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HUNGARIAN AGRICULTURAL RESEARCH

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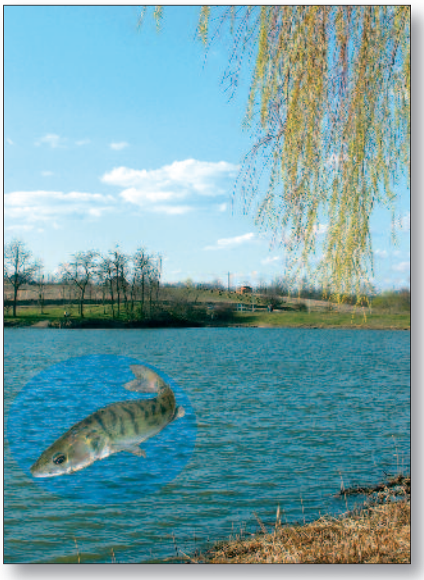
Rural Development, Hungary

New Rector at Szent István University, László Solti

László Solti graduated from the University of Veterinary Science, Budapest, in 1971. First he worked as practicing veterinarian in Kaposvár, then joined to the Department of Animal Reproduction at the university. The activity at the university involved graduate and postgraduate teaching, clinical work on food and companion animals as well as research. His main fields of interest are the detection of steroid hormones by self developed radio- and enzymeimmunoassay, reproduction biotechnology including the superovulation of donor animals for embryo production, cryopreservation of the gametes as well as the in vitro fertilisation of oocytes. In addition to these he was working on the hormonal control of the oestrous cycle, immunological determination of mycotoxins as well as the practical use of assisted reproductive technologies.

On invitation he became director in the newly established Agricultural Biotechnology Center in Gödöllő where he spent 5 years before became appointed to the head of the Department of Animal Reproduction at the University of Veterinary Science Budapest in 1994. Soon he became elected to Rector of this school which was merged with Szent István University in the year 2000, from this time on he was the Dean of the Faculty. When his mandate expired, he went to the University of Veterinary Medicine in Vienna spending there 2 years as vice dean responsible for clinical affairs. By the end of 2005 he returned back to the Faculty in Budapest from where he applied for the Rector's postion of the Szent István University what he received and became nominated in 2007.

Scientific levels: CSc (PhD) in 1984, DSc in 1999, outer member of the Royal Swedish Academy of Agriculture and Forestry since 1998, corresponding member of the Hungarian Academy of Sciences since 2004. National and international activities: Editorial Board member of the Hungarian Veterinary Journal, Acta Veterinaria Hungarica, International Advisory Board member of the Reproduction in Domestic Animals and the Tierärztliche Praxis. Founding Diplomate and Board member of the European College of Animal Reproduction, President of the European Society for Domestic Animal Reproduction, 2001 through 2007 president of the Hungarian Veterinary Society, member of the Veterinary Committee of the Hungarian Academy of Sciences and President of the Local Organising Committee of the International Congress of Animal Reproduction (ICAR-2008).



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Árpád Hegyi[1], Zsigmond Jeney[2], Béla Urbányi[1]

Possibilities of stress reduction in pike-perch during transportation

The process of fish production includes several work phases with a risk of injury to fish (Hegyi et al. 2006). During these work phases in most cases fish come in direct contact with humans and/or several types of fishing equipment (net, sorting table etc.) (Hegyi et al. 2004). One of the most serious stress problems for fish is caused by transportation since the animals have to go through the process of fishing, sorting and transportation. This procedure is even more important in case of fish species which are especially sensitive to physical stress. Pike-perch (*Sander lucioperca* L. 1758), one of the most important Hungarian carnivorous fish, also belongs to these species. Stress caused by transportation has already been examined by several researchers.

Picture 1

Barton et al. (2003) observed an increase in the level of plasma cortisol during transportation with a decrease of plasma chloride concentration at the same time. One hour after transportation the level of plasma cortisol increased from 12–49 ng/ml to 138–174 ng/ml while the level of plasma cortisol decreased by 19%. Experimental results proved that the young pike-perch individuals obtained a significant amount of physiological stress during fishing up, transportation and stocking.

Forsberg et al. (2001) examined transportation in water buffered with natrium chloride when the chemical structure of blood and ethology (orientation ability) were monitored during a five-hour-long transportation period. The buffered transporting media proved to be efficient both when blood composition and ethology were considered.

Brown et al. (2001) examined the transporting media for pike-perch. A sudden replacement of pike-perch into brackish water resulted in a significantly higher concentration of plasma chloride after 24 hours when compared to individuals from fresh water. After six days osmolality slightly increased but the glucose and cortisol concentrations of the plasma and the haematocryt and haemoglobin quantity of blood did not change. When pike-perch were suddenly placed to a salty environment osmolality and chloride concentration of the plasma increased. The initial osmotic disturbance was accompanied by an increasing amount of plasma glucose and blood haematocryt while the concentration of haemoglobin decreased though these parameters were completely restored within six days after the treatment.

We made our experiments on stress sensitivity in pike-perch (*Sander lucioperca* L. 1758) on the

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premise of Öko 2000 Ltd. The experimental fish stock was collected by the help of a pull net from a storage pond with the size of a wintering pond *(Picture 1)*. During the experiment fish were placed on two transporting vehicles in six tanks altogether. The weight of fish varied in the range of 400 grams. Thirty odd pike-perches were placed in each tanks. Control and treated groups were adjusted as follows (*Table 1*).

In the experiments the effects of salt and the two kinds of anesthetics were examined in pike-perch in a simulation of transportation (*Picture 2*). During the transportation period the amount of oxygen was always above the normal limiting value.

Picture 3

Influences of transport stress were tried to be reduced with the application of anesthetics and table salt. In the 1st tank there was no anesthetic/narcotic or salt treatment for fish. In the 2nd tank salt was stirred into the transportation media, in the 3rd tank it was clove oil, while in the 4th norcaicum anesthetic.

Table 1. *Chemicals with quantities used in different tanks*

Tanks	Treatments
Tank 1	No treatment
Tank 2	0.5% table salt
Tank 3	150 ml clove oil/100 l
Tank 4	150 ml norcaicum/100 l

At the beginning of the experiment a 0-hour blood sample was taken and all samples gained at later periods were then compared to this one. At every sampling time 5–5 blood samples were collected from each of the tanks. In every case only one sample was taken from each fish (*Picture 3*). 1–1.5 ml blood was taken from an animal by adding heparin anticoagulant into it. Samples obtained were then centrifuged on 3000 rpm for 10 minutes. From the plasma gained this way the amount of blood glucose was determined.

Results of blood plasma glucose are shown on the following Figures (*Figure 1–4*). On the first figure – where the transporting media was simply pond water – a statistically significant difference ($P < 0.01$) was observed in blood plasma glucose during the 7 hours of transportation compared to the 0 hour blood sample.

In the other three tanks (tank 2: table salt, tank 3: clove oil, tank 4: norcaicum) no statistically significant difference ($P > 0.05$) could be detected in either of the sampling times. It means that both of the anesthetic/narcotic agents and also table salt was able to reduce stress level appearing as a result of transportation.

Picture 2

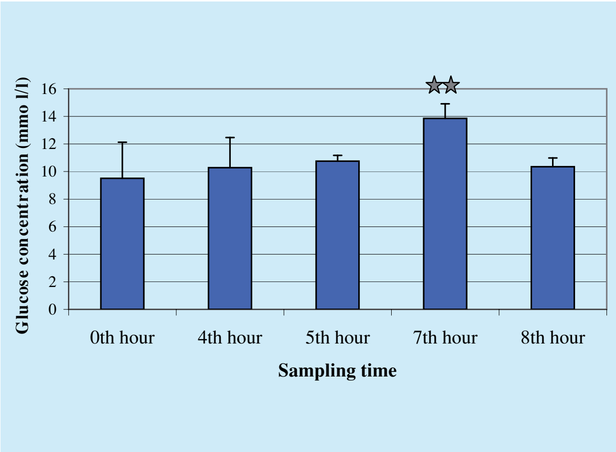

Figure 1. *Results of blood glucose if transported conventionally*

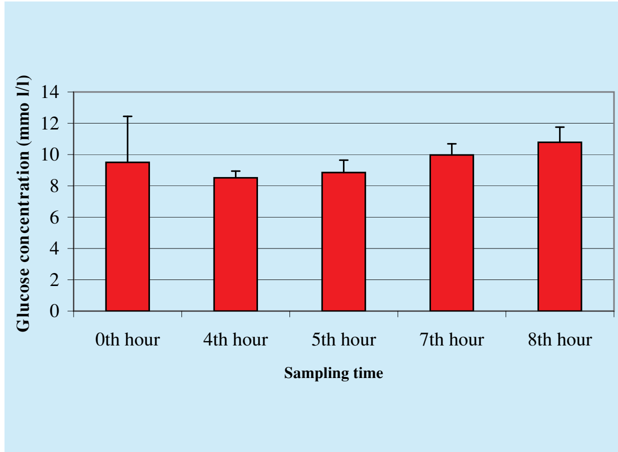

Figure 2. *Results of blood glucose if transported with table salt*

Acute transportation stress of the pike-perch stock could be adequately monitored by measuring the amount of glucose since with no additives the level of blood plasma glucose showed a statistically significant accession. Either the two kinds of anesthetics or the salt reduced the impact of stress on fish since glucose values did not differ significantly from the control ones.

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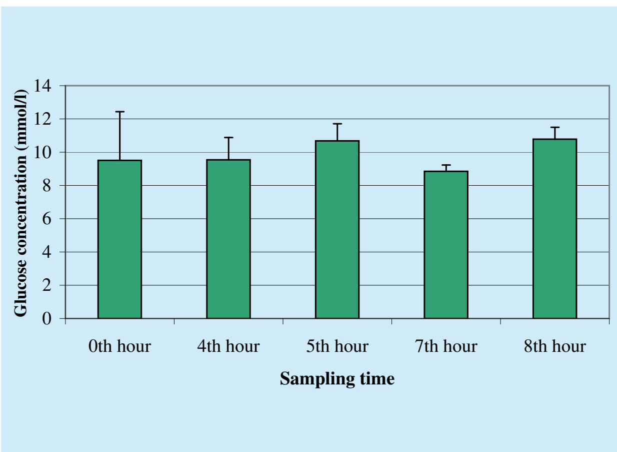


Figure 3. *Results of blood glucose if transported if transported with clove oil*

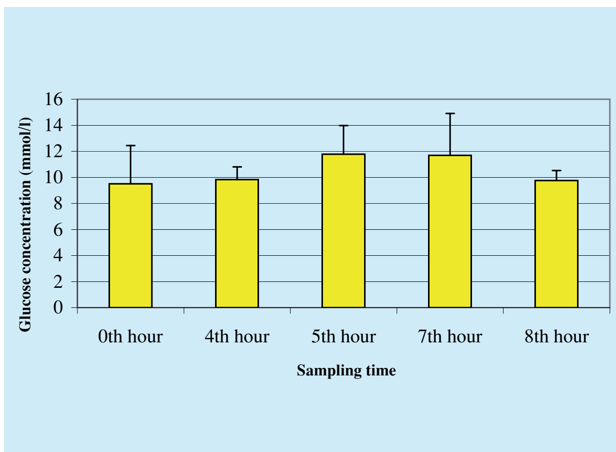
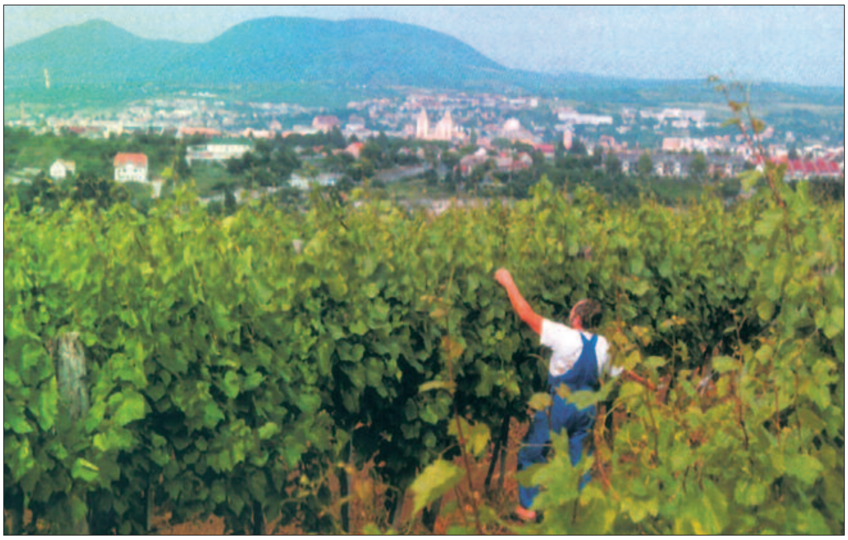


Figure 4. *Results of blood glucose if transported with norcaicum*

Zoltán Naár, Attila Kiss, Péter Biacs[1]

A method for technology risk assessment of grape and wine production in the Eger wine region



EGERFOOD Regional Knowledge Centre (funded by Pázmány Péter Programme of National Office for Research and Technology) was established to support innovation of small and medium size enterprises in the Heves region. The North-Eastern Region in Hungary is one of the industrially developed areas, where food production and processing was a bit neglected. Only a limited number of food companies are innovative enough to survive challenges of new food safety requirements on the market. The leading scientific institute in Heves County, the Eszterházy Károly College has formed a local consortium of 15 enterprises, among them grape breeders and wine processing companies. The EGERFOOD Regional Scientific Centre elaborated a lot of projects to increase safety in production and decrease risk in consumption of local food products, including grape and wine. The project includes not only risk assessment studies, but the application of traceability systems. Horticultural production from fertile soil to harvest and grape pressing to fermentation were investigated according hazard analysis and exposure. Risk assessing results are presented in all steps of the procedure. Local enterprises are successfully using this systematic approach to control documentation of paperwork and electronic data.

Wine is considered as a safe drink because of lack in safety problems, in exceptional cases. The increasing request of consumer for food safety could seriously damage a food and drink company even in the case of a failure of low probability. Wine was not investigated by risk assessment and measured from expected effects of a known failure because of its reputation as a safe product.

During our investigations a relatively simple method was elaborated to assess risks occurring at technological steps of grape harvesting and wine processing. Data were obtained from food safety documentations and by consultation with several specialists in the field of enology in Hungary. Risk factors were multiplied according to their character, weighing and frequency. Ranking of hazard and possible risk was compared for grape harvesting and for wine processing using the same table for illustration.

The Failure Mode and Effect Analysis (FMEA) method was chosen as general method for risk assessment. Today FMEA is widely applied in machine manufacturing and different standards have been worked out to describe its basic principles (for example MIL-STD-1629A and DIN 25448). The aim of the analysis is to recognize the sources of problems in the earliest possible stage of a product's life-

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cycle, to prevent an error to occur and, when an error does occur, to correct it, and by these means to reduce costs and to preserve the company's reputation. This method can be used not only before production starts, but with already running systems and processes as well.

FMEA is more and more widespread in food industry, too, though in this branch the HACCP system is prescribed by the law for quality-control. With regard to the call for application the main difference between these two methods is that FMEA provides a sufficiently more complex picture of the potential hazards. It not only tries to explore all possible problems in the widest panorama, but weighs them as well since besides a certain product it looks at the whole process of production.

As a first step of the customary industrial solution is taken into component parts, then with every component the possible sources of error, and their causes and consequences are identified and the so called component-error-cause-consequence chain is established. This chain connects the related pieces of information together. Every chain is assessed according to three points of consideration and is given a score between 1 and 10, taking into account the current conditions, that is, the already in-built controlling and preventive measures. There are several ways to analyze the information represented by the three risk-level numbers. The most common is based on the calculation of a risk factor (RF) which in turn is used for a Pareto analysis.

The FMEA realized during our work was departing from that customary industrial solution in two respects (Nádasiné, 2005).

1. When searching for possible errors we intend to examine the whole chain from the materials used and the technology applied through to the product, the marketing and the end-user (consumer). Meanwhile we could point out where to improve technology to decrease risk on the consumers' part.
2. To provide a better-founded risk factor that reflects scientific findings more timely, in the analysis we used 10 instead of 3 numerically measurable points of consideration based on scientific literature and international food safety databases.

At the first step, the hazard list of wine making reported by Christaki and Tzia (2002) was used for exploration of possible hazards in a group meeting of grape growers, wine producers, food chemists and microbiologists without considering the probability and the severity. At the second step, three groups of

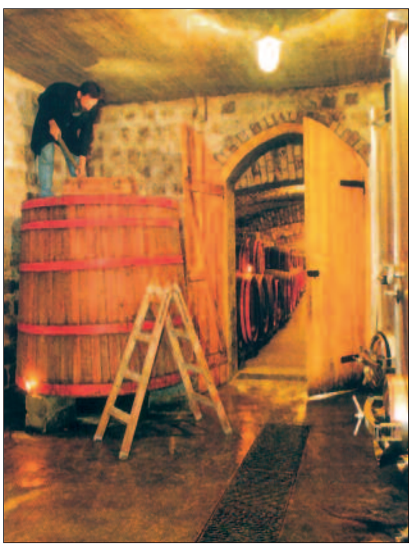
Table 1. The rank of possible hazards identified in a regular red wine production technology according to their risk factors

Grape production		Wine processing	
Hazard	Risk factor	Hazard	Risk factor
microbes on wounded grapes	437,400	harmful microbes on vessels	87,480
residues of insecticides	108,000	biogenic amines	69,120
residues of pesticides I. (benzimidazoles, carbamates)	86,400	methanol in wine	48,600
residues of pesticides II. (botryticides, azols, piretroids)	64,800	chromium from stainless steel	21,600
traces of heavy metals from soil (Cd, Pb)	45,000	sulphur-dioxide	8,640
traces of copper from pesticides	21,600	asbestos fibers from filter layer	7,200
mycotoxin (ochratoxin A)	19,440	ethylocarbamate in wine	6,912
dioxin from contaminated air	13,500	residues of clarification agent	6,000
Cadmium from fuel additives	7,200	cleaning agent in glass bottles	4,800
Lead from fuel additives	2,400	residues of disinfectants	2,304
Mineral oil from grape harvesting machine	240	organic acids, volatile acids	1,296
Piece of iron (steel) from machines	24	mineral oil from hydraulic press	240

considerations (see below) were evaluated with 1–5 values. The risk factors were calculated with multiplication of separate values within a group, and a subsequent multiplication of group values. A programmed Excel datasheet was prepared for data management.

Several aspects were considered for assessing risk factors:

- Aspects for determining how serious are the effect of the hazard: weighing, timing, and cross-compliance.
- Aspects for determining the frequency of hazard: environmental effect on the concentration of a hazardous component in an agricultural product, effect of the technology and distribution of the contaminant.
- Aspects for determining the possibility of the discovery of a hazard: identification and local distribution, observation



We were able to identify 10–15 sources of hazard both in harvested grapes and in processed wine, where risk factors were determined by assessment. Harmful microbes are representing the highest risk with the highest incident was found on wounded grapes. Microbial infection is more frequent at wine processing steps (storage tank, fermentation vessels, wine yeast cultures, biological malo-lactic fermentation, cleaning of glass bottles, contaminated cork), therefore several assessed values were taken into consideration more times. Such identified hazards are representing certain risks, however we are able to control and eliminate most of them. Contamination level of environmental hazards (pesticides, insecticides, manure or fertilizers) could be decreased by integrated plant protection systems. We call your attention to the hazard of mycotoxins (ochratoxin A) which could be produced as consequence of mould infection on the surface (skin) of grapes.

Methods were elaborated and scientifically verified for evaluation of the full process and used for comparing technologies. We were able to evaluate several factors of hazard according to their character, weighing and frequency.

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Acknowledgement

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János Bakk, István Gyürk, Ferenc Kaifás, Gyula Csapó[1]

Seed Treating Machine Development and Manufacturing in Hungary

The treatment of seeds to protect them from pests and deseases before sowing has been known for more than two thousand years. Roman authors write: *Semina vidi equidem multos medicare et nitro prius et nigra perfundere amurca.* (I have seen many people soaking seeds in thick juice of black lees of olive oil and soda)[1] Elsewhere: *Vino autem semina perfusa minus aegrotare existimant*. (It is said that seeds spilled over by wine will be less infected).[2] Seed treatment for bunt by the use of brine is described as early as 1637.[3]

There are Hungarian sources, too from the seventeenth century: *Smear seeds of cabbage with lard …..before sowing let it through guts of wolf.*[4]

It appears in the Hungarian Ethnographical Encyclopaedia under the headword *seed dressing:*

Seeds to be sown were separately stored and treated not long before sowing. Seed treatment was done on pavement or well stamped soil. In case of smaller quantities in manger or in wash tub. In bigger farms special seed treating tub was used. Seed treating basket was in use, too when seeds in the basket were soaked in seed treating liquid. Smaller or bigger shovels were used to protect the hand. Seed treating was done by two or three persons. One of them sprinkled the seed dressing liquid consisting of blue vitriol or caustic lime, while the other one (and third one) mixed the seed with wooden shovel. Seed treatment stroke root at the Hungarian farmer most likely in the eighteenth century. Some places thin dung water was sprinkled on the seed against blight.[5]

***Figure 1.** Dressing bucket* *Dressing manger*

Seed treatment technology went trough a considerable development during the past centuries. The first equipment which could be considered a seed treating machine was probably the drum mixer.

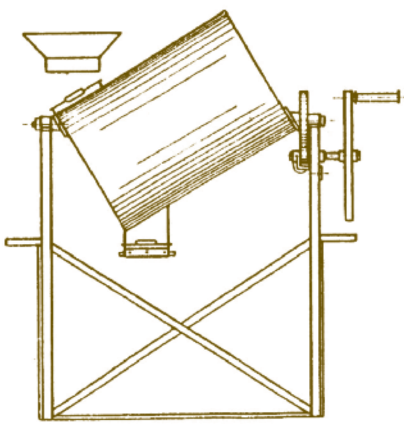

***Figure 2.** Drum mixer*

Nowadays seed treatment is effected by efficient chemicals and sophisticated machines. Novel methods are developed to protect crops, the application of pesticides to seeds has an important and well established place in agriculture. There is a continuing demand of novel pesticides and efficient machinery for applying them.

The development and manufacture of seed treating machines has always been of great importance in Hungary's agricultural machine industry. It is worthy of note the MOBITOX self propelling seed treater and other members of that machine family of a series of more than 60 000 units.

Currently; FARMGEP Ltd carries on the tradition. Hereinafter the authors describe their joint research and development activity aiming the development of a new seed treater model.

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The design of the seed treater machine is largely defined by the characteristics of the chemical to be applied onto the surface of the seed. Formerly the chemical was applied to the surface of the seed in a high rate and distributed by further mixing. Chemicals are used nowadays in such a small rate, that they cannot be further spread, since the chemical immediately adheres to he surface of the seed. Thus the mixing principle is not effective in this case. However with the new spinning disc principle, grain falls in a curtain peripheral to a rapidly spinning disc. A liquid formulation is fed onto the disc, which leaves it in the form of mist of droplets. The grains fall through the mist, picking up a uniform dose of the formulation. The distribution is basically defined by the geometry of the seed curtain and by the mist of droplets. Further mixing is neither possible nor necessary.

In the experimental machine the geometry of the seed curtain was studied by digital scanning. The space where the grains meet the droplets was defined by a set of photos. The volume of this space (a ring) is determinant in respect of the quality of seed treatment. The relationship between the size of the seed curtain and the mass flow was determined and the relationship between the collisions and the given geometrical parameters in case of two planar granulous sets was studied by computer simulation. The results of simulation is to be controlled by the instrument on *Fig. 4*.

To ensure the proper quality of treatment it is important to synchronize grain and chemical metering. Grain shouldn't pass the dressing chamber without chemical, and chemical metering should start only together with grain metering. Furthermore always the properly set quantity of chemical should be fed onto the seeds.

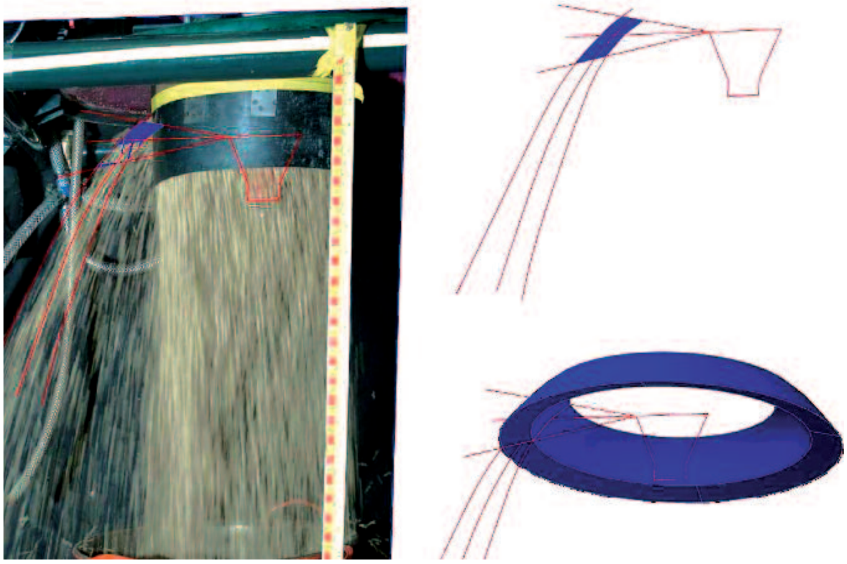

Figure 3. *Geometric model by digital scanning*

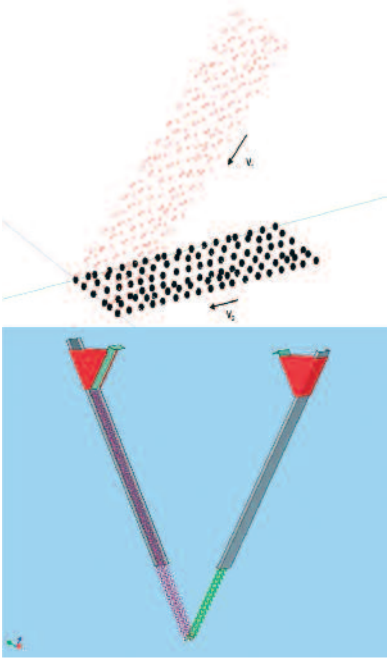

Figure 4. *Computer simulation model and the design of the measurement unit*

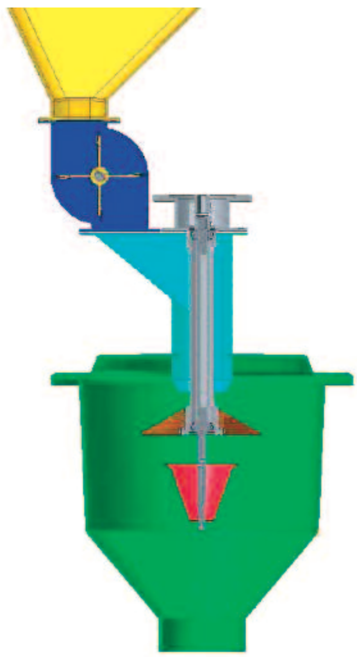

Figure 5. *Section of the dressing chamber*

***Figure 6.** Motion of seeds in the dressing chamber*

***Figure 7.** Computer model of the experimental machine*

The aim of the project was to develop and design a seed treater suitable for the liquid dressing of cereal and vegetable seeds.

According to the spinning disc principle in the dressing chamber grain falls in a paraboloid curtain peripheral to a concentric, rapidly spinning disc disintegrating the liquid into a mist of fine droplets. The grains fall through the mist picking up a uniform dose of the formulation.

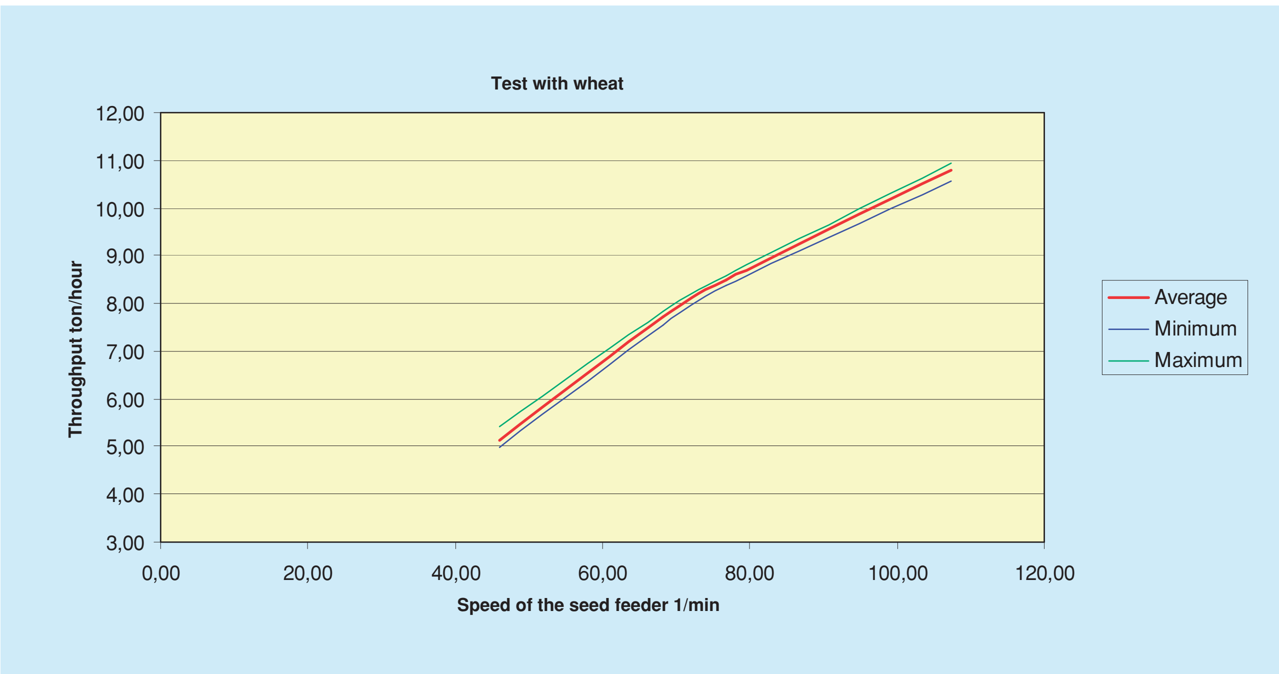

***Figure 8.** Calibration curve of the seed feeder of the prototype machine. (Wheat, volume weight: 0,7 litre/kg)*

A basic improvement compared to the previous GRAMAX design is a cell feeder metering the grain into the dressing chamber. The speed of the feeder is controlled by frequency converter. Calibrating the feeder with various seeds, knowing the volume weight of a given seed the throughput of the machine can be controlled accurately.

The required dose of the formulation can be set by a specially designed electronic unit. This control device receives the signal of the tachoscope fixed to the shaft of the feeder. The instrument stabilizes the required dose in the range of operation.

The harmony of a novel seed treating principle and of an improved grain and chemical metering system was realized in a new machine design. The authors hope that the prototype machine represents not only the first but also a successful member of a new seed treating machine family.

The project was supported by GVOP-3.3.3.–05/3.–2006-04-0023/3.0 .

Figure 10. *The prototype machine*

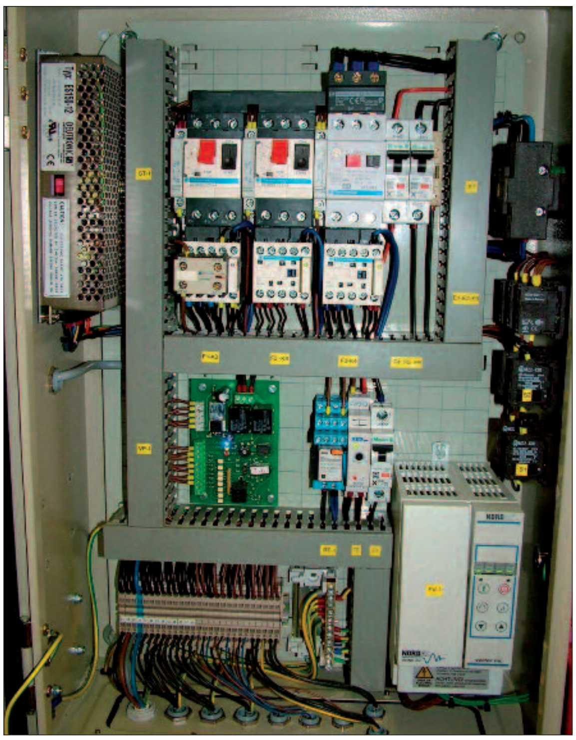

Figure 9. *The control box of the machine with the frequency converter*

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Mónika Forgó PhD[1], Edit Kónya[2]

Analysis of measures of rural development programmes in the EU and in SAPARD Programmes (period 2000–2006)

SAPARD (Special Pre-accession Programme for Rural Development) was established by the Community in order to help with measures for agriculture and rural development in the applicant countries of Central and Eastern Europe (CEE).

To achieve this objective, SAPARD, unlike PHARE and ISPA, specifically requires each applicant country to draw up a development plan in accordance with the principles of the programming approach used by Member States (MSs) for rural development programmes. The plan can include up to 15 measures set out in the SAPARD Regulation. Many are similar to those available to MSs under Community co-financed agricultural and rural development programmes. Under the SAPARD Programme the following pre-accession measures were available to support the agricultural and rural development in the applicant countries of CEE in the pre-accession period: (1) Investments in agricultural holdings, (2) Improving the processing and marketing of agricultural and fishery products, (3) Improving structures for quality, veterinary, plant-health, foodstuffs controls, (4) Agricultural production methods designed to protect the environment and maintain the countryside, (5) Development and diversification of economic activities, (6) Setting up relief and management services for farmers, (7) Setting up producer groups, (8) Renovation and development of villages and the protection and conservation of the rural heritage, (9) Land improvement and re-parcelling, (10) Establishment and updating of land registries, (11) Improvement of vocational training, (12) Development and improvement of rural infrastructure, (13) Water resources management, (14) Forestry, (15) Technical assistance.

First of all, it is interesting to analyse which measures were chosen by the applicant countries such as Hungary under their SAPARD Programme during the programming period, when they submitted their SAPARD Plans to the European Commission.

The content of each SAPARD programme reflected the needs and priorities established by the national authorities, (the managing authorities) depending on the special circumstances of the countries, within the limits set under the basic Council Regulation. The SAPARD programmes were in line with Member States' agriculture and rural development programmes. For every country, the SAPARD plans contained a SWOT analysis which pointed out the strengths and weaknesses of rural areas and food industries. The chosen measures had to be consistent with these weaknesses and needs. According to comparison analysis there were three measures that all countries put in their SAPARD Plans 2000-2006:

1. *Investment in agricultural holdings*. This was essential since the goals were to modernise low revenue-generating agricultural production, increase competitiveness and market efficiency of small and medium sized agricultural enterprises.
2. *Improving the processing and marketing of agricultural and fishery products*. The rationale of this measure is to improve market efficiency in compliance with EU regulations on processing agricultural products (food safety, hygiene, etc.).
3. *Technical assistance measure*. This measure was mandatory for candidate countries (CCs) because it provided funds to main administrative activities for managing authorities, such as monitoring, evaluation of the programme and informing the general public.

There are three measures which were chosen by 90% of all the CCs:

1. *Agricultural production methods designed to protect the environment and maintain the countryside*. It has the objective of developing practical experience of agri-environmental im-

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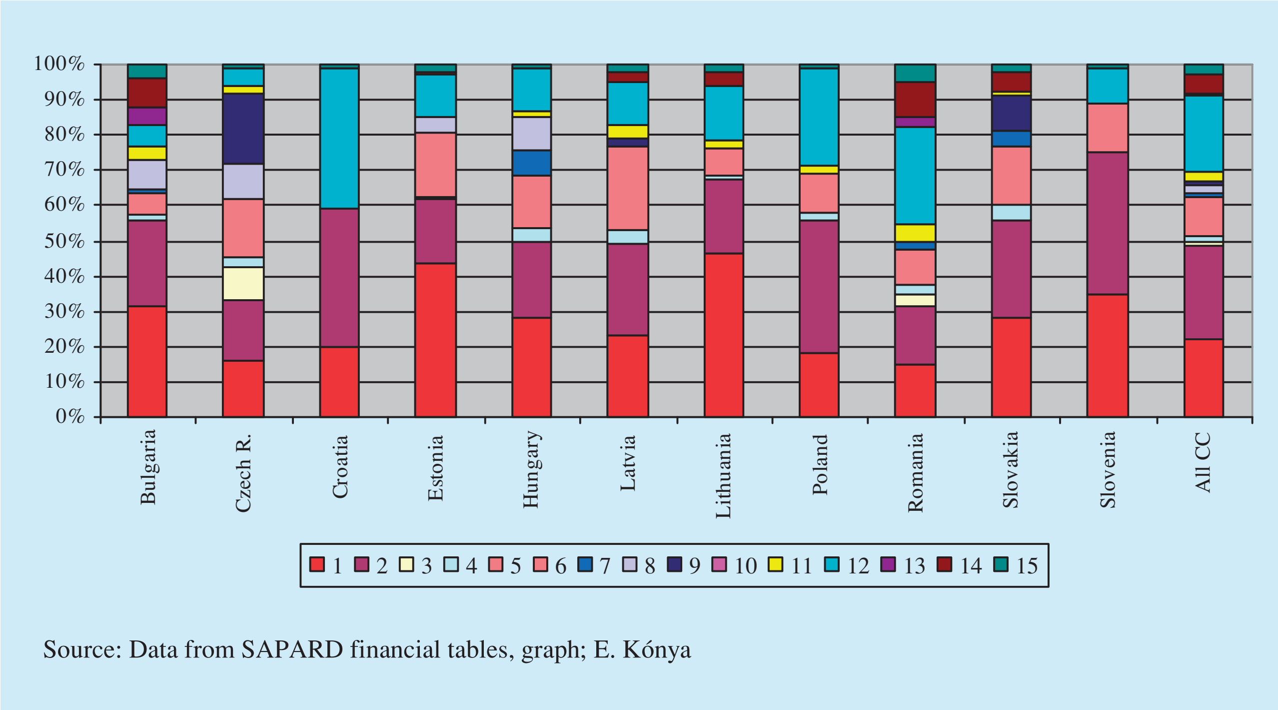


***Graph 1.** Proportion of each SAPARD measure in %*

plementation for both the administrative and farm levels.

2. *Development and diversification of economic activities*. This measure helped to develop alternative business opportunities and income in rural areas.
3. *Development and improvement of rural infrastructure*. Generally, in rural areas the most serious problem is the lack of waste water treatment, energy supply for enterprises and telecommunications infrastructure. When these problems arose in small settlements, the other pre-accession program for infrastructure (ISPA) could not help.

It should also be mentioned that two of the measures, setting up relief and management services for farmers and the establishment and updating of land registries have not been included in any of the SAPARD programs. Most likely, these objectives were financed by national budgets or previously by Phare.

Please note that we took Croatia under consideration in order to be comprehensive in our study however Croatia was not a beneficiary country during the program period (1999–2000). The Council Regulation 2257/2000 specifically took into account Croatia's candidate status. Croatia has a shorter implementation time, as well as limited financial sources. These reasons narrow down the measures to those which have a high level effect on the rural economy and living conditions and which meet an urgent need for invention.

After reviewing the indicative allocation and chosen measures country by country, it was necessary to analyse the proportion of each measures in the SAPARD plans. The method was the following: the financial tables of each country contain the maximum EU contribution[3] per year from 2000–2006, and total of measures. The total amounts were divided by the total of measures, and graph 1 shows the results in percentage. The axis x shows the countries, including a "new" one, Croatia, and the average proportion (all CC). The numbers refer to the measures – like in the table – and the y axis shows the proportion of a measure in percentage.

According to the graph, the main priority in most of the countries is to strengthen the competitiveness in the agricultural sector. We can create groups in line with the proportion of "competitiveness/investment" or "rural" type of measures. ***Group 1***: countries in which the rate of 1) and 2) measures is 50% or more of the total EU contribution for 2000–2006: Bulgaria, Croatia, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia and Slovenia. Their rates are over the average. Consequently, these countries had less emphasis on the other measures, such as "rural" types, 5), 8), and 12). In the case of Hungary, this strategy was considered in the Agricultural and Rural Development Operational Programme. The biggest share of

[3]Figures given in prices 2000, €

investment measures is in Slovenia, Estonia and Lithuania (more than 60%). ***Group 2:*** the Czech Republic and Romania, where the rate of "investment type" of measures is less than 50% of the total contribution, and this group converges to the CC average. These two countries had the most moderate measure-choosing.

If we are taking into account all the measures and all the countries, public aid for investment in processing and marketing leads with 26% of the total Community contribution[4], followed by investment in agricultural holdings and investment in rural infrastructure, each just over 20%. Next is diversification of activities with around 11%. Of the nine other measures in the programmes, none of them averages more than 4% of the total. Even though the agri-environment measure was not obligatory under SAPARD and was only considered as a pilot implementation, unlike for rural development programmes in the Member States, all applicant countries, except Slovenia, had included this measure in their programmes.

According to the number of measures in the CC's plans, 10 or more out of 15 measures had been chosen by Bulgaria and Romania (the average is 8). Croatia and Slovenia had the least number of measures, 5 and 4. This fact shows that where the annual allocation was high, the chosen measures were broader. The Czech Republic was exceptional, 10 measures were included in the SAPARD plan but their annual allocation is less than Hungary for instance. According to rural development financial plans of Hungary – including EAGGF-Guidance, National Rural Development Plan and SAPARD – for the programming period 2000–2006 1 075 168 000 Euro was indicated. If we focuse on the implementation of the Hungarian SAPARD Programme; by 31 December 2005, contracts had been concluded for 2640 applications, the total costs of the projects amounted to HUF 131.9 billion, while their total support need was HUF 62.5 billion. With respect to the SAPARD payments it can be established that in the case of the contracted applications, payments from SAPARD sources were effected in 3686 cases, in a total amount of HUF 53.5 billion (EUR 209.9 million), of which HUF 40.1 billion (EUR 157.4 million) was covered by Community contribution.

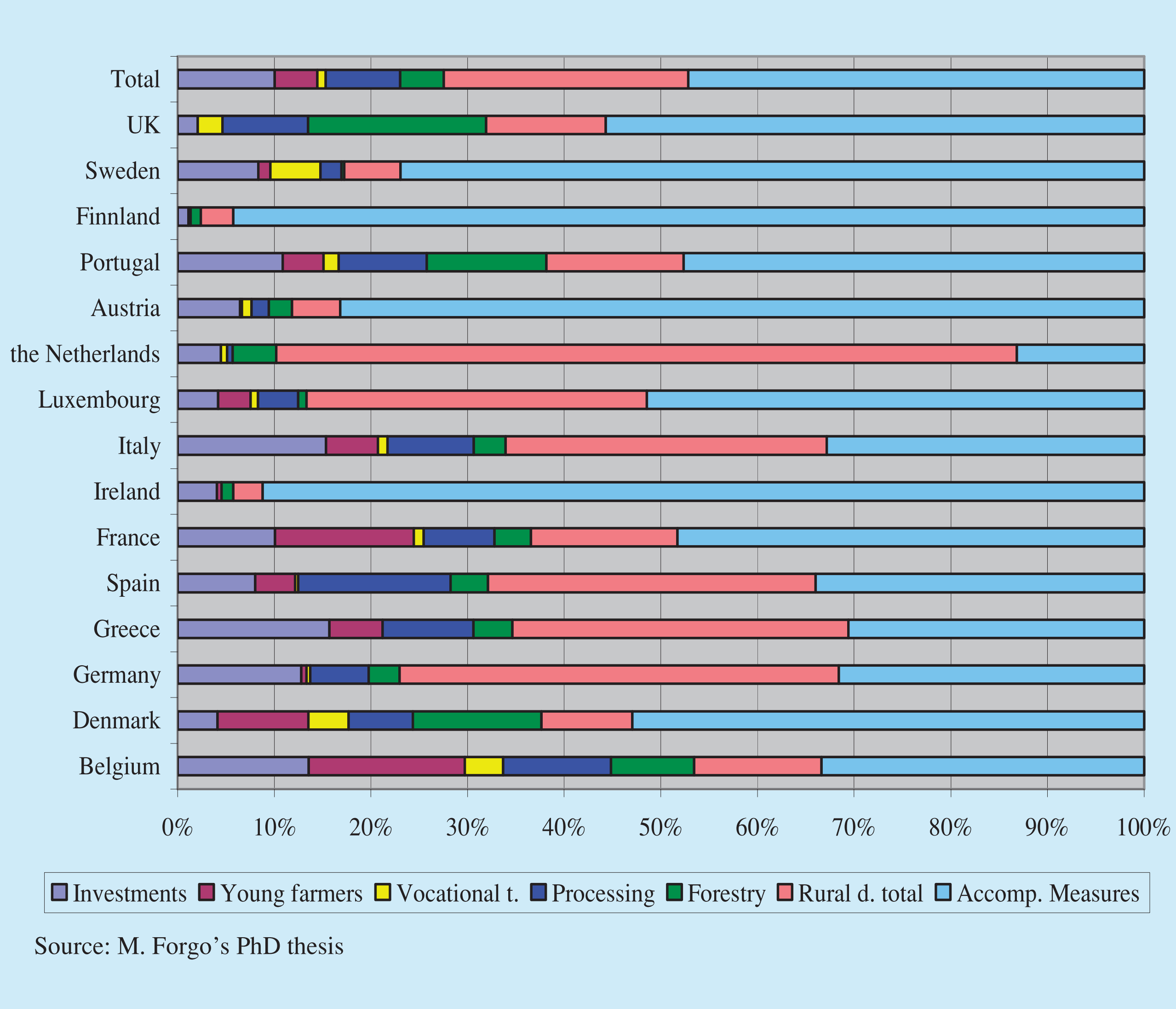
Apart from the SAPARD planning and implementation in candidate countries before accession, we examined the tendency of rural development in the EU MSs between 2000 and 2006. After AGENDA 2000, the following measures were co-financed by EAGGF in the MSs: (1) agri-environment (compulsory), (2) less-favoured areas and areas with environmental restrictions, (3) early retirement, (4) afforestation as accompanying measures, (5) investment in agricultural holdings, (6) improving processing and marketing of agricultural products, (7) setting up of young farmers, (8) training, (9) forestry and (10) promoting the adaptation and development of rural areas (Article 33). The last measure includes 13 sub-measures such as the development and improvement of infrastructure, diversification of agricultural activities, renovation and development of villages, protection and conservation of rural heritage, etc.

If we reviewed the rural development programs of each of the EU-15 MSs to analyze the financial priorities they gave to each measure and objective for the period of 2000–2006, we see that the accompanying measures take more than 47% of the total rural development budget, of which, 84% is spent on agri-environment and less-favoured areas and areas with environmental restrictions. The ratio is different in each MS. The Benelux countries put big emphasis on *agri-environment*, however if we compare the funds to the agricultural area in these countries, they are under the EU average, while Austria and Finland are way above it. Austria is the country, where the agri-environment programme is very successful, since 70–75% of the agricultural holdings somehow participate in it. In Finland and in France, the support of *less-favoured areas and areas with environmental restrictions* is very important. About 85% of the land under agricultural cultivation of Finland and 50% of France belongs to less-favoured areas. *Afforestation* is a high priority in Spain and Portugal. The Spanish Government gives this high priority, because of the high proportion of land affected by erosion and desertification. Also, there are many forest fires causing damage every year. *Early retirement*, among all measures, is the most controversial. Six MSs have not introduced it at all. It can highly affect the social and pension system and the results are not guaranteed.

Almost 50% of the financial allocation spent on the "investment" type of measures (the non-accompanying measures), is taken by Article 33, 19% on investment in agricultural holdings and almost 15% on improving processing and marketing of agricultural products.

In MSs, there are big differences in the planned budget of each measure:

[4]In this section references to the Community contribution relate to the expected total Community co-financing contribution for SAPARD of 3 703 million euro for the entire programming period 2000–2006.



***Graph 2.** The distribution of EAGGF contribution between the accompanying and the "investment" type of measures by Member States (2000–2006)*

- *Article 33* has the biggest share of the rural development budget in The Netherlands, which is almost 88% and 66% in Germany. The lowest one, about 20%, is in Belgium and Denmark. The average is 48%. Germany spends the most on Article 33 as it is included in their national Framework programme, which means it has to be introduced in all its' regions.
- The measure of *investments in agricultural holdings* has 20% of the budget on average, the highest being in Ireland (46%). Italy spends the most on this measure, which is not a surprise considering 32% of the agricultural holdings in the EU can be found there. In particular, the southern regions put emphasis on investments. France invests in increasing storage capacity and its' quality, as well as in modernizing agricultural machinery on highlands.
- The measure of *processing* takes an average of 14,6% of the rural development budget. Spain spends 24%, as the highest. Because of high demand for quality Mediterranean food products, many Spanish regions support modernizing food processing, introducing quality assurance systems, packaging and marketing.

The analysis shows that the accompanying measures take almost 50% of the budget. Graph 3 demonstrates that these compensatory measures, based only on eligibility, have an important role in four countries. In Finland and in Ireland, the proportion of the accompanying measures is over 90%, in Austria and in Sweden this rate is around 80%. The only country where the percentage is low is the Netherlands, where it is under 20%. There is a danger that **the high proportion of the accompanying measures may decrease effectiveness, because this system only redistributes incomes and "only" helps to maintain the landscape**

Table 3

	Group 1	Group 2
Description	Where the rate of investment-type measures is around or more than 50% of the financial allocation	Where the rate of investment-type measures is less than 50% of the financial allocation
Countries (MSs)	The Netherlands, Italy, Greece, Germany, Belgium, France, Portugal, Spain	Denmark, Ireland, Luxembourg, Austria, Finland, Sweden, UK,
Countries (CCs)	Bulgaria, Croatia, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia and Slovenia	Czech Republic, Romania

and reduce the loss of population. These two goals may be achieved if the proportion of the grant is high enough in relation to the farmers' income.

Summary

Taking everything into consideration, we can say that in the case of SAPARD there is a similarity among the programmes, because most of the candidate countries put the emphasis on the investments in agricultural holdings and improving the processing and marketing of agricultural and fishery products. On one hand, it is obvious that their agriculture and food industries had the same problems. In particular these included outdated assets, especially buildings, facilities, technological equipment. Most of the breeding facilities did not meet the EU requirements on animal welfare. On the other hand, it would have been useful had they implemented several measures in order to gain practical experience before starting their agricultural operational programmes and national rural development programmes. Indeed, there were similar tendencies in CCs and MSs.

The above analysis and examples show that there is no significant coherence among the rural development plans of MSs with the same state of development. The countries define their support policy on the basis of their needs and endowment. Some similarity can be seen. For instance, the countries with the worse capability for agricultural production, mostly northern countries, spend proportionately the most on agri-environment and on less-favoured areas. The southern countries typically follow the "little from everything" principal with different emphasis.

According to the graphs and analysis of the measures, we can separate the MSs and CCs into two groups.

It is quite obvious that the CCs focused on investments in the agricultural sector in order to quickly apply EU requirements. By doing so they put the emphasise on implementing investments (e.g. machinery, processing agricultural products). In parallel, the MSs implemented a wide range of measures which could cause a decrease of efficiency. In these counties, a wide range of agricultural and rural development measures were available. In many cases they were different for example, young farmers, etc. In the case of the SAPARD Programme there were not many differences as there were only 15 measures for CCs.

References

[1] COMMISSION REGULATION (EC) No 1268/1999 laying down rules for the application of Council Regulation (EC) No. 1268/99 on Community support for pre-accession measures for agriculture and rural development in the applicant countries of Central and Eastern Europe in the preaccession period. [2] The SAPARD Plans of CCs [3] SAPARD Annual Report – year 2000, Report from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions [4] Mónika Forgó: Rural development in EU-15 and in Hungary. PhD thesis. Regional Policy and Economy Doctorate School, Pécs University, 2006 {5} Hungary's annual report for 2006 and final report.

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DNA Ampelography: Grapevine variety characterization using DNA barcodes

Introduction

The term 'DNA fingerprint' was originally described by Jeffrey et al. in 1985 for the variable regions of the human genome which have since become an indispensable means also for the DNA-level characterization of plant species and varieties. The polymorphisms in the DNA molecule are particularly useful for genotyping and in this aspect, all plant genome components (nuclear, mitochondrial and chloroplast) are suitable objects. Within the three components of the plant genome, the non-coding repetitive gene sequences and also the ones encoding ribosomal RNAs and proteins are sources of DNA fingerprints in the different species, this way in the grapevine (Vitis vinifera L.), too. Consequently, PCR (Polymerase Chain Reaction) based molecular marker systems of different kinds have been elaborated, of which the microsatellite or SSR (Simple Sequence Repeat) method is of particular importance.

The plant nuclear genome is characterized by containing, depending on the species, 10 to 90% or even over 90% of short, monotonously repeated motifs. The repetitive sequences can be di, tri, tetra and penta nucleotides (SSR unit < 6 nt). While the mammalian genome is characterized by $(AC)_n$ or $(AG)_n$ and $(CG)_n$ motifs, plants typically contain $(TA)_n$, $(AG/TC)_n$ and $(AC/TG)_n$ motifs. These repeats are distributed evenly throughout the genome and can be mapped on the chromosomes.

The border sequences of the repeats show a high level of conservatism and the repeated motifs can by amplified by PCR primers designed for these regions. If the number of repeats of the monotone motif differ between two genotypes, the size of the of the PCR product will also differ from one another and show the so-called length polymorphism. This way the SSR method permits the separation of the lines and the identification of unique DNA fingerprints. The SSR markers are also widely used for genotyping because they are codominant, the results can be reproduced and the 1 or 2 alleles (fragments) amplified in the respective microsatellite loci of diploid species can be described with an exact size. The numeric data expressed in bp also make it easier to exchange of information between laboratories, compared to the patterns fixed on geal photoes.

The preservation of genetic sources and their characterization requires the maintenance of old varieties and the reliable and objective characterization of the latter, together with the new ones. Besides the morphological characteristics one must take into consideration the DNA marker systems as subsidiary traits in the description of grapevine varieties. The varieties, the accessions of the gene bank collections should be described with DNA ampelography parameters, too. The objective of our investigation was to determine the DNA fingerprints of 97 varieties grown for many centuries in the Carpathian Basin, of 3 international varieties (Heunisch weiss, Muscat Ottonel/Ottonel Muskotály and Pinot noir) and of the Hungarian variety Csabagyöngye known for its earliness, as well as to find out whether a distinction could be made between the varieties based on the allele patterns obtained in 6 microsatellite loci. The 97 varieties could be separated form one another in a clear manner based on the allele composition obtained with the 6 microsatellite primers and on the allele sizes. In the case of a few grape colour variants the SSR fingerprints were identical (Kozma et al. 2004, Halász et al. 2005). The unique alleles of the 6 SSR loci were used to construct DNA barcodes which make it even more easier to compare microsatellite patterns and distinction between the varieties on the DNA level can be carried out at a single glance.

DNA fingerprints of varieties: determination of microsatellite allele sizes

Genomic DNA was isolated from young grapevine leaves using the kit DNeasy® Plant Mini

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(Qiagen, Biomarker kft., Gödöllő) according to the protocol of the manufacturer and with the method of Lodhi et al. (1994).

The PCR reactions and the determination of microsatellite allele sizes were carried out as described by Halász et al. (2005) at 6 SSR loci using the primer pairs Scu08vv, Scu10vv VVMD21, VVMD36, ssrVrZag64 and ssrVrZag79. Forward primers were labelled with Cy-5 fluorescent dye (IDT Inc., BioSciences). PCR products were separated on a denaturing polyacrylamide gel of 8% (Amersham Biosciences, AP Hungary Kft. Budapest). Allele sizes were determined using the ALF-Express II DNA Analyzer (Amersham Biosciences, AP Hungary Kft. Budapest) employing ALFexpress™ Sizer molecular weight standard.

Microsatellite DNA barcodes were constructed with the Microsoft. Office Excel 2003 program.

The analyses of the 101 varieties resulted in individual discriminative microsatellite fingerprints in 97 cases *(Table 1)*. Thus, varieties can be characterized unambiguously based on their SSR allele structure (HALÁSZ et al. 2005). Only the berry colour variants of Bakator (red – light red), Gohér (red – white – varying) and Lisztes (red – white) produced identical allele patterns with the 6 primer pairs chosen. These proved to be indistinguishable even when using other microsatellite primers which were polymorphic with other genotypes. Similar results were reported by Sefc et al. (2000), too, only failing to separate the colour variants when analysing 100 varieties with 10 SSR primers.

The allele sizes of the varieties of the Carpathian Basin have the same size ranges as the international varieties at the 6 microsatellite loci. Literature data available for these 4 varieties permitted a comparison with the results of other laboratories (Halász et al. 2005). The data that we obtained for the samples Heunisch weiss, Pinot noir and Muscat Ottonel using the VVMD36 primer were exactly the same as the results reported by Bowers et al. (1999), Regner et al. (2000/a) and Crespan and Milani (2001). In the application of the VVMD21, VVMD36, ssrVrZAG64 and ssrVrZAg79 primers to the 4 varieties (Csabagyöngye, Heunisch weiss, Muscat Ottonel, Pinot noir) we obtained results that were 1 to 4 bp higher compared to the international data (Bowers et al. 1999, Sefc et al. 1998, Lefort and Roubelakis-Angelakis 2001). Similar disagreements are also encountered between the observations of other laboratories (Creaspan and Milani, 2001, Regner et al. 2000/a). The difference may derive from the difference in the DNA fragment analysis used for allele size determination. On the other hand, differences can be observed even when applying the same method, which is not easy to explain. According to This et al. (2004) these differences in size might result from the fact that certain types of *Taq* polymerases introduce extra nucleotides into the DNA molecule.

The reason for the widespread use of SSR markers consists in the high level of polymorphism and good interpretability, as well as in that the size is indicated in numeric form. Table 1 contains a total of 1212 data relative to the 101 varieties analysed with the 6 SSR primer pairs which provide an objective characterization of the genotypes and are really suitable for the distinction of the varieties, but require profound study. The identity or non-identity of allele sizes at the individual loci can be checked by pair-wise comparison also when data are made more picturesque applying various colours, as shown in Table 1. In this article the illustration of the barcode representation of the DNA fingerprints is limited to 12 varieties but we have already characterized 150 varieties at 12 loci using this method. The display of the microsatellite allele sizes according to this form offers a solution that is easy to understand and to handle in variety protection and identification, which can be applied in a quick and reliable manner also in commercial disputes.

Hungarian Vitis Microsatellite/SSR Database

Based on the data from the microsatellite analysis we have established the Hungarian Vitis Microsatellite/SSR Database which contains at the moment the data of 103 varieties analysed with 6 SSR primer pairs (Kiss et al. 2006), but which is being continuously enlarged. The Database was established with the aim of permitting all specialists, students and all those interested who deal with grapes either in teaching or in research to utilize the data of SSR allele size. The database can be accessed on the homepage of the Institute of Genetics and Biotechnology of Szent István University, Gödöllő (http://www.mkk.szie.hu/dep/gent/).

Distinction of varieties using DNA (SSR) barcodes

In order to see at one glance that really genotype-specific unique DNA fingerprints were obtained by the microsatellite markers, we constructed variety specific barcodes from the data. Of these, 12 varieties (Csabagyöngye, Csókaszőlő, Furmint, Heunisch weiss, Királyleányka, Kövérszőlő, Leányka, Lisztes fehér, Lisztes piros, Ottonel muskotály/Muscat Ottonel, Pinot noir) are shown in Figure 1. Based on the DNA barcode, difference between the varieties (Furmint-Csabagyöngye–Ottonel muskotály) or sameness (Lisztes piros–Lisztes

__Table 1.__ Microsatellite allele sizes of grapevine varieties at 6 SSR loci

Number	Variety	Allele sizes at loci					
		Scu8vv	Scu10vv	VVMD21	VVMD36	VrZag64	VrZag79
1.	Alanttermő	185-185	202-208	250-259	254-276	161-165	254-260
2.	Aprófehér	185-185	202-208	250-250	264-266	141-145	246-254
3.	Ágasfark	185-192	202-202	244-250	254-264	145-165	252-262
4.	Bajor, kék	185-192	202-208	250-257	252-252	145-165	252-262
5.	Bajor, szürke	185-192	202-208	250-257	254-254	145-165	252-262
6.	Bakarka	185-185	214-214	244-250	264-266	141-145	254-254
8.	Bakator kék	185-185	202-208	250-250	264-264	141-165	252-262
7.	Bakator, piros	185-185	202-208	244-257	266-288	145-165	254-254
9.	Bakator, tüdőszín	185-185	202-208	244-257	266-288	145-165	254-254
10.	Bakszem	185-192	202-208	250-250	252-264	141-165	240-262
11.	Balafánt	185-192	202-208	244-259	276-288	145-165	240-254
12.	Balafánt, fekete	185-192	202-202	250-250	254-276	161-165	252-252
13.	Bálint	185-192	208-214	250-259	264-276	141-145	252-252
14.	Bánáti rizling	185-185	208-211	250-257	254-288	161-161	254-262
15.	Beregi	185-185	208-214	244-250	254-288	139-145	254-262
16.	Betyárszőlő	185-185	202-214	250-257	264-266	139-165	262-262
17.	Bihari	185-185	202-205	250-250	264-264	161-161	250-262
18.	Bogdányi dinka	185-185	214-214	244-250	264-266	139-145	254-262
19.	Bőségszaru	185-185	202-205	244-250	276-296	145-165	248-252
20.	Budai	185-192	202-208	250-250	244-254	141-165	254-254
21.	Cudarszőlő	185-185	208-214	250-250	244-254	145-145	242-254
22.	Cukorszőlő	185-185	202-208	257-257	254-276	141-161	254-262
23.	Csíkos muskotály	185-185	208-217	257-257	244-264	143-161	254-258
24.	Csókaszőlő	185-185	202-208	257-257	288-288	161-165	240-254
25.	Csomorika	185-185	208-211	257-257	288-288	141-145	240-262
26.	Czeiger	185-185	202-208	250-250	264-288	139-165	254-254
27.	Demjén	185-185	202-202	244-257	254-288	141-165	254-262
28.	Erdei	185-185	202-214	244-250	264-264	145-165	246-254
29.	Ezerjó	185-185	202-202	244-250	258-276	139-139	240-254
30.	Fodroslevel	185-192	202-214	250-257	264-266	139-165	262-262
31.	Furmint	185-192	202-208	250-259	254-276	161-165	240-252
32.	Furmint, piros	185-192	202-208	250-257	254-276	161-165	240-252
33.	Fügér	185-192	208-208	244-244	254-276	145-145	252-252
34.	Fügeszőlő	185-192	208-208	244-244	264-288	145-145	240-252
35.	Fürjmony	185-192	208-211	250-257	254-264	141-161	250-254
36.	Gergely	185-185	208-214	244-250	266-276	159-165	240-254
37.	Gohér, fehér	185-192	202-208	244-257	254-288	141-145	252-262
38.	Gohér, piros	185-192	202-208	244-257	254-288	141-145	252-262
39.	Gohér, változó	185-192	202-208	244-257	254-288	145-145	252-262
40.	Gorombaszőlő	185-185	208-214	250-259	254-266	139-145	252-252
41.	Halápi	188-188	208-217	244-267	244-254	141-157	252-258
42.	Hamuszőlő	185-185	208-208	250-250	264-276	139-141	248-254
43.	Hárslevelű	185-185	202-208	244-259	264-276	145-165	240-254
44.	Hosszúnyelű	185-185	208-214	244-257	254-288	141-145	240-254
45.	Izsáki	185-185	208-214	244-250	254-276	139-161	240-246
46.	Járdovány	185-185	208-214	244-250	254-276	141-161	240-254
47.	Juhfark	185-185	208-208	250-257	264-276	141-165	240-252
48.	Kadarka	185-185	208-214	250-250	266-276	145-165	252-252
49.	Kéklőpiros	185-185	202-208	250-257	264-270	159-165	252-262
50.	Kéknyel	185-185	202-208	244-250	252-264	159-165	252-254
51.	Királyleányka	185-185	208-214	244-250	254-266	161-161	252-252
52.	Királyszőlő	185-185	202-208	250-259	266-288	145-165	254-262

53.	Kolontár	185-192	202-208	244-250	254-264	141-145	252-262
54.	Kovácsi	185-192	208-208	257-257	264-288	161-161	254-254
55.	Kovácskréger	185-185	202-211	250-257	254-264	145-161	252-254
56.	Kozma	185-185	202-208	257-267	254-264	141-145	262-262
57.	Ködös	185-185	208-208	257-259	254-276	145-165	252-252
58.	Kőporos	185-185	208-214	257-259	264-266	145-165	254-260
59.	Kövérszőlő	185-185	208-208	250-259	264-266	145-161	240-254
60.	Pécsi dinka	185-185	202-208	244-244	254-288	141-145	252-254
61.	Kövidinka	185-185	208-214	244-250	264-264	139-141	254-262
62.	Ürömi dinka	185-185	214-214	250-250	266-276	145-161	246-254
63.	Vörösdinka	185-185	208-214	244-250	254-264	139-145	254-262
64.	Zöld dinka	185-185	202-208	244-257	264-264	145-145	254-254
65.	Kübeli	185-185	208-214	257-259	264-266	161-165	254-260
66.	Lányszőlő	185-185	208-211	250-257	254-276	161-161	252-254
67.	Lágylevel	185-185	202-214	250-250	254-254	165-165	252-254
68.	Leányka	185-185	202-208	250-250	266-276	161-165	240-254
69.	Lisztes fehér	185-185	208-208	250-257	276-288	141-161	250-258
70.	Lisztes piros	185-185	208-208	250-257	276-288	141-161	250-258
71.	Magyarka	185-192	208-208	244-250	264-288	145-165	250-256
72.	Mézesfehér	185-192	208-214	250-257	266-276	141-165	254-262
73.	Mustos	185-185	208-214	244-250	254-276	145-161	246-252
74.	Pettyesszőlő	185-185	202-208	244-244	254-288	145-165	250-250
75.	Pécsi szagos	185-185	208-211	257-267	264-288	161-161	254-258
76.	Piros gránát	185-185	208-214	244-250	254-264	139-145	250-254
77.	Piros tökös	185-185	202-214	244-250	276-288	145-165	252-254
78.	Polyhos	185-185	202-208	244-259	254-288	145-161	252-262
79.	Pozsonyi	185-192	202-214	244-259	264-264	139-145	254-254
80.	Purcsin	185-185	208-214	250-250	254-276	161-165	250-258
81.	Rakszőlő	185-185	208-214	244-244	254-266	139-161	254-254
82.	Rókafarkú	185-185	208-214	250-250	264-276	141-165	240-246
83.	Rohadó	185-185	208-208	250-257	264-276	145-161	250-258
84.	Sárfehér	185-192	202-208	244-250	264-264	139-165	252-254
85.	Sárpiros	185-185	202-208	244-244	264-288	145-165	254-260
86.	Somszőlő	185-185	202-214	244-250	252-256	139-153	254-254
87.	Szagos bajnár	185-185	205-208	250-250	264-288	139-161	250-262
88.	Szeredi	185-185	202-202	250-257	254-276	145-161	252-252
89.	Szerémi	185-185	202-208	250-250	276-276	161-165	252-258
90.	Szőke szőlő	185-185	202-208	244-250	272-276	139-145	254-260
91.	Tihanyi	185-185	208-208	257-267	254-264	145-161	252-262
92.	Tótika	185-185	202-208	250-257	254-276	145-165	252-254
93.	Tökszőlő	185-185	208-214	257-257	264-276	161-165	240-262
94.	Tulipiros	185-185	208-208	244-244	254-288	145-161	246-252
95.	Tükörszőlő	185-185	202-214	250-250	254-264	161-165	246-262
96.	Tüskéspúpú	185-185	208-211	257-257	254-288	145-161	254-262
97.	Vékonyhéjú	185-185	202-208	250-250	264-276	161-165	246-262
98.	Csabagyöngye	185-185	208-214	244-267	264-296	161-161	258-262
99.	Muscat Ottonel	185-185	208-214	244-267	264-276	139-161	258-262
100.	Heunisch weiss	185-185	208-214	250-250	264-276	161-161	240-246
101.	Pinot noir	185-192	205-217	250-250	254-254	141-165	242-248

fehér) is easy to discern either with the naked eye or using a barcode reader.

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Figure 1. *DNA ampelography – DNA (microsatellite) barcodes suitable for the identification of grapevine varieties. The lines in the case of the Furmint represent the following allele sizes (from left to right) determined at the microsatellite loci (Scu08vv, Scu10vv, VVMD21, VVMD36, ssrVrZag64, ssrVrZag79): 161; 165; 185;192; 202; 208; 240; 250; 252; 254; 259; 276 (bp).*

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