PANNONIAN PLANT BIOTECHNOLOGY WORKSHOPS

THE BIOENERGY QUESTION: REALITY OR WISHFUL THINKING?

TULLN/DONAU

DEPARTMENT OF AGROBIOTECHNOLOGY IFA -TULLN

UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES,VIENNA(FORMERUNIV.BODENKULTUR)

BOKU

MAY 16TH – 18TH 2011

PANNONIAN PLANT BIOTECHNOLOGY ASSOCIATION

HUNGARIAN BIOGAS ASSOCIATION

MONSANTO

Martonvásár

ORGANIZERS

Peter Ruckenbauer ***Austria*** *and* *Ervin Balázs* ***Hungary***

This year workshop of the Pannonian Plant Biotechnology Association will be held at IFA Tulln Austria, between 16th and 18th of May 2011. We cordially invite scientists and experts from the region to give reports on their experiences with bioenergy, its economical social and scientific achievements. This subject will be presented by recognised experts in the region on first day of the conference. The second day will be devoted also for other significant research results on green biotechnology and plant breeding in general.

Invited speakers will include:

Miklós G. Fári (Debrecen),**Werner Fuchs** (Tulln), **Jiri Kopecky** (Trebon) **Kornél Kovács** (Szeged), **Csaba L. Marton** (Martonvásár), **József Popp** (Budapest) **Vince Ördög** (Mosonmagyaróvár) **Erwin Schmid** (Vienna)

The tentative schedule of the three days workshop

Arrival on Monday May 16th

Registration starts at 13.00 p.m. at IFA and the welcome reception will be hosted at 7.00 p.m. in the lecture hall of the IFA -Tulln.

Tuesday May 17

Workshop starts on 17 th of May at 9.30 and will be ended at 6 pm

Wednesday May 18

Workshops will continued on 18 th of May at 9.30 am till 12.30. The visit to the Bio - Ethanol Plant in Pischlsdorf /near Tulln will start at 14.00 pm.

May 18th departure after the visit.

Your abstract will be put into an abstract book so please provide it till **April 15 and send** to balazs@mail.mgki.hu
Organizers are providing publication possibilities for presented papers in the Acta Agronomica a peer reviewed scientific quarterly. Please bring your ms for the workshop and hand it to the organizers on CD.
Our local organizer prof. Peter Ruckenbauer and his secretary

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Participation support of PhD. students from Romania and from the Slovak Republic could be obtained from the organizers after applications. Supported students will be informed in due time. Please send your CV and your research interests for supporting your applications.

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PROGRAMME

16 May Monday

13.00 p.m. ***REGISTRATION***

14.00 p.m. ***WELCOME***
PROF. DR. PETER RUCKENBAUER, IFA TULLN

BIOENERGY SESSION I.

CHAIR: *PETER RUCKENBAUER*

14.15 p.m. *BERNHARD DROSG, WERNER FUCHS, GÜNTHER BOCHMANN* ***(AUSTRIA)***

BIOGAS - STATUS AND POTENTIAL IN AUSTRIA

14.45 p.m. *KORNÉL L. KOVÁCS, Z. BAGI, N. ÁCS, E. KOVÁCS, R. WIRTH, G. RÁKHELY* ***(HUNGARY)***

BIOHYDROGEN AND BIOGAS BIOTECHNOLOGY

15.15 p.m. *ERWIN SCHMID* ***(AUSTRIA)***

EFFECTS OF EU BIOFUEL TARGETS ON GLOBAL GHG EMISSIONS FROM LAND USE CHANGE

15.45 p.m. *JÓZSEF POPP* ***(HUNGARY)***

FOOD SECURITY AND BIOFUELS: TIME TO RE-EVALUATE BIOFUEL POLICIES?

16.15 p.m ***COFFEE BREAK***

16.30 p.m. *JIŘÍ KOPECKÝ* ***(CZECH REPUBLIC)***

BIODIESEL FROM MICROALGAE – MISSION IMPOSSIBLE?!

17.00 p.m. *VINCE ÖRDÖG, P. BÁLINT, CS. LOVÁSZ* ***(HUNGARY)***

LIPID PRODUCTION IN GREEN ALGAE DEPENDING ON N-SUPPLY

17.30 p.m. *ELENA RAKOSY–TICAN, RALUCA MUSTATA* **(ROMANIA)**

WHY NOT TRANSGENIC POTATO FOR BIOETHANOL PRODUCTION – THE CASE OF MARKER-FREE PVY RESISTANT CULTIVARS

19.00 p.m. *WELCOME RECEPTION IN THE LECTURE HALL OF THE IFA -TULLN*

17 May Tuesday

BIOENERGY SESSION II.

CHAIR: *IVO BRANTS*

9.30 a.m. *CSABA L. MARTON* ***(HUNGARY)***

CORN AS A RENEWABLE ENERGY SOURCE

10.00 a.m. *ANDREA HLINKOVÁ, MILAN ČERTÍK, MICHAELA HAVRLENTOVÁ*

LIPID QUALITY OF SLOVAK POPPY SEEDS AND THEIR PLACE ON THE WORLD-MARKET

10.30 a.m. *ZUZANA ADAMECHOVÁ, LUCIA GUOTHOVÁ, KATARÍNA ŠÁGYOVÁ, MILAN ČERTÍK* ***(SLOVAK REPUBLIC)***

SOLID STATE FERMENTATION AS A NATURAL WAY TO ENRICH CEREALS WITH POLYUNSATURATED FATTY ACIDS AND PIGMENTS

11.00 a.m. *MARCELA GUBIŠOVÁ, PAVOL PORVAZ, JOZEF GUBIŠ, ALŽBETA ŽOFAJOVÁ, DANIEL MIHÁLIK, JÁN KRAIC* ***(SLOVAK REPUBLIC)***

GROWING TECHNOLOGY AND BIO-TECHNOLOGY OF MISCANTHUS IN SLOVAKIA

11.30 a.m. *MIKLÓS G. FÁRI, LÁSZLÓ MÁRTON* ***(HUNGARY)***

SOME NEW BREEDING AND PROPAGATION STRATEGIES OF THE FUTURE BIOENERGY CROPS FOR CELLULOSE FARMING

12.00 p.m. ***LUNCH***

NEW ACHIEVEMENTS IN PLANT BREEDING AND GREEN BIOTECHNOLOGY

13.30 – 18.00 p.m. ***POSTER SESSION***

T. ALSHAAL, É. DOMOKOS-SZABOLCSY, L. MÁRTON, M. FÁRI AND J. KÁTAI ***(HUNGARY)***

PHYTOREMEDIATION POTENTIAL OF ARUNDO DONAX L "SYN-PLANTS" GROWTH IN HEAVY METALS CONTAMINATED SOILS

ÁKOS BOLDIZSÁR, ZSOLT GULYÁS, DÁNIEL CARRERA, GABRIELLA SZALAI, ILDIKÓ VASHEGYI, GÁBOR GALIBA, GÁBOR KOCSY ***(HUNGARY)***

CHROMOSOME 5A OF WHEAT AFFECTS GENE EXPRESSION AND THIOL LEVELS DURING VERNALZATION

ISTVÁN CSÓTI, AMBRUS BAKÓ, ESZTER KISS,CECÍLIA TAMÁS, LÁSZLÓ TAMÁS ***(HUNGARY)***

THE MOLECULAR BACKGROUND OF WHEAT HARDNESS: A LOOK AT STARCH GRANULE SURFACE ASSOCIATED PROTEINS

MAGDOLNA DŐRY, LÁSZLÓ BÖGRE, RÓBERT DÓCZI ***(HUNGARY)***

THE ROLE OF STRESS-ACTIVATED MAP KINASE PATHWAYS IN REGULATING PLANT DEVELOPMENTAL PROCESSES

ATTILA FÁBIÁN, KATALIN JÄGER, BEÁTA BARNABÁS ***(HUNGARY)***

EFFECTS OF DROUGHT ON KERNEL DEVELOPMENT OF STRESS SENSITIVE AND TOLERANT VARIETIES OF WHEAT

GYÖNGYVÉR GEL, ENDRE SEBESTYÉN, ERVIN BALÁZS ***(HUNGARY)***

DETECTING INTER- AND INTRASPECIFIC RECOMBINATION EVENTS IN PLANT RNA VIRUSES WITH THE TOPALI AND RDP3 SOFTWARE PACKAGES

MICHAELA HAVRLENTOVÁ, ALŽBETA ŽOFAJOVÁ, IVANA PŠENÁKOVÁ, SOŇA GAVURNÍKOVÁ, ĽUBOMÍR RÜCKSCHLOSS, JÁN KRAIC ***(SLOVAK REPUBLIC)***

COLORED WHEAT AS A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS

ALENA GAJDOŠOVÁ, JOZEF FEJÉR, GABRIELA LIBIAKOVÁ AND ANDREA HRICOVÁ ***(SLOVAK REPUBLIC)***

BOOSTING THE SEED PRODUCTION OF AMARANTH THROUGH MUTATION BREEDING

MARTINA HUDCOVICOVÁ, JOZEF GUBIŠ, LEONA LEIŠOVÁ-SVOBODOVÁ, PAVEL MATUŠINSKY, MARCELA GUBIŠOVÁ, DANIEL MIHÁLIK ***(SLOVAK REPUBLIC)***

MOLECULAR DIAGNOSTICS AND QUANTIFICATION OF RAMULARIA COLLO-CYGNI PATHOGEN IN BARLEY TISSUE

MILÁN IVANICS, ÉVA VÁRALLYAY, GÁBOR TÓTH, GÁBOR GICZEY, ANDREA BALOGH, AID S.OREIFIG, JÓZSEF BURGYÁN, BARNABÁS JENES ***(HUNGARY)***

POSSIBILITIES TO PRODUCE FUNGUS RESISTANT GM WHEAT

JÄGER K., FÁBIÁN A., TOMPA G., DEÁK C., HÖHN M., OLMEDILLA, A., BARNABÁS B., PAPP I. ***(HUNGARY)***

EPIDERMAL CHARACTERISTICS OF THE ABSCISIC ACID HYPERSENSITIVE *ARABIDOPSIS THALIANA CBP20* MUTANT

IMRE MAJLÁTH, GABRIELLA SZALAI, ISTVÁN PAPP, RADOMÍRA VANKOVÁ, TIBOR JANDA ***(HUNGARY)***

***ATNOA1* MUTATION MAY INDUCE TEMPERATURE ACCLIMATION MECHANISMS IN ARABIDOPSIS THALIANA**

JOPCIK MARTIN, MORAVCIKOVA JANA, BOSZORADOVA EVA, MATUSIKOVA ILDIKO, LIBANTOVA JANA ***(SLOVAK REPUBLIC)***

INFLUENCE OF THE CAMV35S PROMOTER ON ECTOPIC ACTIVITY OF FOUR TISSUE SPECIFIC PROMOTERS IN TRANSGENIC PLANTS

KATARÍNA ONDREIČKOVÁ, HANA DRAHOVSKÁ, DANIEL MIHÁLIK, JÁN KRAIC ***(SLOVAK REPUBLIC)***

IMPACT OF GENETICALLY MODIFIED MAIZE MON 810 ON SOIL BACTERIA BY T-RFLP

ISTVÁN MONOSTORI, FRUZSINA SZIRA, GÁBOR GALIBA, ANDRÁS FERENC BÁLINT ***(HUNGARY)***

IDENTIFICATION OF LOCI AFFECTING GRAIN MICRONUTRIENT CONTENT IN WHEAT USING ASSOCIATION MAPPING

ELENA RAKOSY-TICAN, FLAVIU ROSCA ***(ROMANIA)***

GFP* AN ALL PURPOSE REPORTER IN THE MODEL PLANT *NICOTIANA TABACUM

SALAJ, TERÉZIA., MATUŠÍKOVÁ, I., FRÁTEROVÁ, L., SALAJ, J. ***(SLOVAK REPUBLIC)***

CHARACTERISATION OF CONIFER EMBRYOGENIC TISSUES AND THEIR STORAGE BY CRYOPRESERVATION

ELENA RAKOSY-TICAN, RAMONA THIEME, GYONGYI SZEKELY, HILDA SPETH ***(ROMANIA)***

USE OF LABORATORY BIOASSAY AND RAPD MARKERS FOR BETTER INTROGRESSION OF COLORADO POTATO BEETLE RESISTANCE INTO FUSION HYBRIDS AND THEIR PROGENIES

F. RÁCZ, TAMÁS SPITKÓ, G. HADI, C. SZŐKE, Z. TÓTH-ZSUBORI, Z. HEGYI, D. OROSS, E. BODNÁR, L. C. MARTON ***(HUNGARY)***

BIOGAS PRODUCTION USING SILAGE MAIZE HYBRIDS BRED IN MARTONVÁSÁR

ELENA RAKOSY-TICAN, CRISTINA TUTA-POPESCU ***(ROMANIA)***

DIRECT OR INDIRECT IN VITRO REGENERATION IN KALANCOE COMMERCIAL HYBRIDS

18.30 p.m. ***DINNER***

18 May Wednesday

9.30 a.m. *THE VISIT TO THE BIOGAS PLANT „ROHKRAFT" IN 3454 REIDLING, FELDGASSE 2.*

ABSTRACTS

BIOGAS - STATUS AND POTENTIAL IN AUSTRIA

BERNHARD DROSG, WERNER FUCHS, GÜNTHER BOCHMANN

Institute for Environmental Biotechnology, Department IFA Tulln
University of Natural Resources and Life Sciences Vienna, Tulln, AUSTRIA

The Austrian efforts to promote green energy ("Ökostromgesetz 2002" and successive legislation) have created a strong boom in biogas production. Only in the last few years stagnation in the number of biogas plants is observed due to increasing prices for commodities serving as substrates. Currently in Austria there are 344 biogas plants in operation providing 525 GWh of electric power (data from 2009). This figure corresponds to ~10% of the green energy production (with the exception of large-scale hydro power). An even higher amount of energy is available from the thermal energy produced during the conversion of biogas into electrical power in the combined heat and power process. The efficiency of the transfer is approx. 35 - 40 % only. However, the thermal energy surplus is currently used in an insufficient way. In future an improved utilization of thermal energy and moreover the direct injection of purified biogas into the gas grid will improve the overall energy efficiency.

Biogas production in Austria is based on agricultural residues, in particular manure, energy crops and organic waste. Lately, the use of purpose grown energy crops has faced strong criticism. The competition with food crops, widely known under the slogan "fuel or food", is certainly an unwanted side effect. To prevent this discussion, it is necessary to fully exploit the potential of biogas recovery from waste materials and organic byproducts. This is not only of benefit in terms of renewable energy production but also contributes to the general waste management strategy with its principle aims of optimum utilization and recycling of wastes and residues. Significant amounts of organic residues are available from several sources. These include source-separated organic waste, gras and green cuttings, expired food products, supermarket waste, and organic residues from food industry and biofuel production.

In Austria, the total amount of such substrates is roughly 800.000 tons per year of which 550.000 tons are already in use. Therefore the readily available extra potential for biogas purposes is about 250.000 tons corresponding to a theoretical 10 % increase in biogas production. In addition, there is even a wider amount of organic residues which are principally suitable for anaerobic digestion but can not easily be utilized yet e.g. due to logistic reasons. Examples are kitchen waste jointly disposed with municipal waste or animal by-products currently treated in rendering plants. Through exploitation of this extra potential a 30 - 50 % increase in biogas production is possible. Generally, these figures show that the degree of utilisation of biowaste as source of green energy is already quite high in Austria. However, at the European level the latent potential is much bigger and it is estimated that only 2.8 % of the organic waste is currently used for biogas recovery.

While with the aim to change energy supply from fossil to renewable resources a certain competition between energy and food crops seems inevitable, it is necessary to gain best possible benefit from the resources available. With respect to that, research at the Institute of Environmental Biotechnology – IFA Tulln is devoted to improve the efficiency of the anaerobic digestion process and energy recovery. Among others, the topics investigated include process monitoring and control, optimization of microbial consortia, improvement of biodegradability of ligno-cellulosic material and integration of biogas production into green bio-refinery concepts. To generate and disseminate knowledge and best practice in anaerobic digestion the Inst. of Environmental Biotechnology is not only engaged in national projects and co-operations (e.g. Bioenergy 2020) but also in numerous EC-projects and European network activities.

BIOHYDROGEN AND BIOGAS BIOTECHNOLOGY

K. L. KOVÁCS[1,2,#], Z. BAGI[1], N. ÁCS[1], E. KOVÁCS[1], R. WIRTH[1], G. RÁKHELY[1,2]

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[#] *Corresponding author*

Biogas and fermentative hydrogen production are the only renewable energy carrier production biotechnologies associated with double benefits: the elimination of environmental pollution problems is coupled with the generation of useful energy. Moreover, biogas can be used in several ways. After removal of trace contamination H_2S, xyloxanes and water, biogas can be burnt to generate heat or can be used as fuel in gas engines, coupled to a generator to produce electricity and heat. If the CO_2 is withdrawn from biogas, the remaining gas (often called biomethane), has the properties of purified natural gas, and can be utilized as a transportation fuel, a raw material for the chemical industry, or in fuel cells, which convert it to electricity with high efficiency.

Biogas technologies commonly apply natural anaerobic consortia of microbes. This is partly due to the fact that, from a microbiological aspect, this is a very complicated and complex system. Moreover, the population dynamics of the natural ecosystems could not be properly studied before the introduction of molecular biological techniques. Research on the diversity of these microbial communities is needed for the optimization of biogas production technologies as their economic viability is closely related to the efficacy of the concerted microbiological actions. One of the rate-limiting factors in biogas-producing consortia is the actual level of H_2 in the system. In natural ecosystems a very low partial pressure of H_2 is maintained, which may be a limiting factor for the methanogens.

We demonstrated earlier that reductant accessibility is indeed a limiting factor in biogas production and presented data supporting the hypothesis that the introduction of H_2-producing bacteria into the natural biogas-generating consortium effectively increases biogas production both in batch fermentations and in a scale-up anaerobic digestor. Systematic, continuous experiments were conducted in 5 litre fermentors, constructed for biogas research on a laboratory

scale. These devices model the real-life, large scale biogas production plants much better than the routinely used batch systems, and the first results are reported here. Thermophilic conditions were selected because biogas yields are usually higher at elevated temperatures and thus the economic feasibility of the process is improved. The microbial diversity in the thermophilic natural consortia is lower, which requires a thorough inspection of the microbiological profiles. Introduction of a H_2-producing new member into such consortia is therefore somewhat more challenging than altering the composition of a microbial consortium under mesophilic conditions. *Caldicellulosiruptor saccharolyticus* is a good H_2 producer and the beneficial effect of adding this strain to the biogas-producing system has been demonstrated. An important question remained before the implementation of the large-scale application: the conditions under which *C. saccharolyticus* becomes a stable member of the biogas-producing microbial consortium. This can be determined only in systematic experiments with digestors functioning in continuous operation mode. The first results indicated that, under standard feeding conditions (2-3 g organic total solids litre^{-1} day^{-1}), addition of the *C. saccharolyticus* culture at the time when stable and reproducible daily biogas production was reached, brought about an intensification effect similar to that observed in the batch experiments. However, the bacteria in the continuously fed fermentors gradually diluted out and disappeared within 2-3 weeks. By that time, the daily biogas production had returned to the level of the control and no *C. saccharolyticus* was detectable using the DNA molecular marker method. Essentially the same behaviour was observed on the use of various biomass sources, e.g. bagasse, kitchen waste, waste water sludge or DGS (distillers grains with solubles) from starch-based ethanol fermentation. A systematic study has been launched in order to elucidate the reason for the discrepancy between these results and those of the scale-up experiment carried out earlier. As the first important parameter the loading rate of organic total solids (OTS) was identified as responsible for the effect. When OTS was elevated, the beneficial effect of the added H_2 producer *C. saccharolyticus* was not merely obvious, but lasted substantially longer. Other factors influencing the long-lasting intensification of biogas production in this system are currently being studied. It should be noted, for example, that the temperature of 55 °C routinely used in thermophilic anaerobic digestors is not ideal for growth of the extreme thermophilic *C. saccharolyticus.*

Acknowledgement

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EFFECTS OF EU BIOFUEL TARGETS ON GLOBAL GHG EMISSIONS FROM LAND USE CHANGE

ERWIN SCHMID

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One of declared objectives of biofuels is the reduction in greenhouse gas (GHG) emissions from fossil fuel combustion (Directive 2009/28/EC). The net savings of GHG emissions from biofuels depend critically on the type and the location of biofuel feedstock production (Havlík et al., 2010). Although there is a consensus around the fact that ethanol produced from sugarcane can allow significant reductions of GHG emissions, estimated GHG savings of biofuels based on other feedstocks vary a lot and are usually lower (Scharlemann and Laurance, 2008; OECD, 2008). Moreover, CO2 emissions from the conversion of native ecosystems to cropland are likely to lessen the net savings of the majority of first generation biofuels compared to fossil products, which would be compensated only after many years of continued biofuel production (Fargione et al., 2008).

If biofuel feedstocks are grown on currently cropped areas, indirect land use change (iLUC) will occur to produce crops for other uses elsewhere, especially in the context of population growth and increasing demand for food (Searchinger et al., 2008). The US Environmental Protection Agency estimates that the iLUC effect

of growing corn would negate the direct savings if pastureland is replaced with overall savings between -40% and 0% (Federal Register, March 2010). On the EU side, detailed studies also emphasized more specifically the strong land use effects of biodiesel use, showing even worse results than for ethanol (Al Riffai et al., 2010). Legislation in the EU is currently limited to direct land use change (LUC) emissions in the biofuel GHG balance assessments.

Furthermore, trade barriers influence both the choice and the location of biofuel feedstock, and consequently the capacity of biofuels to mitigate climate change. Except Brazil, most of producing countries impose high tariffs on ethanol. For instance, the ad valorem equivalent tariff on European bioethanol imports is 52%. For biodiesel, tariffs are usually not prohibitive but domestic support to farmers and to biofuels industry provide a strong element of protection (Steenblick, 2007). Moreover, biofuels must comply with fuel standards which also influence the choice of the feedstock.

The aim of the presentation is to focus on indirect land use change effect of biofuel development and trade policies of the EU. On the one hand, trade liberalization is more prone to increase biofuels or biofuels feedstock imports in Europe which will induce GHG emissions from agriculture and LUC in those regions where the biofuel feedstock are produced (direct trade effects). On the other hand, European Union is the biggest exporter of agricultural products: the diversion of crops from food to energy may affect global agricultural markets and create indirect emissions from agriculture and land use change elsewhere due to substitution imports (indirect trade effects). The optimal trade policy with respect to climate change mitigation will largely depend on which of the two trade effects is stronger.

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FOOD SECURITY AND BIOFUELS: TIME TO RE-EVALUATE BIOFUEL POLICIES?

JÓZSEF POPP

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Food security is basically a matter of means versus ends. With one billion people hungry, one billion undernourished, another billion obese, and some three billion people trying to move up the food chain, the challenge of feeding nine billion people by 2050 is a real challenge. It is also likely that food prices will remain volatile around their trend in the near future. The race by some countries to secure farmland abroad risks creating a neocolonial system Political problems may eventually arise if a country hosting foreign investment in farming faces a serious food crisis at a time when rich foreigners export all the food produced for the exclusive benefit of richer and better fed people abroad.

However, energy is another key issue in the current international debate. There is a clear relation between energy consumption and development since there is no possible economic and social development without the increase of the demand for energy. The ever growing demand for energy drives countries to look for securing privileged access to energy resources. The production and use of energy is the main source of GHG emissions, especially those related to fossil fuels.

Bioenergy represents approximately 13% of total world energy supply. Traditional unprocessed biomass accounts for most production, but commercial bioenergy is assuming greater importance. Liquid biofuels for transport garner the most attention and production has expanded rapidly. However, their quantitative role is only marginal: they constitute only 1-2% of total transport fuel consumption and less than 0.5% of total energy consumption worldwide. Liquid biofuels can therefore be expected to displace fossil fuels for transport to only a very limited extent. Although liquid biofuels supply only a small share of global energy needs, they still have the potential to significantly affect global agriculture and agricultural markets because of the volume of feedstocks and the land areas needed for their production. While the precise impact of biofuels on agricultural commodity prices is a matter of debate, analyses have concluded that biofuel support policies have an impact on international commodity prices. On the other hand the price of oil should include the real cost of foreign oil including all external costs (national security, environmental and national economics – lost opportunity of jobs – costs. The challenge to offset the dependency of foreign oil is to unlock the potential of agriculture to meet the needs of food, feed and fuel, ensure a clean and sustainable environment and create economic opportunity.

About 98% of transport depends on petrol and 60% of raw petrol is "burnt" by transport. Transportation, including automobile, is thus an important source of GHG emissions. Reduction of GHG emissions requires improvement of vehicle fuel efficiency and biofuels emitting less CO_2. Biofuels reduce climate change by lowering CO_2 emissions in transport, reduce oil dependency by replacing fossil fuels and provide a liquid and high energy alternative to diesel & petrol and support the agricultural sector. Biofuels are not only energy alternatives. They can be associated with income generation, job creation, rural development, GHG emissions reductions, and increased access to energy. The establishment of a global market for biofuels can contribute to address global challenges.

Fixed mandates and subsidies for grain and oilseed-based biofuels can amplify price volatility by drawing down stocks but the implementation of public policies that include mandates are needed as part of a long-term strategy to incorporate biofuels in the global energy supply chain. Nevertheless, policy should introduce greater flexibility and more price-responsiveness in the supply of biofuels. Removing trade distortions and investing in research and development of

advanced and second-generation biofuels will contribute to more effectively reducing reliance on fossil fuels without jeopardizing food security.

BIODIESEL FROM MICROALGAE – MISSION IMPOSSIBLE?!

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Due to their potentially high productivity, microalgae are now receiving exceptional attention as a potential source of biofuels, particularly biodiesel. It is commonly assumed the biofuels from microalgae are structurally the same as biofuels from other biomass such as soy, canola, rapeseed, palm, etc. However, the chemical structure of fuels derived from microalgae can be quite different from that of terrestrial plants because of lipid differences. The chemical pattern of algal lipids showed that the main part of neutral lipids consisted of diglycerides, triglycerides, hydrocarbons, free sterols, and sterol esters. The glycolipids are mostly monogalactosyl diglyceride, digalactosyl diglyceride, esterified sterol glycoside, and sterol glycoside. In phospholipids, phosphatidyl choline, phosphatidyl glycerol, and phosphatidyl ethanolamine are the main compounds. Fatty acid composition patterns are strictly species specific. The microalgal fatty acid profile include components with carbon chain lengths ranging from 10 to 24 carbon atoms, with saturation states ranging from saturated to highly unsaturated. The lipid profile can also vary with species, growth conditions (light, temperature, nutrients etc.) extraction and processing techniques. All these properties could have significant implications for the suitability of microalgal-derived fuels for a variety of applications, including as diesel fuel and aviation fuel. Within the presentation the basic structure of algal lipids, including both storage lipids and membrane lipids, will be discussed. The presentation also includes a discussion of processing techniques and the subsequent impact on fuel properties.

LIPID PRODUCTION IN GREEN ALGAE DEPENDING ON N-SUPPLY

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Mankind is currently faced with two global challenges - global warming and an increasing price and unsafe supply of fossil fuels. Microalgal biotechnology has the potential to provide solutions for both these challenges as well as producing unique metabolites not found in other organisms. Carbon dioxide uptake by microalgae decreases the emission of this green house gas and microalgae are able to produce a higher quantity of biofuel and biomass per time and area than any other photosynthetic organism. Nevertheless, the success of this new biotechnology depends on improving the energy balance and decreasing the costs of the microalgae biomass production. Intensive research is being carried out all over the world, among others in Mosonmagyaróvár, to find the most promising algal strain(s) and to develop the best technology. This presentation will show that the lipid content and lipid production of some *Chlorella* strains can be manipulated by changing the nitrogen and glucose supply as well as by carefully selecting the harvest time.

Synchronous and non-synchronous batch cultures of 40 *Chlorella* strains were investigated. The strains were cultivated in complete and nitrogen-limited nutrient medium, with or without glucose addition, in 500 mL aerated (+1.5% CO_2) Erlenmeyer flasks. Samples were collected from cultures of different ages. Lipid, protein, chlorophyll-a and –b, as well as carotenoid content, of fresh or freeze-dried *Chlorella* biomass were measured using standard methods.

In all type of experiments the N-supply showed principal importance on the lipid content of the algae biomass. The highest lipid content of the investigated strains in nitrogen-limited Tamiya nutrient medium (10% of the original nitrogen content) varied between 10 and 29%. The decrease of nitrogen supply to 3% increased the lipid content up to 44% in the MACC-870 *Chlorella minutissima*

biomass. The best lipid-producing strains were all *Chlorella minutissima* strains. In synchronous cultures of the MACC-870 strain, the decreasing nitrogen availability caused an increasing lipid content of the cells/biomass during the 24 or 48 h cultivation period. The addition of glucose at the beginning of the dark period to the nitrogen-limited synchronous cultures increased the lipid content. The age of the cultures significantly influenced the lipid content of the strains. It was observed that the older cultures had higher lipid content. However, the main reason of the increased lipid content could be explained by the decreased N-availability in the old cultures.

The N-limited green algae has low protein content and are yellow-green rather than dark green. The relations among some relevant parameters were investigated in two *Chlorella* strains. When the relationship between chlorophyll-a, *chl a+b/carotenoids* and proteins were plotted against lipid yields, it was found that the protein levels most accurately reflected the lipid content in both strains. This relationship can be used to determine the optimum harvest time of the cultures. For example, the harvest of *C. minutissima* cultures with a lipid content >38% DW, the protein content should have decreased to <15% DW.

WHY NOT TRANSGENIC POTATO FOR BIOETHANOL PRODUCTION – THE CASE OF MARKER-FREE PVY RESISTANT CULTIVARS

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The new tools of plant genetic engineering can be efficiently applied to bioenergy crops. Potato as a very important crop ranking third at global level in productivity is a good example for biotechnological improvement and can be a good source of bioethanol.

The European Commission has identified biofuels as an environmentally friendly way to contribute to the security and diversity of energy supply for the EU transport. In this context, the Directive 2003/30/EC of the European Parliament and the Council set up target shares of biofuels on the EU transport fuel market at

5.75% by the end of 2010 of all gasoline and diesel. Amongst different biofuel pathways available, producing bioethanol and biodiesel from agriculture-derived feedstock appears to be the most feasible, ready-to-market option. The production of such transport biofuels from agricultural feedstock is however constrained by one core limitation – the availability of land. Reducing the looses in crops yield induced by biotic and abiotic stress factors might contribute to better use of land in these circumstances, including climate change.

Potato ranks second after sugar beet in Europe's bioethanol production with an estimated 86.9 GJ/ha in 2010. Bioethanol can be obtained via fermentation from potato tubers. One of the greatest threats to potato production is potato virus Y, especially its new emerged strain PVY^{NTN}, ringspot virus, able to destroy up to 80 - 90% of the tubers.

Then why not transgenic, "vaccinated", potato resistant to PVY^{NTN} or other diseases for bioethanol production?

As it was shown previously, a two-step simple protocol for potato transformation allows the integration of CP-PVY^{NTN} hairpin construct, inducing post-transcriptional RNA silencing and hence high resistance to PVY(….;Rakosy-Tican et al., 2010). For genetic transformation a first step is based on the transfer and expression analysis of reporter gene *gfp* and marker gene *nptII* (Rakosy-Tican et al., 2007), followed by the transfer of a marker free construct carrying hairpin PVY-CP. Marker-free transgenic potato plants are assayed at molecular level (Rakosy-Tican et al., 2010). The ressistance of such transgenic lines to PVY was also demonstrated in the case of Hungarian cultivars, by using a similar strategy (…). Such a protocol can be applied to commercial potato cultivars and increases tuber yield and quality. The question remains: would the consumers accept fuel made of transgenic plants? We think they most probably will.

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CORN AS A RENEWABLE ENERGY SOURCE

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After the first petroleum slump in the 70's researchers revealed that the petroleum supplies will have run down within the forseeable future, so there is a great need for resources capable to satisfy the growing energy demands. Producing energy based on biomass is a closed cycle, therefore biomass is a greenhouse-neutral energy source. In addition, its great advantage that it can be reproducted year by year, thus it is a renewable energy source. Bioethanol is a fine spirit fermented from agricultural crops with high starch content. In the USA bioethanol is chiefly made from corn. They produce 300 million tons of corn on 36 million hectare per year and ferment bioethanol from 3.7% of it in 1993, and from 13-20% of it in 2007 at 50-60 bioethanol plants in order to be self-sufficient, although they have enough petrol of their own and by import. The countries signed the memorandum at the Kyoto conference in 1997 committed to decrease CO_2 emission by 15% until 2010. Hungary undertook to decrease the emission of harmful substances by 6% during 2008-2012. The researches of the Agricultural Research Institute of the HAS aimed to produce bioethanol from corn with high starch content can help to fulfill these obligations.

In the recent years the chemical quality parameters of almost 100 hybrid corn varieties have been studied at the Agricultural Research Institute of the HAS. It was concluded that the starch incorporation in the grain was growing with greater yields, and it was greater in humid years and lower in dry years. The differences in starch content caused by the locations were greater than the differences caused by the varieties. The genetic determination of starch is weak, though the varieties with greater starch content can be selected by the appropriate method. Improvement of starch yield is mainly achieved by growing starch content in the grain, besides growing grain yields per hectare. The starch content of the maize kernel is 80-82% referred to absolute dry matter of the grain. Since starch represents the largest part of the grain, it can be improved to

a very limited extent. Irrigation is a very important aspect of producing corn for bioethanol, since the insufficient amount of rainfall prevents starch specific for the variety from incorporating in the grain. Fermentability is also a very important characteristic of starch for bioethanol production. Ethanol yield is not closely correlated to starch content: the total starch content influences final ethanol yield by 52%, while extractable starch content by 62%. The composition of the starch (amylose/amylopectin ratio) also influences the bioethanol yield.

A total of 96 hybrids from four maturity groups (FAO 200, 300, 400, 500) were tested in two years (2008, 2009) at four locations in Hungary. Considerable differences were found between the years for the grain yield per hectare and for the grain quality parameters. The grain quality parameters exhibited a close correlation with the grain yield in the individual FAO maturity groups. Hybrids of the flint type, which have a short vegetation period, had high protein and oil contents, but the yield averages were low due to the slower rate of starch incorporation. Hybrids of the dent type have a longer vegetation period and more intense carbohydrate accumulation, but low protein and oil contents. In wet years and locations there was a higher rate of starch accumulation (2008), while dry years are favourable for protein and oil accumulation (2009). The Bravais correlation coefficient was calculated between the yield and the grain quality parameters (averaged over years, locations and varieties). A positive, moderately strong correlation (0.68) was found between the yield and the starch content.

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LIPID QUALITY OF SLOVAK POPPY SEEDS AND THEIR PLACE ON THE WORLD-MARKET

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The cultivation and breeding of poppy seeds (*Papaver somniferum* L.) has a long tradition in the Slovak Republic. However, its areas are very small in comparison with other countries. There are two possibilities for this crop utilization in Slovakia: pharmaceutical and food industry, whereby Slovak poppy seeds were historically used and breeded for edible seed production. From agronomic point of view, they have high seed yield, good agrotechnical properties, and appropriate health state. This study was conducted to examine variability in total lipids content and fatty acids profile in a set of 16 poppy seeds varieties (8 Slovak and 8 from the world collection) which were grown on one locality (Malý Šariš, Slovakia) during one year (2007). Total lipids content ranged in Slovak poppy seeds from 45.0% (Albín) to 51.2% (Bergam), with the average of 49.2%. In the world collection, the average content of total lipids was 44.9% (36.5 – 49.3%). Triacylglycerols, sterolesters, free sterols and free fatty acids were identified as the main non-polar lipid structures. The lowest acid value and therefore low free fatty acids amount was shown in Slovak variety Major. Dominant fatty acids in all samples were linoleic, oleic, and palmitic acids. As a minor fatty acids were detected *alpha*-linolenic, stearic, myristic, arachidic, and gadoleic acids. Slovak variety Opal contained the highest amount of linoleic acid (78.0%) what resulted in the maximal unsaturation index (1.67) of the oil. Compared to the analyzed world collection, Slovak poppy seeds tend to dispose lower amounts of free fatty acids and alpha-linolenic acid. From this point of view these seeds are of better

quality and low sensitive to oxidation. It might indicate that poppy seeds of the Slovak origin are suitable sources for their next end-uses in food industry.

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SOLID STATE FERMENTATIONS AS A NATURAL WAY TO ENRICH CEREALS WITH POLYUNSATURATED FATTY ACIDS AND PIGMENTS

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Solid state fermentations (SSF) have been practiced for centuries to prepare certain types of food. SSF are defined as a process in which microorganisms grow on a moist solid substrate in the absence or near-absence of free water. Interest in SSF has been increasing because of its important applications in producing enzymes, biopesticides, aroma compounds, biopharmaceuticals, organic acids and wide spectrum of other bioactive compounds. Whereas they simulate fermentation reactions in the nature they are often applied for microbial utilization of many agroindustrial materials and their effective valorization to various types of value-added bioproducts with demanded properties and functions.

Cereals represent a major food supply for humanity. However, many of them are deficient in several essential nutrients, such as polyunsaturated fatty acids (PUFAs) and pigments that have a number of uses in biomedical, nutritional and pharmaceutical areas due to their healthy, dietary and functional properties. One possible way how to enrich or modify the content of biologically active compounds in cereals lays on application of lower filamentous fungi because some of them simultaneously decrease anti-nutrient compounds in the substrates (*e.g.* phytic acid) and partially hydrolyze substrate biopolymers. Thus prefermented

mass with a high content of PUFAs may be used as new bioproducts with demanded properties.

Lower filamentous fungi due to their capacity to biosynthesize a variety of useful metabolites were selected to enhance functional characteristics of prepared bioproducts. Our interest is focused on application of Mucorales fungi, such as *Mortierella alpina* (producer of dihomo-gamma-linolenic, arachidonic (AA) and eicosapentaenoic acids), *Thamnidium elegans* (producer of gamma-linolenic acid (GLA)) and *Mucor circinelloides* (producer of gamma-linolenic acid and carotenoids such as beta-carotene, gamma-carotene and lycopene). Depending on the strain, various types of cereal substrates and cultivation conditions were tested and range of bioproducts efficiently enriched with PUFAs were prepared. *M. alpina* fermented mixture of wheat bran/spent malt grain into the bioproduct containing 4.2 % AA. The substrate consisting of wheat bran/spent malt grains was also found as suitable substrate for *T. elegans* and its prefermentation resulted in bioproduct with 0.5% GLA. Optimized fermentation condition finally yield cereal-based bioproduct consisting of 2% GLA. Thus, bioproducts prepared by SSF may provide the opportunity to fill marketing claims in food, feed, pharmaceutical and veterinary fields.

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GROWING TECHNOLOGY AND BIOTECHNOLOGY OF *MISCANTHUS* IN SLOVAKIA

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Energy crops produce biomass as renewable and environmentally friendly energy source with low CO_2 emission. The man constantly looks for new plant species advanced for heat and electricity production. Therefore the breeding and introduction of new plant species originating from tropical and subtropical countries to the zone of mild climate are attractive. *Miscanthus sinensis* and its naturally occurring hybrid *Miscanthus giganteus* are considered as plants with high energy potential. The yield potential of *Miscanthus* exceeds the capabilities of the natural plant species for our country, including fast-growing trees and has a higher energy value than fossil fuel (e.g. lignite).

The polyfactorial long-term field experiment with *Miscanthus sinensis* was established in Slovakia in 2003. Plants were cultivated from rhizomes with planting space 1 x 1 m in fluvisols at experimental station Vysoká nad Uhom (east part of Slovakia, cold winter; elevation 105 m, average annual temperature 9°C, average annual precipitation 584 mm). Experimental plots were fertilised with 60, 40 or 0 kg ha^{-1} of nitrogen without irrigation. Average yield of dry matter in years 2004-2007 was 42.88 t ha^{-1} (at 60 kg N ha^{-1}), 36.60 t ha^{-1} (40 kg N ha^{-1}) and 30.14 t ha^{-1} (without fertilization). Return of investment in tested variants was 4.0, 4.6 or 5.1 years respectively. For total energy inputs from 3.82 to 9.73 GJ ha^{-1}, energy gain was from 430.12 to 799.95 GJ ha^{-1}.

Miscanthus sinensis and *M. gigantheus* are perennial grasses originated from Southeast Asia belonging to C4 plants with high photosynthesis efficiency. Seed production in our climatic conditions is not possible due to low viability of seed of *M. sinensis* and sterility of *M. gigantheus* (*M. gigantheus* is triploid hybrid of *M. sinensis* and *M. Sacchariflorus*). Species of *Miscanthus* are usually propagated by rhizome division. Because the production of rhizomes is slow, other techniques including biotechnology approaches as *in vitro* propagation are demanded.

Micropropagation is possible by two ways – direct shoot induction from axillary buds and rhizomes followed by *in vitro* tillering or adventitious regeneration via callus. The drawback of direct regeneration is high frequency of contamination especially when rhizomes are used. The second serious problem is explant browning. In our experiment with *Miscanthus giganteus*, cultivation of explants in darkness, reduction of cytokinine content in nutrient medium, use of antioxidants (150 mg l^{-1} ascorbic or citric acid) and adsorbents (50 mg l^{-1} PVP, 3 g l^{-1} activated charcoal) as methods for elimination of brown exudates production were not effective. Only addition of cysteine-HCl recommended by Lewandowski (1997) solved this problem. Adventitious regeneration from immature inflorescences proved to be the most effective *in vitro* system for multiplication. In our experiments, contamination of explants from immature inflorescences was negligible. Even though Lewandowski (1997) and Glowacka et al. (2010) mentioned that there is minor problem with explants browning for immature inflorescences, we did not confirm this fact and cystein-HCl had to be added to the media. Three categories of immature inflorescences (up to 5, 5–15 and 15–25 mm in length) were inoculated to callus induction media containing 5 mg l^{-1} 2,4-D and 0.1 mg l^{-1} BAP. Calluses were transferred twelve weeks later to regeneration medium supplemented with 2 mg l^{-1} BAP. Regenerants were counted after 2 and 3 months for each immature inflorescence. The most effective regeneration was observed for the category 5-15 mm – from 21 to 71 regenerants, in average 42 per inflorescence (cluster of shoot kept together was counted as 1 regenerant). *In vitro* tillering of shoots can be achieved by adding 1 mg l^{-1} BAP and 0.1 mg l^{-1} NAA to the basal medium. Except the multiplication, the effective *in vitro* system is also usable for *Miscanthus* breeding by chromosome doubling, somaclonal variation, selection and genetic transformation.

Preliminary results from the biotech propagation of *Miscanthus giganteus* indicate that seedlings production could be solved by this approach and this perspective energy crop can face forward application in agriculture aimed to generation of renewable biomass.

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SOME NEW BREEDING AND PROPAGATION STRATEGIES OF THE FUTURE BIOENERGY CROPS FOR CELLULOSE FARMING

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During the first decade of the 21st Century we experienced that Mankind is still strongly dependent on fossil fuels. At the same time, concerns are increasing about climate change and the potential economic and political impact of peak oil production. To address these issues and reduce its dependency on fossil fuels the EU has adopted measures to encourage the production and use of sustainable new sources of bio-energy involving the next generation bio-fuels. The Hungarian EU presidency of 2011 is a suitable background to promote these efforts. The EU Commission announced the priority area coordinators for the EU Strategy for Danube Region. Together with the Czech Republic, Hungary will be coordinator for priority area 2: To encourage more sustainable energy. It can be expected that this development will generate high-level scientific and political interest in the Pannonian Region: the cheap transport route of agriculturally produced lingo-cellulosic raw materials for bio-energies / bio-fuels and of final products on the river Danube is an outstanding potential in Europe.

Responsible professionals and non-professionals agree that the natural resources accumulated during millions of years cannot be further destroyed, not even in the present intensive era of energy demand. As a result of the above theoretical calculations, agro-economists drew the attention of the scientific world

to the fact, that the further demolition of natural vegetation can only be avoided if the world's energy request is produced on only the 320-702 million hectares of marginal agro-ecosystems. For this purpose, as stated by American scientists, the most effective biological, genetic and other breeding methods should be used and new and adequate agricultural cultivation and biomass processing methods should be developed. The predictions is that breeding, propagation and growing of the future biomass and energy crops in the next fifty years will be done by applying the combination of the most modern techniques, among which the GMO method should also be used.

Plant photosynthesis is the most efficient process for CO_2 removal which is fuelled by 150.000 TW solar energy hitting the surface of the Earth every year. Presently, we use 15 TW energy yearly, which may reach 20 TW by 2030. The series of energy conversions in the photosynthesis are intensively studied not only for CO_2 fixation, biomass production, which is a central area, but also for more direct utilizations such as direct electricity and H_2 production (natural fuel cells) in artificial and half artificial systems as well as for the direct production of liquid fuels. In the present transitional period, all opportunities must be seized to form positive trends and to avoid an environmental catastrophe. The so-called third generation biomass crops have to be selected and bred for the purpose of intensive and environmentally-benign biomass crop production. As it cannot be avoided that biomass production should be limited to the lands of marginal quality and should not compete with food production, drought tolerance or its opposite tolerance of inundation and water logging can be an important aspect. Crops should be resistant to other extreme growing site conditions such as high salt content, extreme pH, chemical residues, heavy metal pollution, which do not allow for food crop production or the use of the crops grown there is not advisable for food. Actually it seems that some old and new perennial crops can be taken into consideration as the annual soil cultivation reduces CO_2 fixation and the perennial herbaceous crops show considerable, continuous sustainability and can be of ameliorative nature (especially grasses) and can improve the lands of marginal quality. The presentation will summarize some new agbiotech approach investigated by our research team.

POSTERS

PHYTOREMEDIATION POTENTIAL OF *ARUNDO DONAX* L "SYN-PLANTS" GROWTH IN HEAVY METALS CONTAMINATED SOILS

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The use of low cost, fast growing indigenous plants with efficient biomass producing plant species such as *Arundo donax* are highly desirable for phytoremediation of metal contaminated sites and waters.

The present study reports the potential growth of *Arundo donax* and its capacity for phytoextraction of different heavy metals from contaminated soil. *A. donax* plants were grown under greenhouse conditions in plastic pots containing 150g from different types of soil. the first soil sample was from vegetable crops' farm, Debrecen University with two different cover vegetation under both grass (DGS) and *Arundo donax* (DAS), the others were from Kolontár-Devecser area as follow; normal soil(KNS), red sludge contaminated soil (KCS) as well as pure red sludge (KIS). We made up new type of soil through mix between normal soil and pure sludge with percent (50:50). Soil analysis data showed that the highest pH value was found in industrial sludge 8.92 measured in KCl and 9.80in distilled water, as a result for contamination with sludge the contaminated soil followed red sludge and recorded 7.26 in KCL and 8.24 in distilled water. Soil electrical conductivity where the highest values were found in sludge followed by contaminated soil 3.44 and 1.82 dSm^{-1}, respectively. The total concentrations of some heavy metals were measured using ICP wherein KIS recorded the highest values for most metals as follow: (Cd 5.2, Ni 64.0, Pb 210.0, Co 45.2, Mn 1870, Zn 125) mg kg^{-1} and Fe 58.0, Al 25.5 g kg^{-1}, followed by KCS. After one year of plantation of *A. donax* in vegetable crops' farm the concentrations of Ni, Co, Mn,

Zn, Al and Fe were decreased in compare with grass, which planted in the same soil. However, the values were 0.4, 0.0, 560, 75, 13600 and 1700 mg kg^{-1} under *A. donax* plants, while the values were 31.4, 16.5, 620, 125, 16400 and 2100 mg kg^{-1} under grass plants.

CHROMOSOME 5A OF WHEAT AFFECTS GENE EXPRESSION AND THIOL LEVELS DURING VERNALZATION

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Winter cereals require a long non-freezing cold period which increases their freezing tolerance and fulfills their vernalization requirement. During vernalization the transition from vegetative to reproductive phase will be initiated, until which the level of freezing tolerance will be increased. From the appearance of spikelets the freezing tolerance decreases. Chromosome 5A has a main role in the regulation of freezing tolerance and the vegetative/generative transition, since there are the two vernalization (*VrnA1* and *VrnA*2) and two freezing resistance (*FrA1* and *FrA2*) genes on this chromosome.

The effect of chromosome 5A on freezing tolerance, gene expression, thiol levels was compared in two wheat chromosome 5A substitution lines, *Triticum aestivum ssp. aestivum* cv. Chinese Spring (*T. ae. ssp. aestivum* cv. Cheyenne) (CS(Ch5A)) and CS (*T. ae. ssp. spelta*) (CS(Tsp5A)) having different freezing tolerance and vernalization requirement. In the freezing-tolerant CS(Ch5A) the freezing-induced membrane injury monitored by electrolyte leakage measurements was smaller than in the sensitive CS(Tsp5A). A correlation was found between the freezing tolerance and developmental phase in CS(Ch5A) line. The freezing tolerance reached its maximum during the initiation of the vegetative

to generative transition indicated by the appearance of the double ridges, and it decreased during the development of spikelets primordia.

The expression of *Vrn1* gene was high in CS(Tsp5A) even under control conditions, while it reached a similar level in CS(Ch5A) only after cold treatment. The expression of several cold-responsive and antioxidant genes was induced by cold and remained at high level until the double ridge state and decreased during spikelet initiation both in crowns and leaves of CS(Tsp5A). Some of these genes were also induced by cold in the other genotype. Some differences were observed in the transcript levels of certain genes between the two wheat lines.

The amount of glutathione and hydroxymethylglutathione and their ratios to the oxidised forms decreased during cold treatment, except for glutathione levels at the double ridge state and spikelet initiation. Under control conditions the amount of cysteine, glutathione and hydroxymethylglutathione was greater in CS(Ch5A), while the ratio of cysteine to cystine during spikelet formation and the ratio of reduced to oxidised hydroxymethylglutathione during the vegetative phase in the cold was greater in CS(Tsp5A).

By comparison of the two substitution lines it turned out that chromosome 5A effects changes in the freezing tolerance, the expression of stress defence-related genes and the redox state of thiols during the vegetative to generative transition.

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THE MOLECULAR BACKGROUND OF WHEAT HARDNESS: A LOOK AT STARCH GRANULE SURFACE ASSOCIATED PROTEINS

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Kernel hardness in wheat is the single most important characteristic determining the end-use quality of the flour. This trait is mainly influenced by the proteins of the friabilin fraction, coded by the Ha locus on the 5D chromosome, which are commonly found associated to the surface of starch granules. The exact mechanism by which these proteins act to create a soft/hard phenotype is not clearly understood yet, but their ability to bind polar lipids and the surface of starch granules is likely to play a significant role in it. In a series of experiments various proteins able to bind to the starch granule surface were studied, including both wild-type and recombinant proteins and different forms of starch from multiple sources. Two friabilin components, puroindoline-a and GSP-1 were expressed in *Escherichia coli*. Three mutants of puroindoline-a were also produced by recombinant DNA technology, to investigate the effects of certain structural elements that may play a role in the binding process. Methods for starch granule preparation were evaluated, including dry, wet and non-aqueous protocols. Scanning electron-microscopy images of the starch granule surface and extractions of surface associated proteins were used to compare the effectiveness of different approaches. Starch binding properties of the recombinant polypeptides and of various protein fractions extracted from wheat flour were compared *in vitro*. The effects of the presence or lack of surface polar lipids on binding activity were assayed, and also the influence of the starch type and source. The recombinant puroindolines were able to bind to the surface of the starch granule, except for those in which the tryptophan rich domain was disabled. Binding of both wild-type and recombinant puroindoline was largely impaired by the removal of polar lipids from the starch granule surface. In addition to puroindolines, a 30 kDa gliadin-type fraction was found to be able to specifically bind to all forms of starch analyzed, even in the absence of surface polar lipids.

THE ROLE OF STRESS-ACTIVATED MAP KINASE PATHWAYS IN REGULATING PLANT DEVELOPMENTAL PROCESSES

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Plant development is heavily dependent on the continuously changing environmental conditions, since plants are sessile organisms. Plant biomass produced by agriculture is one of the most important resources of human life, therefore better understanding of environmental growth regulation is of paramount importance. The continuous environmental adaptation of growth is provided by the crosstalk between environmental and developmental signalling mechanisms, which results in changes in gene expression. MAP kinase (mitogen-activated protein kinase, MAPK) phosphorylation cascades are well-conserved signalling modules in all eukaryotes, which have key roles in stress signal transduction, regulation of cell division and cell growth in animals. In plants, MAP kinase pathways have been shown to play an important role in stress signalling, but recent data reveal their functions in regulating plant development as well. We found that a stress-activated MAP kinase pathway serves a dual function: suppression of photosynthetic growth coupled with induction of defence responses in the model plant, *Arabidopsis thaliana*. Our work focuses on elucidation of the role of this pathway in meristem regulation.

EFFECTS OF DROUGHT ON KERNEL DEVELOPMENT OF STRESS SENSITIVE AND TOLERANT VARIETIES OF WHEAT

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Drought stress caused by the global climate change has increasingly negative influence on agriculture. Models of the ongoing climate change predict a decrease in available water, therefore drought resistance will have increasing importance in the future, and breeders will need to develop cultivars with a higher level of drought resistance. Better understanding of the stress reactions of tolerant genotypes contributes to the successful breeding of new varieties with elevated drought tolerance.

The aim of our studies was to shed light on the changes occurred at the histological level of developing wheat kernels caused by drought stress during the phenophase of early seed development, responsible for yield losses at harvest. For this purpose, observations were made on the effect of drought stress, applied in a controlled environment from the 5^{th} to the 9^{th} day after pollination, on the kernel morphology, starch content and grain yield of the drought-sensitive Cappelle Desprez and drought-tolerant Plainsman V winter wheat (*Triticum aestivum* L.) varieties.

Morphological changes of the developing embryos, starchy endosperm cells and cell layers surrounding filial tissues were studied by histological methods. We used the method of stereology to calculate the number of the developing endosperm cells, and the amount of stored nutrients in these cells. Number of the A- and B-type starch granules and protein bodies per endosperm cells were determined.

As a consequence of water withdrawal there was a decrease in the size of the embryos and the number of A-type starch granules deposited in the endosperm, while the development of aleurone cells and the degradation of the cell layers surrounding the ovule were significantly accelerated in both genotypes.

In addition, the number of B-type starch granules per cell was significantly reduced either by the 14th day of kernel development or at full maturity.

Drought stress during early seed development affected the rate of grain filling, induced early senescence, and shortened the grain-filling and ripening period. Despite the enhanced embryo development and starch deposition induced by water deficit during early development, drought stress shortened the duration of cell division and dry matter accumulation. The plants could not overcome disturbances in metabolic processes and both the storage capacity of the grain and the yield were severely reduced.

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DETECTING INTER- AND INTRASPECIFIC RECOMBINATION EVENTS IN PLANT RNA VIRUSES WITH THE TOPALI AND RDP3 SOFTWARE PACKAGES

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Recombination among co-infecting plant RNA viruses is a natural phenomenon that appears to have played a significant role in the speciation and evolution of many strains. It also has particular significance for the risk assessment of plants that have been genetically modified for disease resistance by incorporating viral sequences into plant genomes.

In the world of RNA viruses the source of recombination during replication has a widely accepted model. By a process termed 'template switching', the viral replicase (an RNA dependent RNA polymerase) might switch from its viral RNA template to another viral RNA or to the transgenic mRNA and give rise to a recombinant RNA molecule. Recombination has played a significant role in the evolution of RNA viruses by producing genetic variation, reducing mutational load, and introducing new viruses (Worobey and Holmes, 1999). In the genus

Potyvirus, recombination has been reported for a number of different species such as Plum pox virus (PPV) (Cervera et al., 1993), Potato virus Y (PVY) (Revers et al., 1993), Bean common mosaic virus (BCMV) (Revers et al., 1993), Yam mosaic virus (YMV) (Bousalem et al., 2000), Lettuce mosaic virus (LMV) (Krause-Sakate et al., 2003), and only one from the SCMV subgroup, the Sugarcane mosaic virus (SCMV) (Zhong et al., 2005).

Several diferrent software packages exist for the detection of recombination between DNA and RNA sequences *'in silico'*. As none of the statistical methods used by these softwares are completely reliable and optimal for detecting recombination under all conditions, we applied the PDM (Probabilistic Divergence Measure) method from the TOPALi software package and several other methods (RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, Phylpro, LARD and 3Seq) from the RDP3 package.

The TOPALi software found only one interspecific recombination event in the full length MDMV genomes, while the RDP3 package detected 4 breakpoints. The analysis of the SCMV subgroups led to the detection of a large number of recombination breakpoints with the RDP3 package but none were detected with the TOPALi software.

COLORED WHEAT AS A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS

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Wheat grain is considered as a good source of potentially health components such as dietary fiber, phenolics, tocoferols, and carotenoids. Anthocyanins, another group of bioactive phenolic compounds, are found in purple and blue wheat grains. These compounds have gained much attention in many scientific research areas as they have strong antioxidant properties and can protect against many degenerative human diseases.

There has been cultivated no winter wheat variety with higher anthocyanins content in the Slovak Republic till now. In our work, we analyzed a set of 28 purple wheat genotypes created in the breeding program aimed at increasing the level of health-beneficial components. Newly bred genotypes were compared in the total anthocyanins content to control varieties as well as to parental ones. The content was in range from 3.9 mg kg^{-1} (control variety Heroldo) to 146.67 mg kg^{-1} (control variety SG-RU 440). In newly bred wheat genotypes, the anthocyanins content was between 9.47 and 47.87 mg kg^{-1}. The genotype with the lowest anthocyanins content disposed of 1.95 mg kg^{-1} higher amount than the Slovak control variety Ilona (7.52 mg kg^{-1}). Six genotypes showed higher anthocyanins content in comparison with the *Triticum etiopicum araratica* (32.87 mg kg^{-1}). The highest content was detected in genotype 704 (47.87 mg kg^{-1}).

One selected genotype was analyzed for both, milling and technological quality; as well as rheological properties and baking test were investigated. Compared to the control variety Sulamit, milling quality was little bit worse. On

the other side, all other parameters were equal or better compared to the control. This genotype is suitable for baking utilization and according to the norm STN 46 100-2 it meets requirements for grade A (baking wheat).

Our next research will be oriented in total anthocyanins composition and their stability in different environment conditions (years, localities etc.).

This work originated thanks to the support within Operational Programme Research and Development for the project: "Transfer, use and dissemination of research results of plant genetic resources for food and agriculture" (ITMS: 26220220058), cofinanced from the resources of the European Union Fund for Regional Development.

BOOSTING THE SEED PRODUCTION OF AMARANTH THROUGH MUTATION BREEDING

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As a part of the recent renewal of interest in nutritional and economic potential of underutilized and neglected crops, research in amaranth cultivation and breeding has been reinitiated providing a basis for agronomic improvement and successful large-scale reintroduction.

The genus *Amaranthus* contains about 60 species including weeds, garden flowers and crops. They exhibit a high degree of variability, are resistant to diseases, insects and weeds, also known for their tolerance to drought, salinity and high temperature what makes them a good alternative for conditions of the global climate warming. Amaranths, as C4 plants, can contribute to mitigate CO_2 concentration, the major factor provoking greenhouse effects. Because of high biomass production and ability to accumulate heavy metals they can be used as a renewable energy source and for phytoremediation. Amaranth has a very

promising nutritional potential when compared to other grains, whether cereals or food legumes with high impact on human health.

Breeding work on amaranth shows the necessity of further research and goals in improving cultivars are determined by their use: improvement of the grain and biomass yield and quality, elimination of anti-nutritional factors, early/late flowering, inflorescence architecture and colour, seed retention or improving harvestibility. The conventional plant breeding is based on the use of genetic variation and selection of the desired genotypes and requires the screening of relatively large populations. Hence, the improvement of existing cultivars through mutation breeding in combination with biotechnology tools offers several advantages over conventional breeding methods.

We focuse our research on the enhancement of the quality and quantity of amaranth grain by use of radiation mutagenesis in combination with biotechnology approaches. Two grain amaranth assessions were used for the irradiation treatment - *Amaranthus cruentus* genotype Ficha and product of interspecific hybridization (*A. hypochondriacus x A. hybridus)* hybrid K-433, both medium early cultivars with weight of thousand seeds (WTS) 0.85 g and 0.73 g, respectively. During the years 1998 – 2010, twelve generations of mutant lines with their untreated counterparts were established. The phenological observations were performed during all vegetation periods and selection on desired traits was done starting in M_2 generation. The negative plants (plants with weak seedling growth, non-uniform flowering and seed maturation, with abundant leafiness in the inflorescence area, low size of seeds etc.) were removed and only plants with positive traits were collected. The WTS was recorded and statistically evaluated. Finally, 3 mutant lines of *A. cruentus* and 1 line of hybrid K-433 with significantly increased WTS were selected with an obvious tendency to stabilization of this trait when compared to untreated controls and to the samples of the previous generations. At those selected lines genetically fixed WTS can be expected. Detailed analysis of some biochemical traits in these lines showed also improved nutritional quality of the grains over the control samples. Therefore, there is a real assumption that this plant material can be considered as valuable matrix useful in further amaranth breeding programme.

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MOLECULAR DIAGNOSTICS AND QUANTIFICATION OF *RAMULARIA COLLO-CYGNI* PATHOGEN IN BARLEY TISSUE

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Ramularia collo-cygni (Sutton & Waller) as a causal agent of Ramularia leaf spot is an important fungal pathogen which causes yield losses of spring and winter barley from 15% to 25%. *Ramularia collo-cygni* (RCC) is difficult to diagnose visually in the field conditions as it can be easily confused with physiological spotting or other pathogens such as *Pyrenophora teres* f. *maculata* and *Cochliobolus sativus*. In our study we developed and verified specific primers and Taq Man probe for reliable molecular diagnostics of RCC and its quantification in barley tissue by real-time PCR. These primers and probe were designed in specific regions of ribosomal DNA (5.8 rRNA) and internal transcribed spacer regions (ITS2) of pathogen DNA and are protected as "Utility model no. 20883" by Industrial Property Office of the Czech Republic. Real-time PCR with our probe RccSON and primers Rccj1-F and Rccj3-R, allows amplification of 63 bp fragment of pathogen DNA and quantification of the starting amount of pathogen DNA. The reliability and the particularity of Rccj1-F and Rccj3-R primers were confirmed in a group of RCC isolates and isolates of different pathogens as well as in biological material of various origin. We verified the utility and the reliability of our probe and primers for real-time PCR on RCC isolates, healthy barley leaves and seeds, naturally and artificially infected barley leaves (dry and young fresh) and naturally infected barley seeds. In tested material we found the highest amounts of pathogen DNA in dry leaves, considerably lower amounts were found in seeds and fresh leaves and non-detectable amount was in control samples. This study has shown that real-time PCR can be used to accurately monitor the colonization and development of RCC in plants throughout a growing season. Described probe and primers can be useful in plant protection, as quantitative PCR allows rapid and accurate diagnosis of pathogen in the crop already in an early stage before the first symptoms of the disease appear.

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POSSIBILITIES TO PRODUCE FUNGUS RESISTANT GM WHEAT

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The rust disease (*Puccinia spp.)* as well as the powdery mildew (*Blumeria graminis f. sp. Tritici)* cause severe loss of quality and quantity in cultivated wheat (*Triticum aestivum L.)* year by year in Hungary.

When plants are attacked by fungi part of their response is production of PR (pathogenesis related) proteins as chitinases. But the induced self-defence mechanisms does not provide enough protection in most of the cases, because either they are not effective or they are activated too late.

One of our goals is to build the *Tham-chi* gene coding for a 42 kDa endochitinase enzyme of *Trichoderma hamatum* into wheat. Preliminary results obtained with the transgene under control of constitutive promoter show promising effect of the new trait.

Genetic analysis of barley resistance responses to the compatible *B. graminis f. sp. hordei* revealed that the mutant alleles (mlo) of the Mlo gene caused a broad spectrum resistance against this pathogen. Broad spectrum resistance against *B. graminis f.sp tritici* does not exist, though orthologs of barley Mlos were found in all three genomes of the hexaploid wheat. After preliminary experiments silencing of the wheat mlo genes seems to be a useful tool to achive powdery mildew resistant plants.

EPIDERMAL CHARACTERISTICS OF THE ABSCISIC ACID HYPERSENSITIVE *ARABIDOPSIS THALIANA CBP20* MUTANT

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Water loss through transpiration is a major control point of the water balance of plants. Transpiration is a strictly regulated process, and represents a target of biotechnological efforts to improve water-use efficiency of crop plants. Water saving by restricted gas exchange does not necessarily hinder photosynthesis, and thus may prove beneficial in water-limited environments. Some abscisic acid (ABA) hypersensitive mutants display decreased transpiration and drought tolerance. ABA hypersensitivity may affect long-term stress adaptation and development, leading to water-saving morphology and contributing to water retention. Considering the decrease in transpiration in ABA oversensitive mutants, however, additional factors might be involved besides the speed of stomatal closure. The cuticle is a primary barrier against water loss, especially under severe water shortage. More trichomes may help to build a thicker boundary layer of air near the transpiring leaf surface, thus hindering gas exchange.

In the present work detailed phenotypic analysis of Arabidopsis thaliana cv. Columbia (WT), ABA hypersensitive and drought tolerant Cap Binding Protein 20 (*cbp20*) mutant and genetically complemented *cbp20* mutant (*cbp20*+35SCBP20) plants was carried out using light, stereo and electron microscopy combined with image analysis.

Stomatal density on the abaxial leaf surface of the *cbp20* mutant was significantly increased compared to the WT and to the complemented mutant line. The density of pavement cells also increased, while stomatal index remained unchanged. Despite identical ontogenetic stage of all genotypes, as a sign of over-proliferation of cells in the epidermis, there were numerous guard mother cells in

the leaf epidermis of *cbp20* mutant plants, but hardly any guard cell mother cells were present in the epidermis of WT and cbp20+35SCBP20 plants. To explore further changes in epidermal morphology of *cbp20* mutant, we investigated the density of trichomes on leaf surfaces. An increased number of trichomes was find on the adaxial surface of *cbp20* mutant leaves. Increased cuticle thickness was a major epidermal phenotype in *cbp20*. The above-mentioned morphological traits of the mutant can be reversed by introduction of a WT CBP20 cDNA. This proves the action of the mutation in formation of developmental abnormalities.

In summary, some of the new morphological features described above may help to explain the water-saving characteristics of the *cbp20* mutant. Changes in development may thus contribute to the already described dynamic features that prevent desiccation in the nCBC class of mutants under drought stress.

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ATNOA1 MUTATION MAY INDUCE TEMPERATURE ACCLIMATION MECHANISMS IN *ARABIDOPSIS THALIANA*

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The adaptation of plants to the changing environment results from the well coordinated functioning of complex biochemical signalling networks. Nitric oxide (NO) is an important signal molecule in many physiological processes, including defence responses. *Atnos1* (recently renamed *Atnoa1*) Arabidopsis mutant plants were earlier characterised by impaired NO production and organ growth, and by impaired abscisic acid-induced stomatal movements compared with wild-type

plants. The present work was focused on evaluating the effect of the *Atnoa1* mutation on temperature adaptation processes and on changes in the content of stress-related compounds, polyamines and salicylic acid. The F_v/F_m chlorophyll-a fluorescence induction parameter and the actual quantum yield were significantly lower in the *Atnoa1* mutant than in the wild-type Col-0. In the wild-type the fastest increase in the non-photochemical quenching (NPQ) occurred in plants pre-treated at low temperature (4 °C), while the slowest was in those adapted to 30°C. The NPQ showed not only a substantial increase in the light-adapted state, but also more rapid light induction after the dark-adapted state in the *Atnoa1* mutant than in the wild type. Freezing tests indicated that cold acclimation substantially increased the freezing tolerance of *Atnoa1* mutant plants, similarly to the wild type, Col-0, suggesting that the negative changes caused directly or indirectly by the mutation in ATNOA1 do not substantially affect hardening processes. The level of putrescine increased substantially, while that of spermine decreased by the end of the cold-hardening (4 °C, 4 d) period. The quantity of spermidine in *Atnoa1* was significantly higher than in Col-0, at both control and cold-hardening temperatures. A similar trend could be observed in the case of spermine, but only under control conditions. The mutant plants showed substantially higher salicylic acid (SA) contents for both the free and bound forms. This difference was significant not only in the control, but also in the cold-hardened plants. These results suggest that *Atnoa1* mutant *Arabidopsis* plants try to compensate for the negative effects of this mutation. These adaptation processes include the stimulation of photoprotection and alterations in the SA and polyamine compositions.

INFLUENCE OF THE CAMV35S PROMOTER ON ECTOPIC ACTIVITY OF FOUR TISSUE SPECIFIC PROMOTERS IN TRANSGENIC PLANTS

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In plant biotechnology there is a big demand for promoters with a strict specific activity in targeted tissues only. The activity of some plant tissue-specific promoters tested *in vivo* might be changed as a consequence of the nearby located CaMV35S promoter sequence. In this study we focused on investigation of interaction of four embryo and/or pollen specific and CaMV35S promoters in transgenic tobacco plants, individually. In case of non-specific transgene expression directed by tissue specific promoter we tested the length of spacer that was able to minimize non-specific expression pattern in transgenic plants. Our results showed that one out of four tested promoters did not show non-specific activity in transgenic plants. Other promoter did display specific pattern activity only in case when the spacer of length 5 kb was located between the plant specific and CaMV35S promoters.

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IMPACT OF GENETICALLY MODIFIED MAIZE MON 810 ON SOIL BACTERIA BY T-RFLP

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Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis is a method of comparative community analysis. The T-RFLP method includes DNA extraction/purification followed by PCR amplification. The primer set is usually a fluorescently 5'-labelled forward primer annealing to the 3' end of the antisense strand and an unlabelled reverse primer annealing to the 3' end of the sense strand of the template marker gene (16S rRNA gene). The PCR reaction thus results in double stranded DNA fragments all labelled in the 5' end of the sense strand. The PCR reaction is followed by restriction enzyme digestion, whereby each DNA fragment renders one labelled terminal fragment and one or more unlabelled fragments. The DNA fragments are then separated (±1 to 2 bases, depending on the total fragment length) by electrophoresis on the ABI sequence analyser, where the labelled fragments are recognised by the fluorescence detector. Internal standards (labelled fragment length markers) are included in each sample. The resulting chromatogram reveals the size-fragments present in the sample as well as the relative quantitative distribution among them. It is thereby possible to compare samples according to the presence/absence of peaks as well as relative distribution among the peaks.

We apply T-RFLP to analyse the bacterial community in the rhizosphere of genetically modified maize MON 810. Transgenic maize was modified to express insecticidal Cry-protein (Bt-toxins) from *Bacillus thuringiensis*. This genetic modification provides plant resistance to European corn borer (*Ostrinia nubilalis*). We used two different varieties of transgenic maize MON 810: DKC 3512 YG and MEB 483 BT. The maize DKC 3511 without genetic modification was used as control. Collection of soil samples was conducted in two terms: the end of July

2009 and the end of September 2009.We found changes in the number of individual bacterial taxa among transgenic corn and the control. Changes have also recorded a collection between July and September 2009, but these changes are not caused only by genetic modification. Major changes in diversity are caused by environmental factors.

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IDENTIFICATION OF LOCI AFFECTING GRAIN MICRONUTRIENT CONTENT IN WHEAT USING ASSOCIATION MAPPING

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Cereal based foods have great importance because 50 % of the food produced worldwide is made up of cereal grains. Wheat has the biggest importance amongst cereals. However, their grains often contain very low amounts of micronutrients; therefore micronutrient malnutrition is a serious problem worldwide. Traditional strategy is the fortification of food with minerals artificially; however, it is also possible to breed wheat with better nutritional value. In this case the first step should be the identification of loci affecting grain micronutrient contents, and the second the use of the determined markers for marker assisted selection of the favourable genotypes.

The aim of our work presented here was to determine the loci affecting the grain micronutrient contents (Fe, Mn, Mo, Cu, Zn and Se) in wheat in long term experiments using the association mapping approach.

94 winter wheat genotypes were screened for grain nutrient contents and genotypes with outstanding high nutritional value were identified. Diversity Array Technology markers were used to find markers associated with trait of interest and markers showing the most significant association will be presented.

GFP, AN ALL PURPOSE REPORTER IN THE MODEL PLANT *NICOTIANA TABACUM*

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In vitro plants of *Nicotiana tabacum* expressing reporter *gfp* and selectable *nptII* genes were micropropagated by apex transfer for more than 12 years without any observable change in transgene expression. The use of floral buds formed on transgenic plants *in vitro* as a new explant showed variation in *gfp* expression maintained up to *ex vitro* stage, at whole plant level. The segregation of *gfp* expression did not correlate with ploidy level of regenerated plants as determined by indirect tools (number of chloroplasts in stomata or stomata on leaf area); 11% of the regenerated plants did not express *gfp*. During development from seed up to fruit set in the laboratory microscopic as well as macroscopic observations show stability and Mendelian segregation of the *gfp* reporter gene. As previously reported gfp is not expressed in pollen grains and the use of right filters for fluorescent microscopy is critical to avoid errors at this level. The effects of starvation, dark and cold (4^0C), draught (PEG 6000, 10%), low pH (5.00) or osmotic stress (0.4 M mannitol) on plant regeneration and *gfp* expression were also assayed in *Nicotiana tabacum*. The number of plantlets regenerated *in vitro* from leaf segments cultured on RMG media were evaluated after 4 and 10 weeks, and *gfp* expression after 10 weeks by fluorescence microscopy at whole organ level. The results showed that all abiotic stress factors reduced the regeneration of new plants from leaf tissue with the lowest plantlet number for draught, followed by starvation, dark and cold treatments. The expression of *gfp* revealed no viable plants after draught stress, and few (up to 20%) plants with reduced *gfp* expression after starvation or lost of *gfp* in one plant on media with pH =5.00. Under extreme conditions *in vitro*, when plantlets were experiencing gradual senescence and death, the meristems – both apical and axial meristems, maintained their viability longer. This might be a surviving strategy and can be shown by gfp expression analysis at macroscopic or microscopic level.

As an all purpose reporter gfp allows evaluation of:

1) Efficiency of transformation
2) Long term transgene stability
3) Expression during plant development or under abiotic stress
4) Cell and tissue viability
5) Transgene segregation
6) The expression of 35S CaMV constitutive promoter in pollen grains and its role for further use in monitoring the risk of transgene flow by pollen
7) Monitoring senescence and protein synthesis in different plant organs under extreme stress.

CHARACTERISATION OF CONIFER EMBRYOGENIC TISSUES AND THEIR STORAGE BY CRYOPRESERVATION

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Somatic embryogenesis in *Pinus nigra* and *Abies* hybrids (*Abies alba* x *A. cephalonica*, *Abies alba* x *A. numidica*) has been initiated from immature or mature zygotic embryos as well as from cotyledons dissected from emblings or seedlings (Salajova et al. 1996, Salajova et al. 1999, Salajova and Salaj, 2001). Somatic embryo maturation of mentioned species or hybrids occurred on media containing abscisic acid and 6-9% maltose (*Pinus nigra*) or abscisic acid and polyethylene glycol 7.5 to 10% (*Abies* hybrids). After partial desiccation the somatic embryos germinated and emblings have been obtained.

The embryogenic tissues are maintained on solid proliferation media. This method of cultivation is time consuming, holds the risk of contamination and loss of regeneration ability. Recently for long-term storage, the method of cryopreservation has been applied with success to embryogenic tissues of several conifer species, since this method seems to be efficient for germplasm storage of many plant species.

Among several cryopreservation methods the slow-freezing procedure was applied to embryogenic cell lines of mentioned conifers. Embryogenic tissues of hybrid *Abies* (cell lines AC1, AC4, AC78-*Abies alba* x *A.cephalonica*, cell line AN72-*Abies alba* x *A. numidica*) were cryopreserved after sorbitol pretreatment (0.4M and 0.8M). All the tested cell lines survived cryopreservation with individual regrowth freqencies 100% (AN72), 83-100% (AC78), 37-100% (AC1), 60-100% (AC4). The post-thaw growth as well as genetic background of tissues were not negatively affected by cryostorage. After cryopreservation the regrown tissues were able to produce somatic embryos that regenerated emblings.

For *Pinus nigra* embryogenic tissues (20 cell lines) the effect of cryostorage duration on the regrowth after thawing has been tested (1 hour versus one year in liquid nitrogen,LN).The regrowth of tissues was not dependent on the duration of cryostorage and the post-thaw growth of tissues was comparable to that of control (non cryopreserved tissues). The correlation between cryotolerance and maturation capacity of cell lines has also been studied and between these parameters no correlation has been found.

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USE OF LABORATORY BIOASSAYS AND RAPD MARKERS FOR BETTER INTROGRESSION OF COLORADO POTATO BEETLE RESISTANCE INTO FUSION HYBRIDS AND THEIR PROGENIES

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The new tools of plant genetic engineering can bypass sexual incompatibility in order to improve plant resistance to biotic and abiotic stress. Potato as a very important crop ranking third at global level in productivity is a good example for biotechnological improvement. The great majority of the wild relatives of the genus *Solanum* growing in Central and South America carry many genes of resistance but are sexually incompatible with the tetraploid potato crop. We present here the development of more methods for analyzing the resistance to Colorado potato beetle (CPB) in different fusion hybrids and their progenies (BC1, BC2).

Cell fusion based somatic hybridization of potato cultivars with well characterized resistant wild relatives aiming to transfer Colorado potato beetle (CPB) resistance is going to be presented along with the development of screening methods for CPB resistance in collaboration with Julius Kühn-Institut (JKI) Germany. One method for the characterization of CPB resistance is a laboratory bioassay that evaluates the influence of feeding CPB larvae with different leaf material from parental lines, their somatic hybrids or BCs. The growth curve of larvae, their development, adult beetle emergence and fertility are all measured. Another assay was based on testing feeding behavior of adult beetles when given the choice to choose either parental leaf or somatic hybrids leaf from the combination potato+Solanum chacoense (chc). In this fusion combination both *S. chc* accession with the highest leptine production or the same accession transformed with *msh2* genes were used. Leptines are glycoalkaloids, produced

only in the leaves of *S. chc*, known to be repellent for CPB. RAPD markers linked with leptine biosynthesis were previously described.

The results indicate that more wild species may exhibit resistance to CPB and can transfer this trait through somatic hybridization to potato genepool. Such species are *S. cardiophyllum* (Thieme et al., 2010) or *S. tarnii* (Thieme et al., 2008). The somatic hybrids or their progenies show resistance to CPB although the mechanism of this resistance is not yet understood. In the case of *S. chc* the RAPD markers along with choice feeding test allows the selection of resistant fusion hybrids. Moreover, the use of DNA mismatch repair deficient transgenic *S. chc* might increase the introgression of CPB resistance into potato cultivars. The importance of different methods to assay CPB resistance and better understand the mechanisms involved are also discussed.

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BIOGAS PRODUCTION USING SILAGE MAIZE HYBRIDS BRED IN MARTONVÁSÁR

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One important component of up-to-date environment protection and energy management aimed at achieving long-term sustainable development is the widespread use of renewable energy sources, including bioenergy. It is particularly

important for Hungary, which has few fossil fuels but good agricultural potential to exploit this potential as much as possible.

At present silage maize is grown on an area of approx. 100.000 ha in Hungary. This area has declined over the last 15-20 years due to the reduction in the livestock numbers. In many countries of Europe the market for agricultural products intended for biogas production has expanded rapidly. Maize is a very favourable crop as a raw material for bioenergy as its production can be easily integrated into the farm structure.

Experiments were set up in Martonvásár in 2008 on eight silage maize hybrids grown in a random block design with two replications. Four of these were normal hybrids (Maros, Mv NK 333, Mv 437, Maxima) and four were leafy (Lfy) hybrids (Mv Siloking, Mv Massil, Mv Dunasil, Mv Limasil).

The aim of the work was to determine the quantity of biogas that could be produced from unit dry matter for the most important Martonvásár silage maize hybrids.

The biogas yield of eight Martonvásár silage maize hybrids was examined in 2008. In terms of 1 kg dry matter the greatest quantity of gas ($LSD_{5\%}$ = 15.7) was produced by the hybrids Mv Limasil (494.5 l/kg dry matter) and Mv Dunasil (490 l/kg dry matter) and the least by Mv NK 333 (409 l/kg dry matter). The grand mean of the experiment was 451.88 l/kg dry matter.

The hybrids with the highest biogas yields all belonged to the early maturity group. A correlation of –0.64 was found between the gas yield and the FAO number. However the greater yield potential of varieties with longer vegetation periods was able to compensate for this disadvantage. No correlation could be found in the present work between the *Lfy* gene and the biogas production.

Averaged over the eight hybrids tested the mean percentage gas production was 87.37%. Significant differences were observed between the hybrids, the highest values were achieved for Mv Limasil (89.5%) and Mv Maros (88.63%).

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DIRECT OR INDIRECT *IN VITRO* REGENERATION IN *KALANCHOE* COMMERCIAL HYBRIDS

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Kalanchoe blossfeldiana is a very popular indoor ornamental crassulacean plant with almost 100 hybrids. Kalanchoe originates from South Africa, Madagascar and Asia. The flowers present four petals and can be red, purple, orange, yellow, pink and white; the plant flowers in the winter months. Although *Kalanchoe blossfeldiana* can be easilly propagated by stem and leaf cuttings, *in vitro* rapid propagation may be of interest for industrial scale propagation. In this case clonal propagation has to be assured. Another opportunity is to create novelty either by selecting new forms after callus culture or by *in vitro* mutagenesis or transformation.

The goal of our study was to establish an efficient culture protocol for direct or indirect *in vitro* regeneration of flower organs: sepals, petals, anthers and carpels. Different hybrids with orange, purple, yellow, pink or white flowers, either normal or with many petals were used. Moreover spontaneous arised new forms have been also assessed for micropropagation like a ten petals flower of purple (violet) commercial hybrid or a conical flower form of a white-pink multiple petal hybrid.

From all the hybrids tested so far the best response was obtained on RMG medium (MS based) from sepals harvested from opened flowers. The direct regeneration from this explant was very efficient, particularly in the case of the ten petal form of purple (violet) flower where from 5 sepals 250 plants have been regenerated (a mean of 50 plants /sepal). The plants were acclimatized successfully and grown in a greenhouse for two years. During further flowering the proportion of five petal flowers was declining but a change of inflorescence form could be also noticed.

Towards the aim to develop new more spectacular varieties different flower organs were assayed for their response to *in vitro* culture in two culture media. Varieties with flowers having colors like: white, pink, red or yellow, with simple or

multiple corollas were cultivated *in vitro*. All the varieties tested so far regenerated plants either from sepals, that proved the best, or petals, leaves or even pistil or anthers. Mainly from petals and leaves besides direct organogenesis callus tissues were also regenerated and callus derived plants could be induced in some of the hybrids.

Further on, the utility to established *in vitro* protocols for rare mutants production, mutagenesis and/or micropropagation is going to be discussed.

As far as we know this is the first report on direct or indirect organogenesis from sepals and petals in *Kalanchoe blossfeldiana* that opens the opportunity for micropropagation of new mutant forms creating novelty in this popular house succulent ornamental plant.

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