature, ultraviolet B radiation, soil poor in nutrients. These limiting factors have a large effect on plant productivity. Additionally, intentions in grape breeding to improve on fruit quality, taste, and nutritional value by introducing new characteristics or amplifying desirable traits. The common objectives of most breeding programs are to produce locally adapted, high yielding and quality cultivars adapted to enviromental and pest stress. In practice these objectives are complex given the different characteristics needed for table, raisin and wine grape production. In addition, other desirable qualities are considered when breeding rootstocks.

Compare molecular to classical breeding

Over the last 20 years, advances in plant biotechnology have produced new tools for genetically improving crops.

The general aim of molecular grapevine breeding programs is to develope and apply novel gene and plant tissue technologies capable of introducing genes in a carefully targeted manner. Molecular improvement in grapevines can be achieved using genetic transformation to improve stress and disease tolerant or resistant varieties, facilitate transfer of desirable traits through producing transgenic plants. The transfer of a single feature into a grapevine is almost impossible by classical crossbreeding methods due to grape's heterozygous nature. In contrast the genetic transformation permits addition of single traits, largely without affecting the genetic background of existing valuable cultivars. In summary, the key difference is that, instead of randomly mixing genes, which occurs as a result of a sexual cross, a specific gene, which is associated with a desirable trait, is selected and inserted directly into the new plant variety. This can save time and it reduces the chance of undesirable traits in the new plant variety. Although, crossbreeding is quite complicated by a high heterozygosity in grapes, frost tolerant and disease grapevine varieties have been obtained which required long time period. This process takes place over a number of generations, which usually means a number of years and newly processed varieties is

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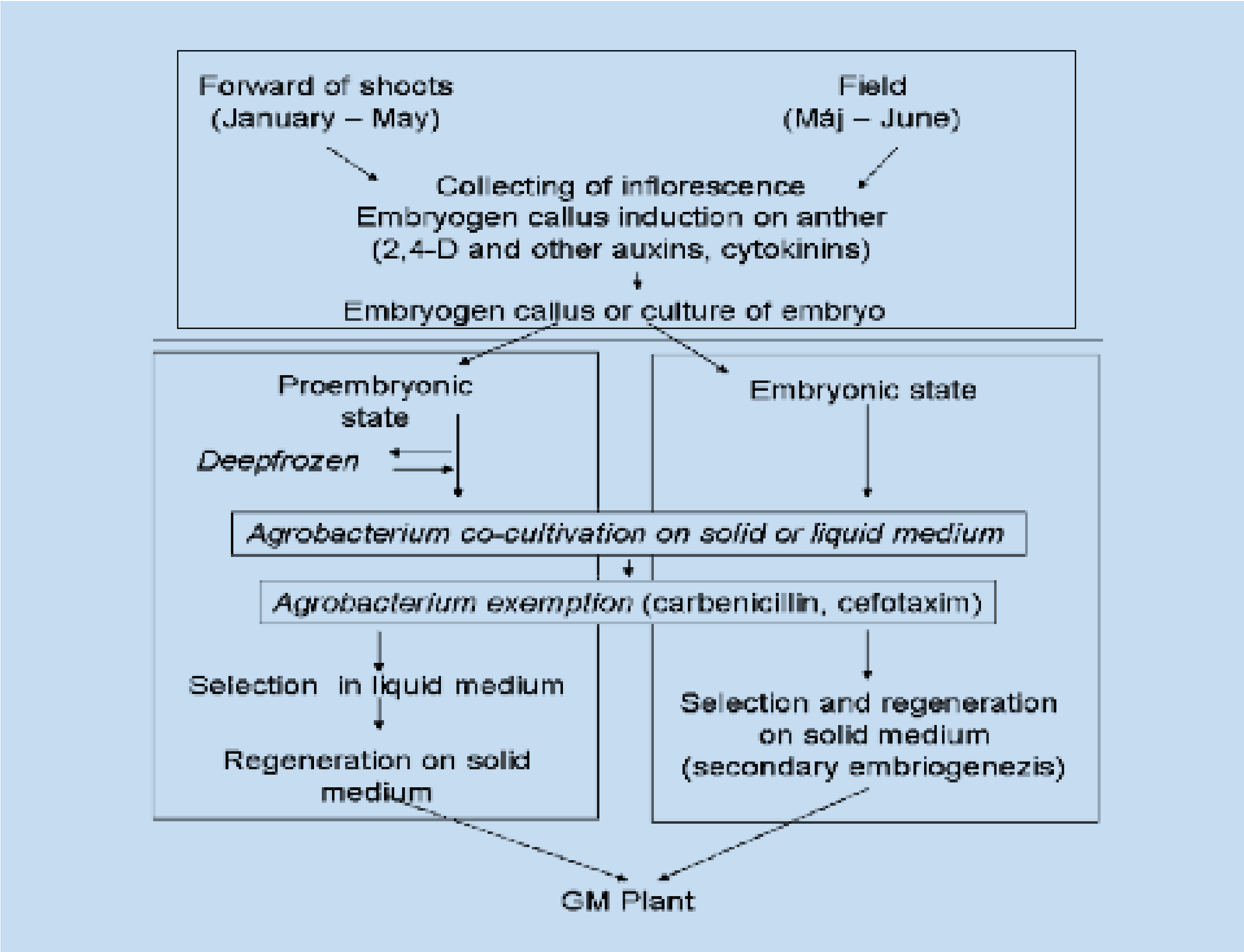

Table 1.

less accepted by growers and oenologists as they essentially prefer traditional ones to unknown, new varieties.

Vitis vinifera grape genom sequencing project was completed last year (Vezzuli et al., 2006; Quetier et al., 2007), the enormous data sets have been organized into searchable databases and made accessible to researchers over the Internet. Grape researchers have also begun to apply this leading science utilizing the resulting information for the advancement of viticulture. Whereas a substantial amount of information enable grape researchers to identify key grapevine genes and understand the function of the query gene due to their over-expression or silencing in GM plants.

How to produce transgenic plants?

Successes have derived from advances in embryogenesis, transformation and regeneration. The foundation of genetic tansformation is the existence of an effective regeneration and transformation system. The first successful results about organogenesis (Rajasekaran et al., 1981) and somatic embryogenesis (Mulins et al., 1976) was reported. Grapevine transformation experiments have already been started in Hungary as well to indruce novel genes into the rootstock variety 'Georgikon 28' (Mozsár et al., 1998). Based on these results the examination of grapevine regeneration via somatic embryogenesis has started and was this transformation method tested on some *Vitis* genotypes with model genes (Oláh et al., 2003) Production of transgenic vines has now become routine in laboratories. In the case of grape the effect of genotype was recognized early on (Stamp et al., 1988). General regeneration technique which can be adapted for every genotype does not exist. (Vivier et al., 2002)

In most plant species, transfering gene is based on *Agrobacterium* mediated transient DNA delivery. Our research team also carry out the transformation experiments with this 'nature vector', exactly *Agrobacterium tumefaciens* strain EHA105, EHA101 of which binary vector plasmid T-DNA harbours the useful transgene and selectable marker genes in construction.

Figure 1: *Forwarding of inflorescence on defoliated grape cuttings*

Figure 2: *Embryogen callus induction on anther*

This method ensures that the introduction of genetic information remains stable genetic transformation whereas a defined transgene is inserted into grape genom.

An effective and repetitive method to operate gene transformation is through the production of somatic embriogenesis and the induction of embryos. *(Table 1.)*

In our laboratory embryogenic callus was obtained from somatic anther tissue, although the use of other tissue types has also been reported. Basically it is a quite simple procedure. First, anthers were collected from inflorescences of different varieties for example from rootstock 'Richter 110' and from scion 'Chardonnay' grapevines in the bud stage. *(Figure 1.)* One of the possible solutions can be defoliation of grape cuttings assisted in their capability for being bloomed under greenhouse conditions.The other possibility is collecting anthers on fields. Secondly, anthers need to be disinfected, for which we used a sodium hypochloride solution (Clorox 10%) keeping in it for 15 minutes then rinsed three times in sterile, distilled water. Excised anthers with filaments were incubated at 26–28 °C in dark on a solid medium in Petri dishes. The embryogenic capacity of the resulting calli was estimated seven months after starting the experiment. *(Figure 2.)*

Embryogenic culture induction medium consisted of Murashige and Skoog (1962) basal salts and vitamins, supplemented with 20g/l saccharose, 70mg/l FeEDTA and were solidified with 7g/l Oxoid agar. The pH was adjusted to 5.8. Mostly used medium for rootstocks is MST1 (Oláh et al., 2003) was prepared by supplementing with 1.1 mg/l 2,4-dichlorophenoxy-acetic acid (2,4-D) and 0.05 mg/l thidiazuron (TDZ). Our research group finds MSE medium (Mozsár et al., 1994) very suitable and effective. It contained 0.1 mg/l 6-benzyladenopurin (BAP) and 1.1 mg/l 2,4-D. Various plant growth regulator (PGR) combinations are effective at inducing somatic embryos in different grape genotypes.

Resulting callus culture were transfered monthly to fresh medium, somatic embryos were induced on hormone-free medium. *(Figure 3a)*

Transformation was achieved by co-cultivating embryogenic

Figure 3.a

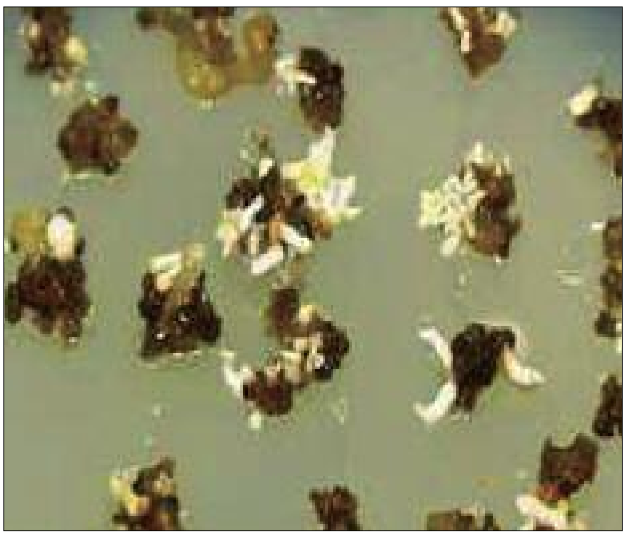

Figure 3.b

Figure 3.c

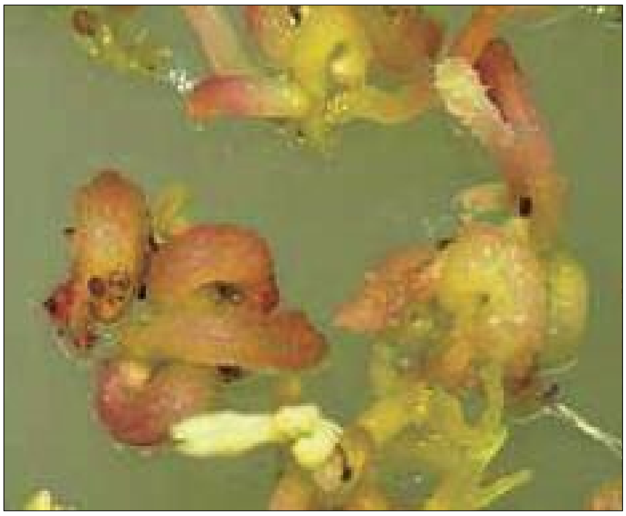

Figure 3.d

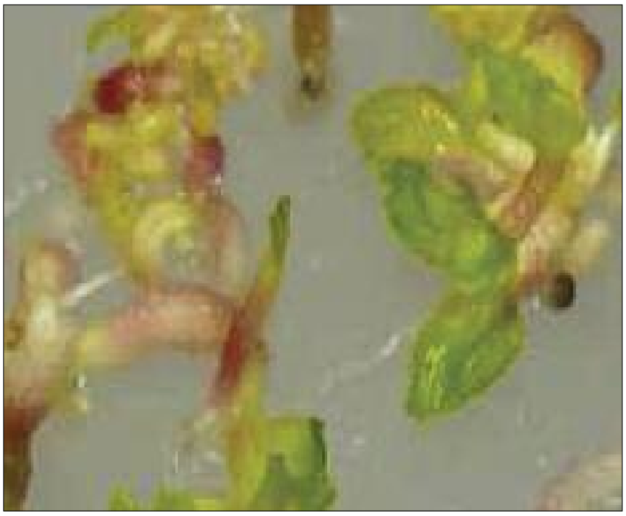

Figure 3.e

Figure 3.f

Figure 3: *a Embryogenic calli derived from anthers b, Newly formed somatic embryos on selective medium c, Different developmental stages of transgenic embryos in dark d, After passing on light e, Plant regeneration f, In vitro transgenic plantlet*

Figure 4.a *In vitro transgenic plant*

4.b *Transgenic grapevines in greenhouse (photo by Ernő Szegedi)* culture with Agrobacterium tumefaciens EHA101 (pTd33). Small amounts, approximately 20-30 µl of bacterial suspension (10^8 cells/ml) were applied to the surface of calli in 5–10mm diameter kept on hormone-free solid medium. Inoculation and co-cultivation with agrobacteria was performed with minimum handling according to Oláh et al. (2003). After 2 days inoculation, the culture was transferred to selection medium, containing 20 mg/l kanamycin for selection among calli, 200 mg/l carbenicillin and 300 mg/l cefatoxin, these antibiotics destroy in the less bacteria, 4 g/l polyvinylpyrrolidone (polyclar AT) which can bind the phenolic-components and 0.1 g/l dithioerythritol antioxidant for reducing damages caused by stress, saving the cells through a transformation process. Viable callus portions and embryos were selected and transfered to fresh selection medium after about six weeks, and this was repeated several times. *(Figure 3.b)*

Then the germinating embryos for plantlet development were isolated and picked to new tubes containing the same medium without antioxidants. In the beginning transgenic embryos grow and develope in the dark *(Figure 3.c),* then are transfered into the light to induce shoot and plantlet development. *(Figure 3.d)*

After the plant regeneration *(Figure 3.e),* picked and passed separately on fresh medium to help development. *(Figure 3.f)*

In summary, our research team is working on some experiments to clarify the importance these factors, study the effects of newly adopted chemicals and optimalize conditions in order to make more effective transformation and improve regeneration method with reducing the time. Based the results we are taking part with different cooperations in applying useful gene constructions for the practice on the following fields: oxidative stress resistance by ferritin, *Agrobacterium* and powdery mildew resistance or tolerant transgenic grapes by using different gene constructions, promoting earlier ripening of berries. In some cases the testing and evaulation of transgenic plants has alread begun and is currently being set up in the greenhouse under direction by Ernő Szegedi.

We hope our resultful work and efforts can be utilizable in practice and increase the efficiency of grapevine cultivation.

Acknowledgements

Thank you to the cooperation partners: Biological Research Center HAS, Institute of Plant Biology, Szeged; CUB, Research Institute for Viticulture and Enology, Kecskemét; Research Institute for Viticulture and Enology, Pécs, Hungary

The authors are grateful to Veronika Tóth for her helpful technical assistant.

This work was funded by grants National Scientific Research Found (OTKA) Grant No. T049438/2005.

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CFRI-DAY on PROBIOTICS

In the series of CFRI-days this was the 4th event. The aim of the symposium was to underline the health promoting effect of probiotic bacteria and to direct attention to statements to be further confirmed and problems to be solved. Organizers invited three experts as speakers to summarise the state of art in: Probiotics as efficient supplements in dietetic treatment of intestinal disorders. Health claim regulations and the Risk assessment of probiotics.

In short communications producers spoke about their product development strategies. Nutritionists showed data concerning the importance of probiotics in the nutrition of adolescents and elder people.

Hungarian researchers cooperating in National Research and Development Programs reported their results in *Lactobacillus* and *Bifidobacterium* strain selection which included their antimicrobial efficiency and immunmodulating activity and ability to grow on vegetable substrate.

The importance of prebiotics was well documented not only as growth substrate but also as protective agent for the probiotics in per os administration.

CFRI= Central Food Research Institute, Budapest

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