

P-ISSN 0551-3677

E-ISSN 1338-4376

AGRICULTURE

POLNOHOSPODÁRSTVO

JOURNAL FOR AGRICULTURAL SCIENCES

VOLUME 61, Issue 3, 2015

The Annex to the Journal Agriculture

**NEW KNOWLEDGE
IN GENETICS AND BREEDING
OF AGRICULTURAL
PLANTS**

Book of Abstracts

The 22th International Scientific Conference

November 10, 2015

National Agricultural and Food Centre



AGRICULTURE (Poľnohospodárstvo)

INTERNATIONAL JOURNAL OF THE NATIONAL AGRICULTURAL AND FOOD CENTRE

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The journal is published in four issues per year by the National Agricultural and Food Centre, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, ID 42 337 402 ♦ Editorial office, orders, subscription and distribution: NAFC – Research Institute of Plant Production, Bratislavská cesta 122, 921 68 Piešťany, phone +421 33 7722 311, e-mail: editorial_office@agriculture.sk ♦ The price is 8.50 € per issue ♦ Printed by Ing. Karol Illý, Vydavateľstvo NOI, Kyjevská 4, 831 02 Bratislava ♦ October 2015 Registered at the Ministry of Culture of the Slovak Republic under the number EV 4946/14

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**NEW KNOWLEDGE IN GENETIC AND
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National Agricultural and Food Centre
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MODIFICATION OF POLYUNSATURATED FATTY ACID BIOSYNTHESIS IN CEREALS

JÁN KRAIC, DANIEL MIHÁLIK, MARCELA GUBIŠOVÁ, KATARÍNA
ONDREIČKOVÁ, LENKA KLČOVÁ, TATIANA KLEMPOVÁ, MARTINA
HUDCOVICOVÁ, JOZEF GUBIŠ, ALŽBETA ŽOFAJOVÁ, MILAN ČERTÍK

Cereals are the main source of proteins, carbohydrates, minerals, fibre, some vitamins as well as other compounds for foods and feeds production. Components that are missing in cereal grains are essential polyunsaturated fatty acids (PUFAs). Their biosynthesis is not possible due to absence of enzymes desaturating and elongating fatty acids and this barrier cannot be overcome by conventional breeding approaches. Nevertheless, specific genetic engineering tools could be used to enrich cereal seeds by self-produced PUFAs. Other organisms including the *Zygomycetes* fungi synthesize PUFAs and could be helpful in this effort. They can provide their own genes encoding enzymes (e.g. fatty acid desaturases) to open biosynthetic pathway leading to PUFAs. Although effective transfer of genes into genomes of cereals is difficult, an introduction and expression of the fungal $\Delta 6$ desaturase gene in barley and wheat plants has already been done in our experiments. Introduction and expression of artificial gene based on the original sequence of the filamentous fungi *Thamnidium elegans* has been confirmed at genomic, transcriptomic, as well as metabolomic levels. Transgenic barley and wheat plants produced both the γ -linolenic and stearidonic acids. This biotechnological strategy offers enormous potential for the natural production of “tailor-made” functional cereals enriched with PUFAs. It could open new ways for development and utilization of improved cereal seeds for production of cereal-based functional foods and feeds. On the other side, this approach could influence parameters of biological membranes in plant cells affected by changes in desaturation of fatty acids in phospholipids and improve tolerance of plant organism to temperature stresses.

Key words: fatty acid, $\Delta 6$ desaturase, cereal

Acknowledgements: *This work was supported by grants APVV-0662-11 and APVV-0294-11 from the Slovak Research and Development Agency.*

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SIGNIFICANCE OF SLOVAK BREEDING IN CHANGING ENVIRONMENTAL CONDITIONS

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The agricultural policy of the Slovak Republic has to be prepared and adapted to the present or future situation in order to limit any adverse effects of climatic and environmental changes. Due to these global changes it is necessary to take the measure our of attitude to plant breeding as a process of development of new varieties for present and future needs with an emphasis on improving their quantitative and qualitative characteristics. Development of new plant genotypes with higher resistance and adaptability to biotic and abiotic factors, differentiated quality and characteristics for multifunctional use of agricultural biodiversity is essential, and significantly affects stability and viability of crop production. The Slovak variety formed under the same conditions in which it will grow is the best adaptable to the agroecological conditions of the Slovak Republic. The Slovak breeder is able to foresee the variety characteristics of importance in 10 to 15 years' time, define them as breeding goals and realize those goals with appropriate measures. Spring barley breeding for resistance is focused on breeding of barley cultivars with effective resistance to *Blumeria graminis f. sp. hordei* based on *mlo* gene, new alleles of the *Mla* locus, *Mli* and *Mlj* resistance from *Hordeum vulgare* subsp. *spon-taneum* with aim to combine two fully effective resistances. PCR analyses of diseased barley leaves confirmed the presence of *Ramularia collo-cygni*, a new disease in the Slovak Republic, therefore *Ramularia collo-cygni* is a new challenge in barley breeding. Variance analysis revealed significant genotype, environment and genotype x environment effects for net blotch (*Pyrenophora teres*) and leaf scald (*Rhynchosporium secalis*). The aim is to select and release spring barley plants with combined resistance to mentioned fungal diseases. Abiotic stresses significantly affect basic technological parameters of malt. Malt produced from less stress tolerant varieties has lower extract content, higher content of nitrogenous substances and β -glucan in wort. As an example of the results obtained most recently, description is made for the Karmel, Kumran, Valis and Exalis spring barley varieties.

Key words: breeding, changing environment, variety, abiotic and biotic resistance

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NEW TRENDS IN BREEDING OF *PETUNIA* × *HYBRIDA*

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Petunia is among the worlds' best-selling annuals. At the beginning, new varieties initially arose through mass selection. In the 1930s' the breeders started to apply individual selection and the varieties were propagated generatively. The first F1 variety 'Ballerina' was launched on the market in 1952 by Pan American Seeds. In the former Czechoslovakia, the first F1 variety named 'Lavina F1' was bred by Ing. Jan Černý in Jaroměř. Hybrid breeding was also a form of intellectual property protection. F1 variety could be stolen just by the physical theft of parental components of which was a hybrid composed of. The current worlds' assortment of Petunias is rapidly changing and is formed predominantly by Young Plants Producers. For them technological aspects are crucial, ie. earliness, compactness, number of transplantable plants. In the early 20th century, the Petunias were bred by many subjects. Currently there are significantly fewer breeders. This is primarily due to substantial expenses of breeding. In order to secure the investment return, the breeding companies protect new varieties by patents. Previously, Plant patents were used, which allowed usage of the variety by other breeders in their programs. Nowadays, so-called Utility patents are applied, they are more expensive, but offer a higher level of protection, so other breeders may not use this material at all. Utility patents are used by big breeding companies, for example Pan American Seeds owns one (US 7,642,436) for unique Petunia groups called 'Debonair', 'Sophistica', 'Easy Wave'. The newest trend in breeding is using biotechnologies, but in Horticulture are still seldom. They could increase the effectiveness of breeding programs. These unconventional methods also give the possibility to create new varieties with unique characteristics (aesthetic as well as technological, for example tolerance to abiotic and biotic stress) that the classical ways of breeding don't allow, for example due to cross incompatibility. Progressive method is the protoplast fusion, through which cytoplasmatic male sterility might be incorporated into the components or new hybrids *Petunia* × *Calibrachoa* might be produced, which are due to post fertilization barriers difficult to cross using the conventional methods. Another method is polyploidy *in vitro*, which might create a unique group of F1 varieties with better resistance to diseases and pests. Dihaploids production from isolated microspores, which is currently not fully solved by Petunias, would bring substantial reduction of duration and costs of the breeding programs. Fundamental changes in Petunia assortment will be in future caused by using Genetics Manipulations in breeding programs.

Key words: petunia, breeding, biotechnologies, patents

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EFFECT OF FERTILISATION ON IMPORTANT CONTENT AND QUALITY PARAMETERS OF COMMON AND NAKED OAT

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The aim of this experiment was to define the effect of fertilisation on important content and quality parameters of oat. In the experiment, 12 liming variants of fertilisation were used. Five oat genotypes (2 common and 3 naked) were grown on each variant, and yield, thousand grain weight (TGW), volume weight (VW), β -glucan, protein and fat content were analysed. In yield, statistically highly significant differences between years 2013 and 2014 were observed, when significantly higher average yield was recorded in 2014. Similarly statistically highly significant difference was observed in fertilisation, when the highest average yield was recorded at variant 026 (5.99 t/ha) and the lowest at variant 011 (2.49 t/ha). Statistically highly significant difference was recorded in yield among genotypes, the lowest average yield 3.79 t/ha was recorded in naked oat 100260CN and the highest 5.28 t/ha in common oat Valentin. TGW was not statistically significantly affected by fertilisation and year. The highest average value of 32.68 g was found in the fertilization variant 013 and the lowest (28.58 g) in unfertilised variant 011. Variety influenced TGW highly significantly, with the highest average of TGW in the variety Valentin (38.57 g) and the lowest (22.57 g) in 100260CN. VW was not significantly affected by fertilization. The highest average VW value of 59.3 kg/100 l was found in the fertilisation variant 023 and the lowest in the unfertilised variant 011 (56.82 kg/100 l). Variety and year influenced statistically highly significant VW with the highest average of total VW (65.1 kg/100 l) in naked oat 100260CN. The lowest average VW had the common variety Valentin (51.71 kg/100 l). The average β -glucan content was not influenced by fertilisation and year. The highest average β -glucan content (3.46%) was found in the variant 024 (120 kg N/ha) and the lowest (2.81%) in the variant 011. Variety influenced the average β -glucan content statistically highly significantly, whereas the highest average content (3.68%) showed 100260CN and the lowest (2.55%) Valentin. The protein content was statistically highly significantly influenced by N fertiliser and variety. This was reflected in all variants fertilised with N from the lowest dose of 40 kg/ha (variants 013, 022) to a high dose of N 150 kg/ha (016). Similarly, statistically highly significant differences in protein content were by genotype. The highest average protein content was found in 100260CN (14.96%) and the lowest in Václav (11.04%). The fat content in naked oats was not statistically significantly affected by fertilisation and year. The highest average value of fat content (7.60%) was found in the variant 011 and the lowest in the variants 015 (6.57%) and 016 (6.82%), both by the highest dose of NPK. Variety influenced the fat content statistically highly significantly and the highest content (7.69%) showed naked oat 100260CN.

Key words: fertilisation, quality parameters, common oat, naked oat

Acknowledgements: *The study was supported by Slovak research and development agency, Slovak republic, project number APVV-0398-12.*

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POSSIBLE DIRECTIONS FOR WHEAT BREEDING

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Breeding is vision, discovering new directions, development of new and unconventional materials that can significantly shift the development of crop forward. In breeding, there is a lot of “blind alleys” which do not lead into a progress. The goal of research was to suggest ways in which breeding can set out for the future. According to our ideas, increasing grain yield through the number of spikes has its limits and so only one way remains to increase the spike productivity. One of breeding directions in the Research and Breeding Station at Vígľaš-Pstruša is to increase productivity through modified morphological structure of spike characterized by greater number of spikelets growing up from each node of rachis (multirow spike, MRS). Meanwhile breeding in this direction is difficult, as greater number of grains per spike is linked with increase of spike diseases as well as reduction of 1,000 grain weight (TGW). Another possible way to increase the productivity of spike is utilization of the Chinese material with more pistils in floret (three pistils mutant, TP-mutant), where up to three fertile grains per floret can be formed. We work currently with this material to improve winter hardiness and TGW. Risks and benefits can be opened flowering. The risk is to maintain the cultivar stability, the advantage can be utilization of this material in the creation of hybrid cultivar. Increasing spike productivity can also be achieved by increasing spike assimilation by prolonging the glumes, which assume full assimilation at the time of ripening. Wheat grains with unusual color (purple, blue, violet) have been included in the breeding process due to high content of anthocyanins with beneficial effects on the health. Thirty lines of winter wheat of F5 and F6 generations were evaluated: 1 – blue, 2 – purple, 3 – violet grain color, 4 – yellow endosperm, 5 – MRS, 6 – TP-mutant, 7 – long glume, and 8 – standard grain color (common wheat cultivar Viglanka). TP-mutants had in average the lowest TGW and the highest number of grains per spike with potential to increase TGW. Long glume wheat dispose of the highest weight of grains per spike caused by relatively high TGW and number of grains per spike. Modified morphological structures of spike are a potential to achieve increased grain yield. Wheats with multirow spike were characterized by high number of grains per spike and grain weight per spike was at the level of Viglanka, however lower TGW caused a reduction in grain yield. During the experiment, low diseases pressure was recorded, therefore wheats with different spike morphology had good health. However, colored wheats of violet and purple color were damaged by stripe rust; seeds had lower TGW influenced by genotype and worse health condition. In general, mentioned winter wheats disposed of lower grain yield due to worse winter hardiness as their origin is in spring wheats. Blue wheats were characterized by good health, high TGW and good grain yield potential. In terms of health benefits in colored wheat, to obtain lines with higher content of anthocyanins compared to purple wheat grains is a vision of breeding. Yellow endosperm wheats had an average grain yield and good health. Progress in this grain group is needed in the future. Our results suggest that in the wheat breeding, many opportunities to improve qualitative and also quantitative traits are still existing. These are, for example grain yield and its quality, quality of grain ingredients, improving resistance to biotic and abiotic environmental factors, which still open up new breeding possibilities and directions.

Key words: multirow spike, TP-mutant, grain color, long glume, genotype

Acknowledgements: *This research was supported by the Ministry of Agriculture and Rural Development of the Slovak Republic in the Research and development project “DOMAPLUS”.*

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THE TECHNOLOGICAL QUALITY OF WHEAT, SPELT AND CROSSBREED PS LUBICA

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Winter wheat PS Lubica is crossbreed of *Triticum aestivum* (winter type) × *Triticum spelta* (winter type) of varieties Estica × Renval. PS Lubica was registered in Slovak Republic in the year 2014. This variety achieves high grain yield (90% in comparison to standard variety), the grain is not necessary to hull. PS Lubica is late variety, the spike of this variety is long, sparse, coloured, 1000-kernel weight is 50 g. PS Lubica is less resistant to lodging, has average resistance to leaf spot, leaf rust, powdery mildew, spike diseases (fusarium). This variety is suitable for growing in all growing regions. Good yield is achieved after better foregoing crops. Recommended seeding rate is 4.5 million germinated seeds per ha in maize production area and 5.0 million germinated seeds per ha in sugar beet and potato production area. The aim of our study was determine and compare the technological quality of PS Lubica, wheat (variety Josef) and spelt (variety Öko 10). We determined qualitative parameters such as: volume weight, extraction of the flour, ash content, protein content, wet gluten, gluten index, swelling of gluten, extension of gluten, elasticity of gluten, falling number, sedimentation index, rheological properties of dough (farinograph evaluations) and the final quality of bakery products. PS Lubica was characterized by the low volume weight, the high protein content and the high water absorption in comparison with winter wheat and spelt. After complex evaluation we can state that the crossbreed of spelt and winter wheat, PS Lubica, is more similar to spelt than to winter wheat with regard to technological quality. The practical aim of our study was to give more detail data for breeders, farmers and bakeries too, to facilitate decision for variety PS Lubica. Therefore we determined wide spectrum of the qualitative parameters for better identification of the technological and bakery quality of this variety. PS Lubica is more similar to spelt from the qualitative point of view, but more similar to winter wheat from the morphological point of view.

Key words: PS Lubica, winter wheat, spelt, technological quality, bakery test

Acknowledgements: *Authors thank the projects: DOMAPLUS (Research and development project of the Ministry of Agriculture and Rural Development of the Slovak Republic) and APVV-0758-11 (Scientific Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic) for financial support of the research.*

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ANALYSIS OF RELATIONS BETWEEN CROP TEMPERATURE INDICES AND YIELD OF DIFFERENT SUNFLOWER HYBRIDS FOLIAR TREATED BY BIOPREPARATES

DÁVID ERNST, MAREK KOVÁR, IVAN ČERNÝ

Exogenous application of biological active anti-stress compounds and remote-sensing control in management of agronomic intervention are an important part of successful crop cultivation. To study effects of foliar application of biological active compounds (Terra-Sorb and Unicum) on yield and yield-forming and quality parameters, as well eco-physiological traits calculated from infrared (IR) thermographs data (crop water stress index – CWSI and index of stomatal conductance – Ig) of three hybrids of sunflower (NK Brio, NK Neoma, NK Ferti), poly-factorial experiments with 3 replicates realized during two vegetation periods (years 2012 and 2013) was conducted. The climatic conditions in chosen experimental years were different in quantities and distribution of precipitation at main growth period of sunflower plants (June to August) and allows evaluating the yield stability between used hybrids and treatments. Results showed that the application of selected biologically active preparations (BAP) has contributed to an increase of sunflower yield, in particular through increase the weight of thousand seeds (TWA; $r_p = 0.761$, $p < 0.001$). Similarly, oil content in achenes was significantly higher in treatments with BAP, mainly with preparation Terra-Sorb. The study also describes the quantitative relationship between sunflower yield and quality of production ($r_p = -0.41$, $p < 0.01$). Moreover, plant water status and thus overall plant fitness was evaluated in terms of CWSI and Ig indices. Selected hybrids of sunflower in two growth stages (in both years) showed the significant differences in CWSI and Ig (both at $p < 0.01$), respectively. Analysis of negative linear relation between yield of achenes and CWSI ($r_p = -0.654$, $p < 0.001$) confirmed that higher value of plant stress resulted into smaller yield and vice-versa. The opposite trend was observed between yield and Ig index ($r_p = 0.576$, $p < 0.001$). The data obtained from IR-thermography can be used for monitoring of physiological health of sunflower plants, as well in potential prediction and control of yield.

Key words: infrared thermography, CWSI, yield, oil content, foliar biopreparates, sunflower

Acknowledgements: *The work was funded by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic, project VEGA 1/0093/13 Rationalization of sunflower (*Helianthus annuus L.*) and sugar beet (*Beta vulgaris* boil. *Altissima Doell.*) cultivation system in terms of global climate change, with emphasis on climate change, optimize the production process, the quantity and quality of production.*

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THE USE OF DNA MARKERS FOR DETECTION OF TOMATO AND PEPPER BREEDING LINES RESISTANT TO TOBAMOVIRUSES

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Tobamoviruses such as pepper mild mottle virus (*PMMoV*), paprika mild mottle virus (*PaMMV*), tobacco mosaic virus (*TMV*) and tomato mosaic virus (*ToMV*) can easily be transmitted by mechanical way or by seeds and cause serious losses in tomato and pepper yield and quality. The development of new resistant genotypes is important especially by the use of resistance genes from wild related species. DNA markers closely linked to resistance genes can make breeding process faster and more efficient. The aim of our study was to evaluate the suitability of known DNA markers to detect alleles of resistance genes against *ToMV*, *TMV*, *PaMMV* and *PMMoV* in tomato and pepper breeding lines provided by Zelseed company in Slovakia. The presence or absence of the resistant allele *Tm-2* (conferring resistance to *ToMV* strains 0 and 1) in tomato lines were determined by the allele-specific PCR marker – primer pair *Tm2R-f1c/Tm2R-r3*. The presence or absence of the susceptible allele *tm-2* in tomato lines were determined by the allele-specific PCR markers – two primer pairs *Tm2S-f1/Tm2S-r1* and *Tm2S-f2/Tm2S-r2*. These dominant markers can not distinguish the homozygous and heterozygous genotypes at *Tm-2* locus individually, but combination of these markers can do. Out of 184 analysed tomato breeding lines in 133 genotypes only the fragment for the susceptible allele *tm-2* was amplified, in 33 genotypes the fragments for both - the susceptible allele and also for the resistant allele were amplified and in four genotypes only the fragment for the resistant allele *Tm-2* was amplified. These genotypes were tested by artificial inoculation with *ToMV* strain 0 according to CPVO methodology and all 4 resistant homozygotes showed full resistance (9), while heterozygotes showed full or unbalanced resistance. The presence or absence of the *L³* gene alleles conferring resistance to *P₀*, *P₁*, and *P_{1,2}* pathotypes (*TMV*, *PaMMV* and *PMMoV*) in pepper breeding lines were determined by the codominant SCAR marker pair PMFR11₂₆₉ and PMFR11₂₈₃. Two types of fragments were amplified: the 283 bp fragment for the susceptible allele and the 269 bp fragment for the resistant allele. Out of 62 analysed pepper lines in 58 genotypes only the fragment for the susceptible allele was amplified. In 3 genotypes only the fragment for the resistant allele was amplified and these are genotypes coming from Hungarian cultivars Brill and Brillant, which are declared resistant to tobamoviruses. In one genotype both fragments were amplified and this genotype is coming from Hungarian cultivar Hurricane also declared as resistant to tobamoviruses. These genotypes were tested by artificial inoculation with *P₀* pathotype virus (*TMV*) and all four were resistant. The tested DNA markers proved to be suitable for their introduction into the selection process of breeding material and enable increased efficiency of creation of new tomato and pepper genotypes resistant to tobamoviruses.

Key words: tomato, pepper, molecular marker, tobamovirus, *Tm-2*, *tm-2*, *L³* gene

Acknowledgements: *This work was supported by OP Research and Development: Transfer of effective procedures for selection and identification of plants in breeding (ITMS 26220220142) from European Regional Development Fund and by the Slovak Research and Development Agency under the contract No. APVV-14-0055.*

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CAN TRANSGENIC MAIZE CHANGE THE COMPOSITION OF BACTERIAL COMMUNITIES IN THE RHIZOSPHERE?

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Transgenic plants are worldwide cultivated for several decades, and it is necessary to examine the possible risks of their cultivation for the surrounding environment. In our study we examined the possible impact of transgenic maize cultivation on the composition of bacterial communities in the rhizosphere of maize hybrid NK603 × MON810. This transgenic maize expresses CP4 EPSPS proteins derived from *Agrobacterium* sp. strain CP4, which confer tolerance to Roundup® agricultural herbicide (containing glyphosate) and also expresses the Cry1A(b) protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection from predation by certain Lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp). The overall bacterial communities from two localities of the Czech Republic (Ivanovice na Hané and Probluz) were studied using culture independent molecular technique – Terminal restriction fragment length polymorphism (T-RFLP). The results from T-RFLP analysis did not show statistically significant difference in genetic diversity of rhizosphere bacteria between samples from control and transgenic maize. The differences in the composition of rhizosphere bacterial communities were detected only between collection sites (Ivanovice na Hané vs Probluz), and these differences were apparently a consequence of various soil type and weather conditions.

Key words: bacterial communities, NK603 × MON810, rhizosphere, transgenic maize, T-RFLP

Acknowledgements: *This work was supported by the Ministry of Agriculture and Rural Development of the Slovak Republic under the project BIOTRAMI.*

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THE INFLUENCE OF SEWAGE SLUDGE ON THE GENETIC DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE RHIZOSPHERE OF GIANT REED

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JÁN KRAIC

Arbuscular mycorrhizal fungi (AMF) are enhanced in the natural environment and can provide many benefits to the host plant. These include improved nutrition, enhanced resistance to environmental stress and diseases, and tolerance to heavy metals. In this study, genetic diversity of AMF from the rhizosphere of giant reed (*Arundo donax* L.) was studied using T-RFLP method. The plants were planted in the soil with the addition of dewatered activated sewage sludge in doses of 0t/ha, 5t/ha and 15t/ha. The rhizosphere samples were collected in two dates (August and November, 2014) and 18S rRNA gene was amplified from metagenomic DNA using specific primers for AMF. Forward and reverse primers were labelled at the 5' end with different fluorescent dyes. The PCR products were then separately digested using *Hinf*I and *Hsp*92II restriction enzymes. Microbial biomass was increased with increasing sewage sludge concentration and statistically significant differences between control and samples with sewage sludge were detected. In August, there were detected from 43 to 234 terminal restriction fragments (T-RFs) and in November from 31 to 162 T-RFs, whereby the number of T-RFs detected in August was increased and in November was decreased with increasing dose of sewage sludge. In August, the Gini-Simpson, Shannon's diversity index and Pielou evenness index were higher in sludge samples than in controls. In November, these diversity indices were opposite, with the highest values in control samples. Principal component analysis and cluster analysis did not show sample separation by sludge concentration or by collection dates. In conclusion, the addition of sewage sludge to the soil significantly increased microbial biomass but the composition of AMF communities was not affected.

Key words: arbuscular mycorrhizal fungi, *Arundo donax*, fungal diversity, sewage sludge, T-RFLP

Acknowledgements: *This study was funded by the Project No. HU-SK/1101/1.2.1/0148 within the frame of the Operational programme „Hungary-Slovakia Cross-border Co-operation Programme 2007–2013”.*

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BIOTECHNOLOGICAL APPROACHES TO PROPAGATION OF MISCANTHUS GRASS – AN INTRODUCED ENERGY PLANT

MARCELA GUBIŠOVÁ, ALŽBETA ŽOFAJOVÁ, JOZEF GUBIŠ

Genus *Miscanthus* includes perennial giant grasses belonging to C4 plants with high photosynthesis efficiency. *Miscanthus giganteus* Greef et Deu, which is the most valuable for biomass production, is naturally occurring triploid hybrid ($2n=57$) of *M. sinensis* ($2n=38$) and *M. sacchariflorus* ($2n=76$) sampled in 1935 by A. Olson in Yokohama, Japan, cultivated in Denmark and spread out over middle Europe. It is considered environmentally friendly producer of biomass, biofuel, lignin and lignocellulose. Due to low moisture content during harvesting, biomass is suitable for direct combustion without the need for drying. The yield potential of miscanthus grass exceeds the capabilities of the natural plant species in Slovakia, including fast-growing trees. *Miscanthus giganteus* is triploid hybrid which doesn't produce fertile seeds. Plants are usually propagated by rhizome division, but rhizomes production is slow and the success of establishment of new crops is strongly affected by the age of mother crops. Plant tissue culture is an alternative way to prepare necessary amount of propagules within a relatively short time. Two approaches of *M. × giganteus in vitro* propagation – direct (*in vitro* tillering) and indirect (via callus phase), were tested as alternatives to vegetative propagation by rhizomes. For direct propagation, stem segments with axillary buds were used to establish the explant culture. Regenerated shoots multiplied on MS medium containing cysteine HCl, which prevents browning of explants, and cytokinin Thidiazuron or BAP (6-benzylaminopurine), with multiplication coefficient 2.95 or 2.65 shoots/month respectively. Shoots rooted spontaneously in multiplication medium, but addition of auxins improved rooting and acclimatization after transplantation to soil also. In indirect way of regeneration, callus cultures were established from immature inflorescences. Those of the length 10 mm were the most regenerable. Regeneration started four month after culture initiation, and 64 regenerants/immature inflorescence developed within next three months. Regenerants were then multiplied by direct method mentioned above. First plants propagated by this biotechnological approach (by indirect method) were transplanted to the field in Piešťany in 2011 and compared to rhizome-propagated plants. There were not observed any problem with winter freezing in both groups of plants. In the 3rd year of cultivation, *in vitro* plants produced higher amount of shoots with lower diameter, and the yield of dry biomass were not significantly different compared to rhizome-propagated plants.

Key words: immature inflorescence, *in vitro* tillering, micropropagation, *Miscanthus giganteus*

Acknowledgements: *This work was supported by the Ministry of Agriculture and Rural Development of the Slovak Republic under the project BIOTRAMI.*

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MONITORING AND PROTECTION OF OLD CHERRIES TREES IN THE REGIONS OF SLOVAK REPUBLIC

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IVETA ČIČOVÁ

Plant Genetic Resources for Food and Agriculture is an essential element not only for growing but also for plant breeding. The Slovakia is a country where can still be found old fruit plantation with the appearance of old varieties and landraces of fruit species. In 2014 and 2015 at fourteen locations in Slovakia, old trees of cherry (*Cerasus avium* (L.) Moench) with an estimated age of 60-80 years were monitored. The health status, morphological traits of trees and flowers, flowering intensity and selected characteristics of the fruit according to Descriptor lists of genus *Cerasus* Mill. were assessed. By description, 11 fruit characters were evaluated: fruit (weight, length, width, thickness, shape and colour), flesh (colour, juice and firmness), pedicel length and stone shape. Botanical classification was used by Marhold and Hindák (1999). These trees are today very suitable material for the conservation and preservation of plant genetic resources collections for the reason that today there is any breeding programme of cherries fruit already in Slovakia, and they have been found naturally in the wild, either in the form of remnants of old orchards, alleys and solitary trees. Research workers from Gene Bank of SR in cooperation with other departments developed in recent years activities for their saving. During collecting expeditions, 170 genotypes of sweet cherry fruit of the different quality and health were found. There have been identified large-old cherry varieties and seedlings (*Cerasus avium* (L.) Moench) suitable as a rootstocks. From the fourteen monitored localities, the most valuable genotypes were found in localities of west Slovakia Horna Streda – Cachtice, near the village Krakovany and at the village Brdarka in the central Slovakia. The largest variability of fruit colour was in the locality Brdarka. Fruit colour varied from light red to dark red, but till today there have not been found purely yellow fruits genotypes that were popular and grown especially in the past. The fruit weight of genotypes in locality Cachtice ranged from 5.8 to 9.7 g per fruit, in locality Krakovany from 4.1 to 8.9 g per fruit and in locality Brdarka from 5.4 to 8.8 g per fruit. Results of the monitoring and collection of plant genetic resources have been processed in the database. The most interesting 96 genotypes have been grafted onto rootstocks with different intensity of growth (*Cerasus avium* (L.) Moench., *Cerasus mahaleb*, GISELA5) and will be used for the establishment of experimental plantings and orchards. Activities related to the monitoring of cherry trees will continue in 2016 and 2017 in other regions of Slovakia.

Key words: genetics resources, *Cerasus* spp., monitoring, evaluation, regeneration

Acknowledgements: *This work was created with the support of project no. APVV-0174-12 the Agency for Research and Development (APVV) of Slovak Republic.*

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THE RESPONSE OF SPRING BARLEY ON SELECTED EXPERIMENTAL FACTORS

EVA CANDRÁKOVÁ, EVA HANÁČKOVÁ

In 2012 and 2013, an experiment with spring barley, variety Kangoo was carried out at an experimental basis of SPU, the locality Dolná Malanta. The experimental site of Slovak Agricultural University in Nitra is located in south-western Slovakia (E 18°09' N 48°19') with altitude of 175 m above sea level. The location has warm and moderate continental arid climate with an average annual temperature of 7.9°C and average annual precipitation of 561 mm. The mean temperature during the growing season is 16.2°C. The soil type is Orthic Luvisol with a loamy texture, medium humus content of 1.95–2.60% and a pH of 5.7. There were tillage treatments (O1-medium deep plowing to 0.24 m, O2-disc tools to 0.12 m) and fertilisation treatments H1-control treatment, H2-inorganic fertilisers (P, K, N), H3-inorganic fertilisers and incorporation of forecrop residues. The doses of fertilisers were supplemented on the basis of soil analysis for grain yield 5 t/ha (P and K in the autumn, N in the spring). Preceding crop was grain maize. Size of plot was 20 m². Spring barley variety Kangoo is medium early, higher type (0.75 m), with good resistance to lodging. Grain yield has been influenced by year. In 2013, grain yield was significantly higher (4.00 t/ha) compared to 2012 (3.03 t/ha). Tillage affected the yield no significantly, while grain yield after conventional tillage (O1) was 3.57 t/ha, which exceeded the yield after minimizing cultivation (O2) only by 0.12 t/ha. Fertilisation treatments influenced the grain yield positively. The highest yield was on var. H3 (3.77 t/ha), followed by var. H2 (3.58 t/ha) and the lowest yield was on control variant H1 (3.18 t/ha). A similar influence of experimental factors was reflected also in the thousand grain weight. It was significantly higher in 2013 (50.13 g) in comparison with 2012 (45.55 g). Among fertilisation treatments, the significantly higher values of thousand grain weight were on variants H3 (50.03 g) and H1 (48.96 g). Volume grain weight was significantly higher in 2012 (718.20 g/l), which was in comparison with the year 2013 more by 67.54 g/l. A statistically significant difference was between soil cultivations in favour of the O2 – no tillage (690.56 g/l) versus O1 – plowing (678.30 g/l). Fertilisation variants influenced volume weight no significantly. Year conditions and fertilisation treatments also affected the protein content in grain barley. In 2012 it reached 12.68%, thus exceeding the standard requirements for malting quality. In 2013, grains of barley contained 11.00% protein and complied with the criterion for malting barley. Application of fertilisers caused an increase in the protein content of grain barley, which is in terms of grain quality for malting purposes undesirable. The highest content was on variant H2 (12.20%) and the variant H3 (12.12%). The lowest content was on control variant (11.05%). Yield and grain quality of spring barley in the evaluated period were not optimal as a consequence of less suitable preceding crop.

Key words: spring barley, tillage, fertilisation, yield, quality

Acknowledgements: *Projekt VEGA č. 1/0816/11 „Produkčný proces poľných plodín pri rôznych systémoch obrábania pôdy, aplikácie priemyselných hnojív a zvyškov rastlín s ohľadom na zachovanie a zvyšovanie úrodnosti pôdy“*

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THE CONTENT OF ANTHOCYANINS IN COLOR GENOTYPES OF WHEAT AND INTERACTION WITH SELECTED AGRONOMIC PARAMETERS

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SOŇA GAVURNÍKOVÁ

The anthocyanins are naturally occurring bioactive water soluble compounds that are responsible for the color (orange, red, purple and blue) of the fruits, vegetables, and plants. They belong to the widespread class of phenolic compounds collectively named flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The interest in them has increased in the last decades as they represent alternatives to artificial food colourants and research suggests potential health benefits due to their antioxidant properties. Anthocyanins in wheat grains are expressed in either the pericarp or aleurone layer. Previous studies revealed that different anthocyanins are present in wheat varieties carrying genes for either the purple pericarp or the blue aleurone trait. The aim of the research was to compare the genotypes with different colors of grain, mainly to compare the contents of anthocyanins and a potential relation with other agronomical parameters. The analysed material contains 18 varieties and genotypes of different colors of grains - 7 purple, 5 blue, 4 with yellow endosperm. As control samples we used varieties Ilona and Heroldo, a variety with white grain. The experiment was established by the method of randomized blocks in the vegetation 2012/13 in Piestany. Between a group of genotypes with blue and purple color of the grains, significant difference in the content of anthocyanins was not observed. The mean content was 61.27 mg/kg, respectively 59.03 mg/kg. The highest content of anthocyanins had a variety Scorpion (91.91 mg/kg). Control varieties Ilona and Heroldo had an average content of anthocyanins 20.91 mg/kg and 18.90 mg/kg, respectively. In monitoring of correlation with selected agronomic parameters, there were not observed statistically significant relationship. A negative correlation was found in relation between content of anthocyanins and tolerance to powdery mildew and leaf rust.

Key words: anthocyanins, wheat, content, genotypes

Acknowledgements: *This work was supported by Resort Project of Research and Development „New benefits in primary products of plant domestic production“, acronym DOMAPLUS.*

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ACHIEVED PARAMETER CHANGES IN CZECH AND SLOVAK WINTER WHEAT CULTIVARS (*TRITICUM AESTIVUM* L.) DURING 90 YEARS OF BREEDING

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Wheat breeding has been influenced by several important milestones in the former Czechoslovakia. The first was rediscovery of Mendel's laws of inheritance in the beginning of the 20th century. Thereafter, the first selections made from local landrace populations enabled development of the old Czech varieties Chlumecká 12, Postoloprudská Přesívka and Dobrovická Přesívka in 1922. Just several years later (1927), the first wheat crossbred variety in the Czech Republic, Stupický Bastard, was registered. In the Slovak Republic, the original landraces predominated till 1948 (e.g. Radošínská, Šarišská). Science-based breeding programs began after the Second World War and introduced a number of important genes, such as for semi-dwarfing and photoperiod insensitivity, as well as genes for disease resistance obtained, for example, through alien introgression. The development of molecular biology in the 1980s and 1990s enabled the application of marker-assisted selection, which accelerated breeding of wheat for improved resistance and grain quality. Wheat breeding still faces many challenges including the never-ending pressure to boost growing efficiency, faster changes in environmental conditions and a possible loss of genetic diversity. As a reaction on these challenges, the large set of 262 original Czech and Slovak winter wheat accessions developed in the periods 1920-2008 were gathered in the Gene Bank of Prague in the cooperation with the Slovak Gene Bank in Piešťany and cultivated during 3 years (2010-2012). The objectives of the present research were predominately aimed at essential parameter changes among obsolete and modern wheat cultivars as well as a comparison of their variability or stability and identification of perspective wheat germplasm. Our results indicated that wheat breeding has unambiguously improved spike productivity (from 1.48 g to 1.87 g), lodging resistance (from 5.2 points to 8.9 points) and mildew resistance (from 3.7 points to 5.2 points) compared to wheat landraces and obsolete varieties. The grain-filling period was also significantly the longest for modern wheat varieties. The incorporation of *rht* genes into Czech and Slovak wheat varieties at the beginnings of 1960s significantly decreased the plant height in current wheat varieties and dramatically increased the stability of lodging resistance (from 42% for cultivars developed up to 1930 to 3% for cultivars developed 1991-2008). Simultaneously, the breeding period (1960-1970) is possible to characterize as the key period responsible for essential changes among obsolete and modern cultivars. On the other hand, these economically positive properties of modern wheat varieties were negatively reflected in a lower crude protein content and its annual stability, as well as in stability of Zeleny sedimentation index. Several promising wheat varieties (e.g. Diana II registered in 1965 and Regina registered in 1977) with acceptable spike productivity and high parameter stability were identified as well. Their properties could be suitable for other breeding directions (low input systems, organic farming) where the maintenance of yield stability and grain quality (especially crude protein content) play a key role.

Key words: winter wheat, landraces, modern cultivars, breeding

Acknowledgements: *This work was supported by the Ministry of Agriculture of the Czech Republic (Projects no. QJ1310219 and no. QJ1510163).*

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THE USE OF GAS CHROMATOGRAPHY FOR THE EARLY SELECTION OF GENOTYPES OF RAPESEED (*BRASSICA NAPUS* L.) WITH THE REQUIRED CONTENT OF FATTY ACIDS IN THE OIL

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ZUZANA NAVRÁTILOVÁ

Currently, rapeseed ranks the strong position in the Czech and European agriculture. In the year 2015, it has been grown on the area about 400,000 hectares with the average yield of 3.3 t/ha in the Czech Republic. The aim of production is primarily a profit from vegetable oil which has the high quality. The chemical composition of the oil predicts its numerous possibilities of applications. The oil is used both for food application and for technological purposes. The quality of rapeseed oil is determined mainly by composition of fatty acids (FA). The representation of individual fatty acids in rapeseed oil is conditioned by genotype. It is possible to influence the proportions of fatty acids in the seed by breeding process. The possibility of very early selection of suitable genotypes in the seed could provide important efficiency of the breeding process. The aim of the study was to develop and optimize the method of isolating and determination of FA methylesters by GC / FID from the minimum amount of the initial sample (1 seed or segment of rapeseed). In 2014 and 2015, 24 samples of the seed segments of the selected rapeseed genotypes were analysed, the rest of the segments of the same seed was sown and grown to mature plants. The sampling of twelve seeds according to the defined position of mature plant was performed and the content of FAs in 288 samples of rapeseeds was determined by GC. The contents variability of the main FAs was observed in the selected genotypes. The developed and optimized GC method of determination was validated. The possibility of analysis of the major FAs in the segments of rapeseed was confirmed as well as its possible application for the rapeseed genotypes selection with regard to the FA content.

Key words: rapeseed, fatty acids, gas chromatography, breeding

Acknowledgements: *This paper was supported by project QJ1510172 „The use of non-conventional source materials, biotechnological methods and effective procedures in line and hybrid breeding of winter oilseed rape“ from NAZV.*

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GENETIC VARIATION IN GLUTENIN PROTEIN COMPOSITION OF WHEAT VARIETIES AND ITS RELATIONSHIP WITH QUALITY

EDITA GREGOVÁ, SVETLANA ŠLIKOVÁ, DARINA MUCHOVÁ, CSABA LANTOS,
JÁNOS PAUK

Genetic variability of high molecular weight glutenin subunits at the *Glu-1* loci and presence of wheat-rye translocation in 34 varieties of European wheat *Triticum aestivum* L. was studied using sodium dodecyl sulfate polyacrylamide gel electrophoresis and acid polyacrylamide gel electrophoresis. The end use quality of wheat is mainly influenced by the composition of glutenin protein. Three alleles has been determined on *Glu1A* locus, allele 1 (a) in 19 genotypes (44.2%) allele 2* (b) in 10 genotypes (23.3%) null allele (c) in 14 genotypes (32.5%). On *Glu-1B* locus 7+9 subunit (b) in 17 genotypes (39.6%), 7+8 subunit (b) in 12 genotypes (30%), subunits 6+8 (d) in 7 genotypes (16.3%), subunits 6+8 (d) in 7 genotypes (16.3%), subunits 6+8 (d) in 7 genotypes (16.3%) subunits 17+18 (i) in 3 genotypes (6.9%) and subunit 7 (a) and subunit 20 (e) (4.6%) have been determined in only two genotypes. On *Glu-D1* locus allele 5+10 (d) in 34 genotypes (79%) and allele 2+12 (a) in 9 genotypes have been determined. In this research 17 different HMW glutenin subunit combination were determined. From these genotypes, 9 genotypes (20.9%) have taken highest *Glu-1 score* of 10 out of 10, indicating superior dough quality for bread making. The quality score value of 34 genotypes (79.1%) has been determined varying between 5 and 9. The allelic block *Gli-1B3*, the marker of rye translocation *IRS/IBL* as well as the marker of poor bread-making quality, was detected in 5 genotypes.

Key words: HMW-GS, *Glu-score*, *Triticum aestivum* L., bread-making quality

Acknowledgements: „This work was supported by the Slovak Research and Development Agency under the contract No. SK-HU-2013-0028”.

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CHARACTERIZATION OF GLIADIN AND HMW GLUTENIN SUBUNITS ALLELES AND THEIR RELATION TO BREAD-MAKING QUALITY IN COMMON WHEAT BREEDING LINES

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CSABA LANTOS

Electrophoretic analyses of glutenins and gliadins were applied during 2015 for investigation of 15 Hungarian and 18 Slovak wheat breeding lines, which were tested for agronomic traits in the trials under field conditions in breeding workplaces Malý Šariš and Szeged and to establish the co-operative work of Hungary and the Slovak Republic. The proteins of 33 wheat breeding lines were fractionated by SDS-PAGE and A-PAGE to determine their HMW glutenin subunit composition and 1BL/1RS translocation. On the basis of previously established relationships between individual subunits and measures of bread-making quality, *Glu-score* and *Rye score* were calculated for 33 breeding lines. Fourteen high molecular weight (HMW) - glutenin subunits (GS) were found, three belonged to *Glu-1A*, seven to *Glu-1B* and four to *Glu-1D* locus. The most commonly represented alleles were *Glu-1A1*, *Glu-1B7+9* and *Glu-1D5+10*. Three breeding lines reached maximum *Glu score* and *Rye score* 10. The obtained knowledge from this research helped breeders in appreciating genetic resources for hybridization programs to make high quality, healthy, adapted and high yielding genotypes.

Key words: *Triticum aestivum* L., HMW-GS, *Glu-score*, *Rye-score*, bread-making quality

Acknowledgements: „*This work was supported by the Slovak Research and Development Agency under the contract No. SK-HU-2013-0028*”.

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PREPARATION OF BIOLOGICAL MATERIAL OF ALPINE BELLFLOWER FOR CELLOMIC ANALYSES USING EXPLANT CULTURES

MARCELA GUBIŠOVÁ, DANIEL MIHÁLIK

Mountain ecosystems are an excellent locality for the examination of the impact of atmospheric pollutions. Although such localities are far from pollution sources, they are heavily exposed to atmospheric contaminants. Alpine bellflower (*Campanula alpina* Jacq.) was identified as a candidate for study of environmental contamination, because levels of heavy metals in its tissues exceeded normal levels. Such plant species could serve as a source of genes responsible for tolerance and hyperaccumulation of heavy metals, with potential use in phytoremediation technology. Cellomic analyses enable to obtain important information at all levels of the expression of genetic information. Seeking for specific genes requires preparation of treated plants under controlled laboratory conditions. Alpine bellflower is a plant with small dormant seeds. Seed germination may take several months under low temperature, but dormancy can be broken by using phytohormones such as gibberellic acid (GA). *In vitro* culture enables to treat seeds and multiply emerged plantlets to obtain enough plant material in aseptic and specifically defined condition without effect of any undesirable factors. Seeds of alpine bellflower were germinated on MS medium supplemented with 0, 100 and 250 mg/l GA₃. The highest germination frequency (62.5%) was observed on medium with 100 mg/l GA₃, while on other media seeds germinated only sporadically. To multiply plantlets under *in vitro* condition, basal MS medium or MS medium supplemented with 0.4 mg/l of cytokinin BAP (6-benzylaminopurine) and solidified with agar or Gelrite were used. Based on our result, Gelrite is not suitable for this species, because it caused, especially in combination with BAP, massive plant vitrification (41% of vitrified explants). Addition of BAP elevated multiplication coefficient from 2.89 (MS medium without BAP) to 4.45 per 6 weeks of cultivation. Tiny shoots appeared on BAP supplemented medium, so basal nutrient medium without cytokinin can be recommended for the next subculture to let shoots grow and root. Well grown plantlets were able to multiply again not only via newly formed shoots, but also by cutting the largest ones into several nodal segments. Roots were formed sporadically on 93% of plantlets during 6 weeks on basal MS medium.

Key words: alpine vegetation, *Campanula alpina*, micropropagation, seed dormancy

Acknowledgements: *This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0380-12.*

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CHARACTERIZATION OF SELECTED PARAMETERS IN UP-TO-DATE COLLECTION OF COMMON WHEAT PRESERVED IN PRAGUE GENE BANK

JIŘÍ HERMUTH, VÁCLAV DVOŘÁČEK, ZDENĚK NESVADBA

The main task of the Gene bank of Prague is to preserve and evaluate wide collections of different species of cultural plants in cooperation with the next 12 research institutes in the Czech Republic. The workplace directly focuses on cereals and wheat collections have dominant position here. The evaluation strategy of individual collections has been prolonged for 3 years recently. The winter wheat collection (*T. aestivum*) have included 250 accessions by the 2013 and covered one- and two-year cycle till this time. The accessions were obtained from 18 countries of Europe, Asia, USA and New Zealand. Approximately one third (82 accessions) including 4 check cultivars has already been evaluated for two years (2012–2013) and achieved results of selected field and grain parameters are presented here. The overwintering of the wheat set was in the range from 6 to 9 points. It indicates high ability of most wheats to survive the Czech winter conditions. The only one cultivar 'Severina' (BGR) was more sensitive (6 points). The lowest height of plant was confirmed in the three French cultivars 'Alezan', 'Menestrel' and 'Toison dor' (70–77 cm). On the contrary, the Russian cultivars 'Suzdalskaya 2' and 'Bezenchukskaya 38' were the highest (136–138 cm). Significant differences were identified in the time of flowering in ranges from 143 days ('Bayles' – USA, 'Rata' – NZL, 'Momcil' – FRA) to 156 days ('Nova' – SWE). The shortest time of ripening showed the cultivar 'Rata' (NZL) – 181 days. On the other hand the longest time was detected in the cultivar 'Zappa' (DEU) – 193 days. Thousand grain weight also significantly varied from 36 g in the cultivar 'Orator' (GBR) to 57 g in the cultivar 'Progres' (BGR). A high resistance to wheat leaf rust (*Puccinia triticina*) was generally detected in the Dutch, French, German and Bulgarian cultivars (totally 16 accessions). On the contrary, the cultivars 'Rata' (NZL) and 'Volontaire' (FRA) were the most sensitive (1 points). Fourteen accessions, including two Czech cultivars 'Golem' and 'Forhand', showed high resistance to the stem rust (*Puccinia graminis*). The cultivars 'Rata' (NZL), 'Zlatina' and 'Katja' (BGR) were more sensitive (3 and 4 points). Presumably, a new strain of the yellow rust of wheat (*Puccinia striiformis*) widely divided the tested wheat collection from very sensitive up to resistant cultivars as well. High resistant cultivars to powdery mildew (*Blumeria graminis*) were found in 18 cultivars of the French and German origin. The complex resistances to rusts and powdery mildew were identified in the French cultivars 'Boisseau' and 'Sankara' and the German cultivar 'Zappa'. The favorable weather (water) conditions contributed to achieve very high grain yields in 2013. For example, the converted grain yield was 12.29 t/ha in the English cultivar 'Istabraq' in this year. On the contrary, the high productivity of plants in 2013 reflected in lower crude protein content (by 2%) compared to 2012. In spite of the fact, several cultivars showed high grain parameter stability in crude protein and wet gluten content. It is possible to mention the Russian cultivars 'Suzdalskaya 2', 'Vasylyna' and the Bulgarian cultivar 'Zlatina' with crude protein oscillation in the range from 12 to 13%. Simultaneously, their wet gluten content always exceeded 25% in both years.

Key words: winter wheat, genetic resources, gene bank, phenotyping

Acknowledgements: *This work was supported by National Programme on Conservation and Utilization of Plant, Animal and Microbial Genetic resources (Projects no. 206553/2011-MZE-17253) and the Ministry of Agriculture of the Czech Republic (Projects no. RO0415).*

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SCREENING OF REGENERATION CAPACITY OF WHEAT GENOTYPES FOR GENETIC TRANSFORMATION

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Wheat is one of three most important cereals worldwide. Its production has risen every year. Wheat grains are a rich source of proteins and polysaccharides in human food. Current breeding efforts comprise mainly increasing of production capacity, nutritional value, biotic and abiotic stress resistance. Traditional and marker assisted breeding are unlikely to bring improvement, because of long-time requirement or limited gene-pool. Biotechnological methods, such as genetic transformation, are promising approaches to produce cultivars with improved qualitative and quantitative traits. Successful genetic transformation is largely dependent on *in vitro* regeneration ability of tissues. In wheat, the efficient regeneration system is a key factor, because of its recalcitrance to *in vitro* cultivation. Genotype is the primary factor influencing plant regeneration of wheat. The explant source, media composition and donor plant material also affect tissue cultures. The objective of this work was to screen Slovak wheat varieties with high regeneration capacity suitable for transformation experiments. Donor plants were pot grown under natural conditions. To identify responsive genotypes we tested 9 Slovak (Ilias, Ilona, Torysa, Venistar, Madejka, Víglanka, PS Pintta, IS Karpatia, Astella) and 2 good regenerating (Bobwhite, CY 45) cultivars. Immature scutella, as the most widely used explants, were cultivated at media based on MS salts, optimized for wheat callus induction and plant formation. Frequency of callogenesis (% of explants forming callus from plated explants) and regeneration (% of calli formed shoots from plated explants) was evaluated. All genotypes were able to produce callus. The lowest frequency was recorded at Ilona (19.16%), high values at Ilias, Madejka and Víglanka, and 100% at Venistar and CY 45. Regeneration of shoots was achieved in 6 out of 11 genotypes tested. Cultivars Ilona, IS Karpatia, PS Pintta, Torysa and Venistar didn't regenerate. The lowest effectivity showed Astella, the highest one Ilias (78.3%; 7 shoots per explant plated). Madejka, Víglanka and Bobwhite regenerated in about 15%, CY 45 in 20.8%. Morphogenic ability was independent of the callogenic capacity. Our results indicate the ability of local cultivar Ilias to produce high amount of regenerants and are useful in subsequent transformation experiments.

Key words: wheat, *in vitro* regeneration, immature embryo, genotype

Acknowledgements: *This work was supported by the Slovak Research and Development Agency under the grants No. APVV-0294-11 and APVV-0662-11.*

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ADAPTABILITY OF GRAIN SORGHUM'S VARIETIES (*SORGHUM BICOLOR* L. MOENCH) IN THE CONDITIONS OF THE EASTERN LOWLANDS

LADISLAV KOVÁČ, JANA JAKUBOVÁ

Three years experiments with sorghum (*Sorghum bicolor* L. Moench) have been made on heavy soils of Eastern lowlands. In the experiment five varieties of sorghum were compared with the maize hybrid with FAO 300. Adaptability of grain sorghum bred in Hungary was investigated in the conditions of heavy soils of Eastern lowlands and its grain yield was compared with yield of maize. Yield of maize was 9.99 tons per hectare in average of the years. Crop of grain sorghum were significantly lower and ranged between 3.57 to 5.41 tons per hectare. The experimental results were statistically evaluated by analysis of variance and statistically significant differences in the yield were compared. The significantly highest yield of grain sorghum was reached at variety 24/08 – it was 5.41 tons per hectare. Variety Jami achieved yield 4.57 tons per hectare and variety 28/08 achieved 4.50 tons per hectare. These yields were significantly higher than ones of varieties GK Emesse (3.57 tons per hectare) and Alföldi I (3.57 tons per hectare). The experiment results confirmed that the grain sorghum's yield in terms of the Eastern lowlands are significantly lower than the yield of grain corn with FAO 300. Because of its resistance to drought, the grain sorghum may complete a range of grown crops in Slovakia in terms of global warming. On the basis of the results, the most suitable varieties in conditions of heavy soils are 24/08, 28/08 and Jami.

Key words: grain Sorghum (*Sorghum bicolor* L. Moench), maize, yield, adaptability, heavy soil

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BIODIVERSITY CONSERVATION THROUGH GRAZING WITH HORSES IN THE “MURÁNSKA PLANINA NATIONAL PARK”

JANKA MARTINCOVÁ

Over 2012–2014, a project was performed within the “Hungary – Slovakia Cross-border Cooperation Programme 2007–2013” (www.husk-cbc.eu) in the “Muránska planina National Park”. The project title was “Biodiversity conservation through grazing with a herd of *Hucul* horses and using the traditional grassland management techniques – HUSK/1101/2.2.1/0065”. The project aimed at possibilities for utilization of permanent grassland by grazing with horses, considering the aspect of preserving the biodiversity of pasture swards and applying the traditional methods of grassland management. The overall objective is to care for the natural environment through the Hucul horses breeding and to maintain these horses in their original standard. The Lead Partner of the project was “Občianske združenie Zbojská“ (*Zbojská* Civic Association) in cooperation with the project partners: “Štátna ochrana prírody SR – Správa Národného parku Muránska planina” (State Nature Protection Agency – Administration of the Muránska planina National Park), the Hucul Club at Sihla and the Hungarian partner Bükkvidék Természetvédelmi Közalapítvány. The field research was carried out to study effects of the horse grazing on the botanical composition of sward and to compare the situation at grazing and under cutting. Moreover, the parameters of dry matter production and forage quality as well as the etiological behaviour of horses were studied. The research showed notable effects of grazing on the botanical composition, but overgrazing leads to changes in the species composition of indigenous communities.

Key words: horse breeding, *Hucul* horses, biodiversity conservation, Muránska planina National Park

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SENSITIVITY OF *VENTURIA INAEQUALIS* POPULATIONS TO QOI, DMI AND MULTI-SITE FUNGICIDES IN SIX ORCHARDS IN SLOVAKIA

SYLVIA MATUŠÍKOVÁ, KAMIL HUDEC

In 2014, a study was conducted to evaluate sensitivity of *Venturia inaequalis* to the QoI fungicides, DMI fungicides and multi-site MOA fungicides. Research was carried out in three Slovak orchards under integrated and three under ecological management. Decreased sensitivity to each active compound was found in all orchards. Highest fraction of spores with lowered sensitivity to dithianone at concentration 2 mg/L of active compound was detected in orchards with integrated management and long history of single fungicide use, therefore application of these fungicides should be reduced according to FRAC recommendations. Proportion of less sensitive spores was lower in orchards with ecological management. No date-related differences in sensitivity were determined.

Key words: dithianon, DMI fungicide, dodine, germ tube elongation, QoI fungicide, spore germination, *Venturia inaequalis*

Acknowledgements: *This study was carried out with support from the scientific project of Scientific Grant Agency of The Ministry of Education, Science, Research and Sport of the Slovak Republic No. 1/0539/15*

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USING IMAGE ANALYSIS FOR MORPHOMETRIC EVALUATION OF THE POPPY SEED

DARINA MUCHOVÁ, TOMÁŠ KALATA, JANKA NÔŽKOVÁ, MICHAELA HAVRLENTOVÁ

In cooperation between the NAFC – Research and Breeding Station at Malý Šariš and Faculty of Agrobiolgy and Food Resources of the SUA in Nitra, there were evaluated genetic resources of poppy in the field and laboratory conditions. Six poppy genotypes (3 registered cultivars, 2 landraces and 1 breeding material) were characterized for 24 quantitative and qualitative traits on plants, capsules and seeds in 2014. Novelty in this area, image analysis for evaluation of the morphometric parameters of seeds and the structure of the seed surface at full maturity was used. Seed analysis was performed by using microscope CARL ZEISS DISCOVERY V20 capable to obtain digital video recordings. The morphometric evaluation was done using a software package for image analysis Axio Vision Rel. 4.8. Image analysis of seed size in parameter area (mm²) showed low to medium variability from 9.89% to 12.49%, but in the parameter area (mm²) on seed surface structure it showed to have medium to high variability (16.11% to 20.49%). Statistically significant differences using ANOVA ($p < 0.05$) were observed among experiment files obtained by image analysis of six genotypes of seed poppy. For seed size, parameters such as area (mm²), boundary width (mm) and height (mm), diameter (mm), Feret maximum (mm) and Feret minimum (mm) were selected. Statistically significant difference was confirmed in area and Feret maximum between genotypes: MSGZ-4 – MSGZ-3, Orfeus, and Opal. In Feret minimum, statistical difference between genotypes MSGZ-4 – Orfeus, MSGZ-3 and Orfeus – MSGZ-3 was detected. In boundary height and width, no statistically significant differences among genotypes were observed. The structure of the seed surface was evaluated based on the size of hexagonal pads on the furrowed surface and selected characters were used: area (mm²), boundary width (mm) and height (mm) and perimeter (mm). Opal statistically significantly differs from Major genotype in all evaluated traits. Statistically significant difference was found between genotypes Major – MSGZ-4, MS 423 and Orfeus – MS 423 in the parameter boundary width. Major statistically significantly differs from the other genotypes also in hexagonal perimeter pads. Variability among genetic resources is a good assumption for their potential use in breeding process of poppy.

Key words: poppy, genotypes, seeds, image analysis, morphometric analysis

Acknowledgements: The work was supported by the Project APVV-0248-10 from the Ministry of Education of the Slovak Republic.

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MICROPROPAGATION OF *THYMUS VULGARE*

BOŽENA NAVRÁTILOVÁ, MICHAELA ŠVÉCAROVÁ, VLADAN ONDŘEJ

Thyme (*Thymus vulgaris*) is one of the important aromatic and medicinal genera of the family *Lamiaceae* and one of the best known historical medicinal and spice plant. Stems include plurality of active ingredients of essential oils (including thymol, camphor, cineol, carvacrol, borneol, flavonoids etc.) with antibiotic and antiseptic effect in particular against microorganisms, and also with strong antifungal effect. The aim of this work was to develop effective protocol for the *in vitro* propagation to obtain a large number of identical plants. The explants (2–3 cm long) were isolated from apikal parts of donore plant, washed under running water for 3 hours. Then they were blotted dry on filter paper and subsequently sterilized in a beaker placed on the shaker 80–90 rpm. Surface disinfection was performed in 70% ethanol 1 min, 5% Chloramine-T 20 min and rinsing (3 times) in sterile distilled water. The segments were cut into 1 cm long shoot tips, placed onto hormon free MS (Murashige-Skoog, 1962) medium and cultured for 5 weeks in a growth chamber. For our experiments only non contaminated and growing explants were used. The shoots 23 cm long were placed into Erlenmayer flasks with 30 ml (5 shoots in the flask) with basal medium MS supplemented with 6-benzyladenine (BA: 0.01; 0.1; 1.0 mg/l) in combination with indole-3-butyric acid (IBA: 0.01; 0.1; 1.0 mg/l) and were cultured in a growth chamber at $22 \pm 2^\circ\text{C}$ with photoperiod of 16 h light/8 h dark. In each experiment 30 shoots were utilized. The highest proliferation (after 7 weeks in culture) was obtained on medium supplemented with 1.0 mg/l IBA and 1.0 BA, namely 6.3 shoots produced per explant, on 0.1 IBA and 0.1 BA it was 3.1 shoots and on 0.01 IBA and 0.01 BA only 1.4 shoots per explant. The shoots were rooted on hormon free MS medium and 71.6% of plants had 3–5 roots after 5 weeks in culture. *In vitro* plants were good growing and rooting, abnormalities or vitrification of shoots were not observed. Method of micropropagation leads to obtain sufficient plant material for further experiments, in which we want to use method of *in vitro* polyploidization utilizing oryzaline, and try to increase the content of biologically active substances in plants, in our case in thyme.

Key words: *Thymus vulgaris*, thyme, micropropagation, 6-benzyladenine, indol-3-butyric acid

Acknowledgements: This research was supported by the grant QJ1510160 (NAZV, Ministry of Agriculture, Czech Republic).

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BREEDING OF NEW GENOTYPES FOR DWARF HOPS IN CZECH REPUBLIC

VLADIMIR NESVADBA, JITKA CHARVATOVA, LUCIE STEFANOVA

Hop breeding aimed at new genotypes suitable for growing on low trellises in Czech Republic has been solved since 2008. Within Eureka program we studied dwarf hops in the cooperation with our English colleagues since 2011 to 2014. During this project we managed to carry out 24 crossings, which resulted in 75,502 seeds. Saazer was used as the standard of the best aroma quality as a female plant whereas English dwarf male hops were used as a father plants. They have been the donor of genetic dwarfishness as well as tolerance to fungal diseases. Since 2011 we have evaluated 22,327 plants in breeding trials placed on 6 ha of low trellises. We have selected 91 perspective genotypes, which represent only 0.41% of the origin tested array. On the base of detail description, determination of quantitative and qualitative characteristics, statistical assessment and productivity we have selected twelve very perspective genotypes, which were transferred into registration trials. Brewing tests were carried out with these selected genotypes to find out their suitability for production of high quality beers. Pilot plots have been established to confirm appropriateness of the selected genotypes for practical growing. The crop from these plots will be used for brewing tests. Specific hop protection in low trellises aimed at problems with downy mildew and especially with spider mites (*Tetranychus urticae* Koch) was a part of the project. A great many genetic analyses were made to try to find out markers of a dwarf gene. At present these genotypes are propagated for planting in two production trials, which will be started in 2015 in two farms in CR. Registration of the first Czech dwarf hop cultivars suitable for growing in low trellises is expected in 2017 and 2018.

Key words: *Humulus lupulus* L., hop breeding, perspective genotypes, low trellis, dwarf hops

Acknowledgements: *This work was supported by Czech Ministry of Education within the Research Project EUREKA no. LF 11008. Genetic resources, is a part of "National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity" (MZe 33083/03-300 6.2.1) issued by Czech Ministry of Agriculture.*

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ANALYSIS OF THE SPECIES DIVERSITY OF EARS PATHOGENS IN SLOVAK WHEAT FIELDS

MARTIN PASTIRČÁK

Wheat (*Triticum aestivum* L.) is the most important cereals crop of Slovakia and the source of primary inoculum in wheat systems are still not well documented in disease transmission. A survey was conducted for the assessment of ear blight of wheat in main wheat cropping areas in Slovakia in 2013. Main goals were to describe fungi that sporulate directly on winter wheat ears including glumes. Samples of ears were collected at the maturity stage of wheat crop from 118 locations of 8 districts and wheat ears with glumes were examined for presents of fungal reproduction structure and photographed under an Olympus SZ61 stereomicroscope and an Olympus BX51 light microscope. The taxonomic position of fungi were determined by morphological analysis. Ear rot and blight is a major biotic constraint of wheat in Slovakia. The disease occurs as a complex of glume blotch and head blight caused by *Stagonospora* spp. and *Fusarium* spp., respectively. The pathogen attacks leaf blades, sheaths and spikes, and lesions have been found among the florets, suggesting the possibility of this fungus being seed-borne in wheat. The mycoflora of winter wheat ears consisted primarily of *Deuteromycetes* (17 genera), *Ascomycetes* (8 genera) and *Basidiomycetes* (2 genera). The frequency of occurrence of fungi on winter wheat ears in examined samples was as follows: *Monographella nivalis* (23.7%), *Mycosphaerella graminicola* (5.9%), *Phaeosphaeria* sp. (1.7%), *Pyrenophora tritici-repentis* (13.6%), *Bipolaris sorokiniana* (3.4%), *Botrytis cinerea* (39.8%), *Colletotrichum* sp. (8.5%), *Drechslera tritici-repentis* (25.4%), *Microdochium nivale* (2.5%), *Nigrospora* sp. (2.5%), *Papularia arundinis* (13.6%), *Periconia* sp. (2.5%), *Phoma* sp. (11.0%) and *Stagonospora avenae* (8.5%). The species from genus *Alternaria* (64.4%) and *Cladosporium* (58.5%) belong to common saprophytic mycoflora. The species from genus *Fusarium* (83.9%), *Stagonospora* and *Zymoseptoria* belong to parasitic mycoflora. *Stagonospora nodorum* (68.6%) and *Z. tritici* (34.7%) were occurring with high frequency. The species *Ascochyta tritici* was determined on winter wheat ears in collected samples with low frequency (6.8%). In the 2013 phytopathogenic fungi as *Gibberella zeae* (39.0%), *Phaeosphaeria nodorum* (17.8%) and *Ph. avenaria* (8.5%) on ears of winter wheat were recorded. The species *Epicoccum purpurascens* was abundant sporulating and identified in the middle or over margin of ears glume of winter wheat in 23 collected samples with different percentage of occurrence. From group of obligate, biotrophic parasites fungi two species were identified: *Blumeria graminis* (10.2%) and *Puccinia recondita* (10.2%). Fungi, which directly participate in deformed grains, *Tilletia* sp. (14.4%) and *Claviceps purpurea* (1.7 %), were also detected. Grain is replaced with bunt balls containing masses of black spores or survival structure the ergot sclerotium. They belong to the fungi that cause large economic losses, especially *Tilletia* sp., and pose a risk of contamination of the soil for long periods. The results of this study showed that a considerable proportion of the winter wheat ears were affected by a variety of disease symptoms caused by fungi.

Key words: *Triticum aestivum*, fungi, diversity, ear rot, *Gibberella*

Acknowledgements: This study was supported by the Ministry of Agriculture and Rural Development of the SR, Research and Development project „Genetic improvement of farm traits of important crops“.

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GENOTYPING OF FLAX GENETIC RESOURCES BY MIRNA-BASED MOLECULAR MARKERS AND MORPHOLOGY

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MicroRNAs (miRNAs) are a type of small non-protein coding regulatory RNAs. DNA-based markers corresponding to miRNA genomic sequences are suitable for development of functional marker as microRNA. DNA sequences are common in the genomes of plants. A miRNA-based molecular marker is a type of functional marker exploited so far in very few plants species. For the analysis of 15 genotypes of flax with different geographical origin we applied miRNA-based primers designed from the sequences of two different microRNA molecules, miR408 and miR156b. MiRNA-based primers produced clear bands and the amplified length of most PCR products obtained by miR408-based primer ranged from 40 bp to 300 bp. This type of miRNA-based primer was capable to distinguish some of the analyzed genotypes. The results of molecular analysis were also supported by morphological analysis. Selected genotypes differ in morphological traits of buds, flowers, capsules and seeds depending on geographical region from which they come.

Key words: microRNA, molecular markers, morphology, origin, flax

Acknowledgements: *This research was supported by the project “ECOVA” (ECPUA — Excellent Centre of Protection and Use of Agrobiodiversity) under project no 26220120015 and by European Community under project no 26220220180: Building Research Centre “AgroBioTech”.*

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IMPORTANCE OF RECORDING OF OLD AND REGIONAL FRUIT VARIETIES IN SELECTED LOCATIONS

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Importance of old and regional varieties particularly their conservation, presents many possibilities for their use. The advantage is the high adaptability to climatic conditions, especially in marginal areas. Many local varieties are treated as local speciality and characterizing national identity of the country and the region. The significant aspect is the preservation of the gene pool of fruit trees varieties for a wide area of breeding. There is also possible use in organic cultivation. At selected locations Svitavsko surveying and mapping the occurrence of old fruit trees was realized. Listed trees were compared with the recommended species and varietal composition of this region. Based on the literature and recommendations of witnesses 5 locations were selected for recording: Hradec nad Svitavou - alleys of dirt road, Vodárenská Svitavy s.r.o. – fruit orchard, Gardening area Svitavy, Koclířov - monastic fruit orchard and area of the former village Muzlov. In selected areas the work was focused on the graphic record, pomological determination, followed by the evaluation of growth, health status and harvest values. Varietal composition according to the zoning focuses on the predominant varieties. For apple trees to the collection of the prevailing varieties were included 'Boskoopské', 'Matčino', 'Strýmka', 'Wealthy', for pear-trees 'Červencová pestrá', 'Hardyho', 'Salisburyho', 'Špinka', for cherry-trees 'Germersdorfská', 'Kaštánka'. In orchards predominate varieties 'Strýmka', 'Wealthy', 'Vilémovo', 'Matčino', 'Boskoopské', 'Croncelské', 'Panenské české'. The attention deserve varieties 'Rozmarýnové', 'Panenské', 'Halberstatské', 'Ribstonské'. The volume of the crown of variety 'Vilémovo' reached 162.26 cubic meters.

Key words: old varieties, pomological determinations, field investigations

Acknowledgements: *The study was based on the programme of applied research and development of the national and cultural identity (NAKI) DF11P01OVV006 "Preservation and the cultural heritage of historical Bohemian and Moravian fruit varieties and other traditional and forgotten crops".*

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OCCURRENCE OF OLD VARIETIES OF POME FRUIT IN THE TIŠNOV REGION

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The objective of the study was to record the incidence of old varieties of pome fruit in some localities and to evaluate their growth properties and health status; at the same time to evaluate the endangered and oldest apple and pear trees. Samples were taken continuously and after short storage sensory evaluations were carried out. The focus was on fruit tree orchards, home fruit gardens, farmsteads, alleys, trees along roads and field orchards in cadastral areas of some communities of the Lomnice, Tišnov and Nedvědice regions. We recorded the growth parameters – volume of crown, diameter of the tree trunk, data of the health status and our own classification and pomological description of the variety. The variety 'Blenheimská reneta' ('Blenheim Orange') had the largest volume of the crown (276 m³); the crown volumes of the varieties 'Krasokvět žlutý' ('Yellow Bellflower'), 'Vilémovo' ('Kaiser Wilhelm') and 'Panenské české' ('Pomme des Vierges') were also very large (253 m³, 248 m³ and 207 m³, respectively). Trees of the varieties 'Strýmka' ('Bohnappel') (91 m³), 'Baumannova reneta' ('Baumann's Reinette') (99 m³) and 'Kardinál žíhaný' ('Geflammtter Weisser Cardinal') (104 m³) had the smallest volumes of crowns. Trees of the variety 'Smiřické vzácné' had the best total health status (7.4 points); the varieties 'Míšeňské' ('Edelsborsdorfer'), 'Krasokvět žlutý' ('Yellow Bellflower'), 'Jadernička moravská' and 'Vilémovo' ('Kaiser Wilhelm') scored 7 points. Trees of the variety 'Strýmka' ('Bohnappel') scored the least points for the health status (5.4 points). Pear trees of varieties 'Charneuská' ('Fondante de Charneus') and 'Muškatelka šedá' ('Musqué Grise de Bohême') had the largest volume of the crown (133 m³ and 131 m³, respectively). The varieties 'Křivice' ('Clairgeau de Nantes') and 'Williamsova čáslavka' ('Bartlett') had the smallest crown volumes (36 m³ and 79 m³, respectively). Trees of the varieties 'Williamsova čáslavka' ('Bartlett') and 'Clappova máslovka' ('Clapp's Favourite') boosted the best overall health status (7.8 points and 7.2 points, respectively). The worst health status was that of trees of the varieties 'Děkanka Robertova' ('Doyenné de Comice') (6.0 points), 'Křivice' ('Clairgeau de Nantes') (6.2 p) and 'Muškatelka šedá' ('Musqué Grise de Bohême') (6.3 p). The apple variety 'Velník' reached an age of 200 years in Křížovice; the variety 'Kanefl' in Křížovice 150 years. Old pear varieties on seedy rootstocks reach a high age. The age of the pear variety 'Václavka' in Zvole was over 200 years and of the variety 'Neznámka' in Žernůvka it was 195 years.

Key words: old varieties, pomological determinations, field investigations

Acknowledgements: *The study was based on the programme of applied research and development of the national and cultural identity (NAKI) DF11P01OVV006 "Preservation and the cultural heritage of historical Bohemian and Moravian fruit varieties and other traditional and forgotten crops".*

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SELECTION OF *RPH7* GENE AND EFFECTIVE *MLA* ALLELES IN BARLEY USING DUPLEX MOLECULAR MARKER

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The most important fungal diseases of barley are leaf rust caused by *Puccinia hordei*, and powdery mildew caused by *Blumeria graminis* f. sp. *hordei*. Optimal way to avoid losses caused by these diseases is growing resistant varieties. Concerning practical breeding for powdery mildew and leaf rust resistance, simple, cheap and robust selection methods are required. Marker assisted selection (MAS) is of great potential to fulfil this demand. We present development of a robust duplex marker for simultaneous selection of *Rph7*, and presence of one of the following *Mla* alleles: *Mla16*, *Mla19*, *Mla20*, *Mla21*, *Mla27* or *Mla28*. Ecotilling marker was converted to more breeder-friendly CAPS, and added simultaneous selection for *Rph7* gene in a duplex reaction. Cleavage by *RsaI* endonuclease was shown to be robust enough, and specific for the resistant phenotype, so it is possible to use it for marker assisted selection of effective *Mla* alleles. PCR and cleavage of its products by *RsaI* endonuclease can be successfully run in duplex for effective *Mla* alleles and *Rph7* gene brings along the possibility to select the tracked genes more effectively. This duplex marker can be used in pyramiding strategies in common barley breeding programs.

Key words: leaf diseases, powdery mildew, rust, marker assisted selection

Acknowledgements: *The work was supported by the Ministry of Agriculture of the Czech Republic, Projects QJ1310091 and RO0211.*

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ASSESSMENT OF HLVD PRESENCE IN RECOVERED SAAZER

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Viroids are non-protein encoding, single-stranded RNA molecules, currently considered as the smallest plant pathogens. They cause diseases in economically important crops. Between plants, viroid movement occurs predominantly by mechanical transmission (i.e. physical contact with contaminated sources, such as infested pruning gear). Viroids belong to the pathogens causing important economic losses of quality and quantity of hop cones. HLVD is worldwide distributed in hops, usually without visual symptoms of infection. Dissemination of HLVD in hop gardens is very fast. Assessment of hop plants from hop gardens planted with Saazer – Osvalds' clones no. 31, 72 and 114 was carried out. This monitoring was aimed at the presence of HLVD. These plants are from planting material within the recovery program of Czech hops. The samples were taken from 27 hop gardens in Saaz (Žatec) and 12 hop gardens from Auscha (Úštěk) hop growing region. Original varieties and old hop gardens of Czech hops were completely infected by HLVD. Hop cones yield was reduced by 5-8% and alpha acids by 20-40%.

Key words: hop, *Humulus lupulus*, L., hop gardens, HLVD

Acknowledgements: *This work was supported by Grant Agency of CR in project 13-03037S and by Technological Agency of CR in Centre of competence TE02000177.*

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NATURAL OCCURRENCE OF DEOXYNIVALENOL IN GRAINS OF OATS PRODUCED BY SLOVAK FIELDS

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The deoxynivalenol (DON) is probably the most frequently detected mycotoxin B-trichothecene in grain cereals. It is produced by fungi *Fusarium* spp. which can attack the spike of cereals during growing. The symptoms of infection of oat panicle and groats by *Fusarium* spp. are not clearly visible, therefore the information on oat infection by *Fusarium* spp. is limited. There a lot of the infection of groat and oat contamination with mycotoxins appears quite frequently in climatic conditions favourable for *Fusarium* diseases development. There is more information about natural occurrence of DON in grains of wheat but little of DON content in grains of oats growing in Slovakia. The oat grains were obtained during the 2013 and 2014 growing seasons and were collected directly from growers from locations in Slovakia. These are: Dargov, Most pri Bratislave, Poltár, Plavnica, Prašice, Podolie, Michalovce, Valča (in 2013) and Podolie, Plavnica, Bottovo – Rimavská Sobota, Čataj, Hybe, Malcov, Stropkov, Kežmarok and Prašice (in 2014). The incremental samples were mixed and put in paper bags and stored in a cool place (cca. 2000 g). After grinding the full lot sample, a subsample (5 g) was taken for analysis. A commercial ELISA kit was used to determine the DON concentration in samples with the limit of detection < 0.2 mg/kg (ppm) and limit of quantification 0.2 mg/kg (ppm). The occurrence of samples with DON was 30% in 2013, with maximum DON content of 0.49 mg/kg, and 40% in 2014, with maximum DON content of 0.60 mg/kg. The total mean DON content was 0.18 mg/kg. The limit of 1.75 mg/kg imposed by the European Union (EU) (setting maximum levels for certain contaminants in foodstuffs by EC No 1881/2006) for DON content was not exceeded in the studied samples. The present results indicated that DON content was at a low level in grains from oats grown during 2013 and 2014.

Key words: mycotoxin, *Avena sativa* L., locations

Acknowledgements: *This work was supported by OP Research and Development: Development of new types of genetically modified plants with farm traits (ITMS 26220220189), by the European Regional Development Fund and by the Science and Research Support Agency (No. APVV- 0398-12) of the Slovak Republic.*

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CREATION OF NEW ELITE GENOTYPES WITH IMPORTANT RESISTANT GENES BY USE OF GENETIC MARKERS

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EDITA GREGOVÁ, VALÉRIA ŠUDYOVÁ

Raising of plant resistance against major pathogens by incorporating effective resistance genes is the most appropriate tool how to control the occurrence of diseases, spreading of pathogen, and how to support the reduction of chemical sprays. The genetic markers deployed to assist selection for disease resistance currently help to increase the efficiency of the process of incorporation of effective resistance genes into new breeding lines. Selection of plants based on genetic markers has many advantages over classical phenotypic selection, because it allows selecting plants without the presence of selection environment. The method is suitable for characters which are hardly detectable by conventional methods, and/or traits significantly influenced by environment and also showing up in the late stage of plants. We have cooperated with a commercial plant breeding stations for some years. These have expressed interest in obtaining breeding lines with genes resistance against important pathogens. Breeding program is realized of means backcross breeding with the use of molecular markers.

Key words: wheat, tomato, barley, marker assisted selection, resistant gene

***Acknowledgements:** This work was supported by OP Research and Development: ITMS 26220220142.*

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BARLEY – VIRUS DISEASE AND PROTECTION

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Barley yellow dwarf (BYD) is the most distributed and the most economically important virus disease of spring and winter barley. Barley yellow dwarf luteoviruses are not transmitted mechanically but by aphids. Protection is based on reduction of the vector incidence using application of the insecticides. Nevertheless, this increases the cost of cultivation and adversely affects the environment. Barley mosaic virus complex (BaYMV, BaYMV-2, BaMMV) attacks winter barley mainly in the western Europe and East Asia. Protection with chemicals against the virus itself is not possible, virus particles are transmitted into the root cells of susceptible plants via - the fungal vector *Polymixa graminis*. Therefore, an effective anti-virus strategy is protection based on efficient resistance genes and targeted transfer of these genes including molecular marker based selection. Our research program was aimed to creation of spring and winter barley lines possessing the gene *Ryd2* effective against barley yellow dwarf virus by the conventional hybridization. Genes *rym4* and *rym11* are still effective against BaYMV / BaMMV-2 and these genes were introduced into donor genotypes. Our strategy was to achieve more durable and broad-spectrum resistances by combination of different genes against the same pathogen, but also different pathogens in one breeding line. For this reason, barley lines with gene combinations *rym4* + *rym11* and *rym11* + *Ryd2* were developed. Marker-assisted selection (MAS) has been proved as a valuable tool for different resistance genes in each created genotypes. The lines are currently at various stages breeding.

Key words: barley, BaYMV/BaMMV, BYDV, resistant gene, marker assisted selection

Acknowledgements: *This work was supported by the Ministry of Agriculture and Rural Development of the Slovak Republic through the project Genetic improvement of the economic characteristics of crop.*

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EFFECT OF SIMULATED MICROGRAVITY IN GENE EXPRESSION DURING EMBRYOGENESIS

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Gravity is one of the environmental factors that controls development and growth of plants. The microgravity has been demonstrated to have inevitable impact on living organisms during space flights and should be considered as important factors for estimating the potential health risk for astronauts. During the life, plants are exposed to various kinds of stresses both abiotic and biotic. The changes in gravitational field strengths are most probably another type of stress, which is transduced by signaling chains. The embryos of model system *Arabidopsis thaliana* (cv. Columbia) were used for study in gene expression as a response to altered gravitational field. *Arabidopsis thaliana* was chosen due to its extensively studied and catalogued genome, as well as its germination and growth period. To study effects of microgravity, a laboratory instrument 3D-clinostat (generally called random positioning machine or RPM) was utilized. The studied genes are involved in molecular analysis of embryogenesis, cell proliferation and oxidative stress. In the current study, we examined transcription of *Arabidopsis thaliana* embryos genes that include Short Integument (SIN), Kanadi (KAN), Proliferating Cell Nuclear Antigen (PCNA), Late Abundant Protein (LEA), Catalases (CAT), Superoxid dismutases (SD) and Lipoxygenases (LOX) after 2 days of 3D clinorotation treatment. The control samples (embryos) were incubated at the same conditions as model ones only without RPM. The technique of RT-PCR was applied to determine the expression of studied genes of *Arabidopsis thaliana*. First, total RNA was isolated and treated using DNase. Second, the isolated RNA was transcribed to cDNA according to the manufacturer's protocol using anchored-oligo(dT)₁₈ primers. Finally, the qRT-PCR was carried out on LightCycler Nano Real – Time PCR System (Roche Diagnostic Corporation). We identified seven genes (KAN, LEA, PCNA, CAT2, CAT3, LOX1 and LOX2) which increased transcript levels after 48 hours of exposure to microgravity. Others mentioned genes such as superoxid dismutases and short integument (SIN) tested with the 3-D clinostat did not show a significantly different expression as compared to the respective control. We have concluded, that modeled microgravity using RPM can alter some transcriptional activity of studied genes in *Arabidopsis thaliana* connected with lateral organ identity and auxin transport (KAN), cell division (PCNA), embryo maturation (LEA) and stresses (CAT and LOX genes). We assume that plants under microgravity stress can alter production of secondary metabolites.

Key words: *Arabidopsis thaliana*, embryogenesis, gene expression, random positioning machine, simulated microgravity

Acknowledgements: This work was supported by the grant QJ1510160 (NAZV, Ministry of Agriculture, Czech Republic)

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THE MONITORING OF VARIABILITY OF ALKALOIDS IN POPPY STRAW DURING BREEDING OF POPPY (*PAPAVER SOMNIFERUM* L.)

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The Czech Republic is one of the world's leading producers of poppy (*Papaver somniferum* L.). Currently, the world's largest poppy producer is Turkey where white-seed cultivars are grown mainly, the seeds of which are intended for the Indian market in particular. In terms of blue-seed poppy cultivars for the primary use in the food industry, the Czech Republic is the world's leading producer, accounting for about 60% of the world's total production. The poppy growing has had a long-term tradition in the Czech Republic, and the Czech producers master large-scale production technologies. However, due to its content of alkaloids, morphine in particular, poppy is a valuable raw material for the pharmaceutical industry as well. The morphine content in poppy straw is affected by many factors. Among them, the genetic basis of the variety is the most important one. Therefore, the most efficient tool to intentionally affect this characteristic is a breeding. The assessment of the morphine content in poppy straw is an important part of the breeding process and registration procedure within the process of cultivar approval by the Czech Central Institute for Supervising and Testing in Agriculture. In addition, the final morphine content is affected significantly by the weather during the growth and ripening of poppy and, last but not least, agronomical operations or processes (such as nutrition, protection and harvest technology). Breeding and research of poppy is realized in organisation OSEVA Development and Research Ltd., Czech Republic. As part of these activities, content of alkaloids was analysed (morphine, codeine, thebaine) in poppy straw of 275 genotypes by high performance liquid chromatography (HPLC). Determinations were carried at the registered varieties from European catalogue, older and regional cultivars (maintained in the National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity) and breeding materials created by classical and mutational techniques. Identified concentration of morphine in the examined samples confirmed that the commonly grown and bred poppy in the Czech Republic have a low to medium content of morphine. The biggest proportion (70%) of the analysed genotypes had morphine in the interval 0.00–0.69% of dry matter of poppy straw. The highest concentrations were found in the varieties of Hungarian origin – Botond (1.52%), Buddha (1.54%), Postomi (1.88%) and Tebona (2.53%), and materials created by mutation breeding in OSEVA D&R Ltd. (1.89–1.99%). The contents of the other two alkaloids (codeine, thebaine) were usually very low. Average contents of codeine and thebaine were 0.16%, respectively 0.01%. The highest content of codeine (0.67%) had breeding material OP-P-14. The highest content of thebaine (0.20%) was found in breeding material OP-832.

Key words: alkaloids, poppy, HPLC, breeding

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BIOMASS ACCUMULATION AND TRANSLOCATION IN COMMON AND SPELT WHEAT

ALŽBETA ŽOFAJOVÁ, PAVOL HAUPTVOGEL

In grain crops, both current assimilation transferred directly into the kernels and remobilization of assimilates stored in vegetative plant parts contribute to grain yield. For the assessment of future breeding strategy in wheat it is important to identify sources of breeding progress. Understanding the relationships among grain yield and biomass can assist in achieving high yield through plant breeding and agronomic practice. Five common wheat cultivars (Slovenská B, Renan, PS Karkulka, Naturstar, Capo) and two spelt cultivars (Rubiota, Line 1/17) were evaluated in pot experiment in three replications and two harvests, at heading and at maturity in two vegetative years (2011/12, 2012/13). The purpose of research was to evaluate the dry matter accumulation and translocation in common and spelt cultivars. Spelt cultivars accumulated more dry matter at anthesis and maturity in vegetative parts compared to common wheat. The grain yield showed opposite tendency, in average common wheat cultivars exceeded spelt ones, but difference was not significant. Spelt cultivars had significantly lower harvest index (by 30%) suggesting a lower rate of partitioning of assimilates into the grain compared to common wheat. Plant height was significantly affected by cultivar in both years. Rubiota was by 43% taller compared to average of common wheat, but Line 1/17 was comparable to Slovenská B. Plant height was significantly correlated with total dry matter accumulation at anthesis ($r = 0.688^+$). Plant height is related to lodging tolerance which is permanent one of main goal of spelt breeding. For better spelt adaptation and cultivation, development of early maturing cultivar is necessary as evaluated cultivars were by 8 to 10 days later compared to common wheat. Our results indicated that spelt could be used as alternative crop. Actual interest is connected with increasing demand on unconventional foods and low-input agriculture.

Key words: common wheat, spelt wheat, accumulation, translocation, dry matter

Acknowledgements: Research was supported by the project APVV- 0197-10.

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DETECTION OF GENETIC DIVERSITY OF *PYRENOPHORA TRITICI-REPENTIS* ISOLATES

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Pyrenophora tritici-repentis (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) is the causal agent of tan spot disease of wheat, which is found in major wheat growing areas worldwide. This disease is very destructive and can cause yield losses from 3% to 53%. The study of genetic diversity in pathogen populations is important for evaluation of pathogen capability to rapidly respond to changing environments and to overcome host resistance and fungicides. For this reason, the aim of this study was to develop microsatellite (SSR) primers and use them together with five published RAPD primers to study genetic diversity in a group of isolates of different geographic origin, and to describe the relationship between them. In our case, eight SSR primer pairs were designed for assessment of genetic diversity in a group of 13 Slovak, 1 Czech and 10 Finnish isolates of *P. tritici-repentis*. After PCR optimisation, three SSR primers were monomorphic and five SSR primers showed polymorphism with 21 detected bands. The number of bands per primer varied from 8 to 2 with an average of 4.2 bands and average diversity index 0.454 per primer. By use of five published highly polymorphic RAPD primers in group of 24 *P. tritici-repentis* isolates, much higher polymorphism was found, i.e. 95 bands in total. The number of bands per primer varied from 16 to 23 with an average of 19 bands and an average diversity index 0.912 per primer. Dendrograms with Principal Component analysis based on SSR and RAPD data did not show association between genetic diversity of the isolates and their geographic origin, but proved the suitability of SSR and RAPD markers for genetic diversity studies of this pathogen. Knowledge of the pathogen genetic diversity helps in the development of a successful disease management, especially in the development of resistant cultivars, effective fungicides and biological control agents.

Key words: SSR, Random Amplified Polymorphic DNA, tan spot, *Pyrenophora tritici-repentis*

Acknowledgements: *This research was supported by projects 7AMB14SK198, SRDA project SK-CZ-2013-0175.*

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Title	New Knowledge in Genetics and Breeding of Agricultural Plants The 22 th International Scientific Conference
Editors	Mgr. Katarína Ondreičková, PhD. Mgr. Marcela Gubišová, PhD. Ing. Alžbeta Žofajová, PhD.
Cover design	Jarmila Poništová
Edition	1 th
Publisher	National Agricultural and Food Centre Hlohovecká 2 951 41 Lužianky
Year of publication	2015
Number of pages	47
Print	National Agricultural and Food Centre – Research Institute of Plant Production, Piešťany
Page format	CD
Impression	55 pcs

Not for sale / designated for own consumption.

This book of abstracts was carefully produced. Nevertheless, editors do not warrant the information contained therein to be free of errors.

P–ISSN 0551-3677

E–ISSN 1338-4376