PANNONIAN PLANT BIOTECHNOLOGY WORKSHOPS

ADVANCES IN PLANT BREEDING AND PLANT BIOTECHNOLOGY IN CENTRAL EUROPE

HOSTED BY

THE DEPARTMENT OF PLANT BIOTECHNOLOGY, UNIVERSITY OF DEBRECEN, CENTRE OF AGRICULTURAL SCIENCES

JUN 4TH – 6TH 2012

DEBRECEN

PANNONIAN PLANT BIOTECHNOLOGY ASSOCIATION

BOOK OF ABSTRACTS AND PROGRAMME

Scientific program chair
Prof. László Márton

Local organisers
Prof. Miklós Gábor Fári
Dr. Éva Domokos-Szabolcsy
Tünde Kaprinyák
Erika Kurucz
Pál Szarvas
Tarek Alshaal
Gabriella Antal

Venue:
University of Debrecen, Centre of Agricultural Sciences, Böszörményi street 138, Debrecen

PROGRAMME

SCIENTIFIC PROGRAM

SUNDAY JUNE 3, 2012

15.00-18.00 REGISTRATION

18.00-18.20 WELCOME ADDRESS
PROF. JÁNOS NAGY,
PRESIDENT OF CENTRE FOR AGRICULTURAL SCIENCES

18.30- WELCOME DINNER

MONDAY JUNE 4, 2012

CHAIR: ERVIN BALÁZS

9.30- 9.30 **OPENING THE WORKSHOP BY PRESIDENT OF PPBA**

9.30-10.00 ANDRÁS CSERI, RÓBERT MIHÁLY, LÁSZLÓ SASS, JÁNOS PAUK, IMRE VASS AND **DÉNES DUDITS**

BRIDGING GENOMIC AND PHENOMIC LEVELS IN SUPPORT OF CEREAL BREEDING FOR DROUGHT TOLERANCE

10.00-10.30 **JAMIE COSTA**

KEY FACTORS FOR THE 14 YEARS SATISFACTORY CULTIVATION OF BT-MAIZE IN SPAIN

10.30-11.00 **MAURIZIO BOSELLI**

RECOVERY AND CONSERVATION OF THE GRAPEVINE AUTOCHTONOUS GERMPLASM IN ITALY

11.30-13.00 LUNCH

CHAIR: JAMIE COSTA

13.00-13.30 JÚLIA HALÁSZ, BERNADETT SZIKRISZT, S. ERCISLI, ZOLTÁN SZABÓ, JÓZSEF NYÉKI, MAGDOLNA TÓTH, ANDRZEJ PEDRYC, **ATTILA HEGEDŰS**

RECENT PROGRESS IN TREE FRUIT SELF-INCOMPATIBILITY STUDIES

13.30-14.00 **ELENA RAKOSY-TICAN**

PLANT COMBINATORIAL BIOTECHNOLOGY – THE CASE OF POTATO

14.00-14.30 **LÁSZLÓ MÁRTON,** MIHÁLY CZAKÓ, ERIKA BALOGH, TAREK ALSHAAL, MIKLÓS FÁRI

ARUNDO DONAX: A NATURAL BIOREACTOR SYSTEM FOR ENVIRONMENTAL CLEAN UP AND CROP FOR BIOMASS PRODUCTION

14.30-14.50 **TAREK ALSHAAL**, ÉVA DOMOKOS-SZABOLCSY, LÁSZLÓ MÁRTON, JÁNOS KÁTAI, MIKLÓS FÁRI

RED SLUDGE TOLERANCE AND REMEDIATION EFFECTS OF ARUNDO DONAX L. „SYN-PLANTS"

14.50-15.20 **JÓZSEF PROKISCH,** ATTILA SZTRIK, BEÁTA BABKA, PÉTER ESZENYI, ZSÓFIA MIKA, MOHSEN ZOMMARA

PRODUCTION OF SELENIUM NANOPARTICLES BY BACTERIA AND ITS APPLICATION IN BIOLOGICAL SYSTEMS

15.20-15.30 COFFEE BREAK

POSTER WRAP UP
FIVE MINUTES/POSTER

CHAIR: MAURIZIO BOSELLI

15.30-16.00 **ZOLTÁN MOLNÁR,** VINCE ÖRDÖG, EMESE VIRÁG, PÉTER BÁLINT:
PLANT BIOTECHNOLOGICAL RESEARCH AT THE FACULTY OF AGRICULTURAL AND FOOD SCIENCES, UNIVERSITY OF WEST HUNGARY

TERÉZIA SALAJ, JANA MORAVČÍKOVÁ, ILDIKÓ MATUŠÍKOVÁ, JÁN SALAJ:
EVALUATION OF SOMATIC EMBRYOGENESIS OF *PINUS NIGRA* AND *ABIES* HYBRIDS

JUDIT DOBRÁNSZKI, ILDIKÓ HUDÁK, KATALIN MAGYAR-TÁBORI:
FACTORS AFFECTING *IN VITRO* ROOTING OF *FAGOPYRUM ESCULENTUM* CV. HAJNALKA

GABRIELLA ANTAL, LÁSZLÓ MÁRTON, MIKLÓS FÁRI:
INHERITED FROST TOLERANCE OF ARUNDO DONAX SYNPANTS IN FIELD TEST

DANIELA ZUBRICKA, LINDA PETIJOVA, EVA CELLAROVA:
CAN LOW TEMPERATURE STRESS INDUCE BIOSYNTHESIS OF HYPERICIN IN *HYPERICUM* SPP

PÁL SZARVAS, LÁSZLÓ MÁRTON, MIKLÓS FÁRI:
NEODOMESTICAATION OF ORNAMENTAL AND ENERGY MALLOWES: POLYPLOIDIZATION OF ALYOGYNE HUEGELII

16.00-18.30 SIGHTSEEING TOUR IN DEBRECEN
18.30 DINNER

TUESDAY JUNE 5, 2012

CHAIR: ELENA RAKOSY-TICAN

9.30-10.00 **MIKLÓS G. FÁRI,** MIHÁLY CZAKÓ, LÁSZLÓ MÁRTON

INDUSTRIAL-SCALE HIGH-TECH CLONAL PROPAGATION PLATFORMS FROM SUSTAINABLE SOMATIC EMBRIOGENESIS IN HIGHER PLANTS

10.00-10.30 **GÁBOR SZEMÁN-NAGY**

EXTENDED TIME-LAPSE IMAGING IN BIOTECHNOLOGY

10.30-10.50 **ZSUZSA MAROZSÁN-TÓTH,** ILDIKÓ VASHEGYI, GÁBOR GALIBA, BALÁZS TÓTH

IDENTIFICATION OF SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN COLD STRESS BY GENE EXPRESSION STUDIES IN CEREALS

10.50-11.10 **ZSOLT GULYÁS,** ÁKOS BOLDIZSÁR, DÁNIEL CARRERA, GABRIELLA SZALAI, GÁBOR GALIBA, GÁBOR KOCSY

REDOX REGULTAION OF DEVELOPMENT AND GENE EXPRESSION IN MAIZE

11.10-11.30 **DORINA PODAR,** DALE SANDERS

METAL TRANSPORTERS AS A MEAN FOR BIOFORTIFICATION

11.30-13.00 LUNCH

CHAIR: MIKLÓS GÁBOR FÁRI

13.00-13.30 MILÁN IVANICS**,** ANDRÁS KIS, GÁBOR TÓTH, ANDREA BALOGH, KRISZTINA TAKÁCS, BALÁZS BARNA, KLÁRA MANNINGER, JÓZSEF FODOR, **BARNABÁS JENES**

INVESTIGATION OF TISSUE SPECIFIC TRANSGENE EXPRESSION IN WHEAT

13.30-14.00 **CSABA MÁTHÉ**, GÁBOR VASAS, SÁNDOR GONDA, ZITA DEMETER, ANNA RESETÁR, ÁDÁM SIMON, GYULA SURÁNYI

TISSUE CULTURE OF RED LIST SPECIES FROM CARPATHIAN BASIN AND THEIR POTENTIAL USE IN THE PRODUCTION OF PHARMACOLOGICALLY IMPORTANT COMPOUNDS

14.00-14.20 **FLAVIU ROSCA,** ELENA RAKOSY-TICAN

SEGREGATION OF *GFP* AND *NPTII* TRANSGENES IN T1 AND SELF-POLLINATING GENERATIONS OF TOBACCO

14.20-14.50 **MIHÁLY KONDRÁK,** FERENC MARINCS, FERENC ANTAL, ZSÓFIA JUHÁSZ, ZSÓFIA BÁNFALVI

TRANSCRIPTIONAL AND METABOLIC CHANGES BY DROUGHT STRESS IN *TPS-1-EXPRESSING* POTATO LEAVES

14.50-15.10 **ÉVA DOMOKOS-SZABOLCSY**, MARIANN KATÓ, OTTÓ ZSÍROS, GYŐZŐ GARAB, MIKLÓS FÁRI

PHOTOSYNTETHIC ACTIVITY CHANGES IN TOBACCO CULTURE TREATED BY RED ELEMENTAL SELENIUM NANOPARTICLES

15.10-15.30 COFFEE BREAK

POSTER WRAP UP
FIVE MINUTES/POSTER

CHAIR: BARNABÁS JENES

15.30-16.00 **KATALIN FÖLDESI - FÜREDI,** HELGA AMBRUS, BEÁTA BARNABÁS: **DEVELOPMENT OF CULTURED MICROSPORES OF MAIZE IN PRESENCE OF N-BUTANOL AND 2-AMINOETHANOL**

MARTIN CARACH, TERÉZIA SALAJ, ILDIKÓ MATUSIKOVÁ: **EFFECT OF CADMIUM ON SOMATIC EMBRYOGENESIS OF HYBRID FIRS TYPES IN IN VITRO CONDITIONS**

ENDRE TÓTH, ÉVA KRISTON, KLÁRA NYERGES, CSABA TÓTH, ERIKA KURUCZ, MIHÁLY CZAKÓ, LÁSZLÓ MÁRTON, MIKLÓS FÁRI: **EXAMINATIONS OF PATHOGENES AND PESTS OF PLANT BIMASS SPECIES**

IMOLA MOLNÁR, ELENA RAKOSY-TYCAN: **CYTOGENETIC ANALYSIS OF SOMATIC HYBRIDS DERIVED FROM SOLANUM TUBEROSUM AND LATE BLIGHT RESISTANT SOLANUM BULBOSCASTANUM**

ERIKA KURUCZ, PÁL SZARVAS, GÉZA KOVÁCS, ZOLTÁN KOVÁCS †, MIKLÓS GÁBOR FÁRI: **BIOTECHNOLOGY ASSISTED BREEDING OF SIDA (*SIDA HERMAPHRODITA* L.)**

16.00-17.00 TO VISIT THE FUTURE BIOMASS PLANTS IN THE DEMONSTRATION GARDEN OF DEBRECEN UNIVERSITY, CENTRE OF AGRICULTURAL SCIENCES

17.00- DINNER IN CAULDRON WITH WINE TASTING OUT OF DOORS

WEDNESDAY JUNE 6, 2012

9.00-11.30 SCIENTIFIC TOUR TO "HORTOBÁGY PUSZTA"

11.30-12.30 LUNCH IN THE HORTOBÁGY INN NEAR TO NINE-HOLE BRIDGE

13.30 ARRIVAL TO DEBRECEN

LECTURES

SLUDGE TOLERANCE AND REMEDIATION EFFECTS OF *Arundo donax* L. "SYN-PLANTS"

T. ALSHAAL[1], É. DOMOKOS-SZABOLCSY[1], L. MÁRTON[3],
J. KÁTAI[2] AND M. FÁRI[1]

[1]*Department of Plant Biotechnology, University of Debrecen, HUNGARY;*
[2]*Department of Agricultural Chemistry and Soil Sciences,*
University of Debrecen, HUNGARY;
[3]*Department of Biology, University of South Carolina,*
Columbia-SC, USA

On October 4th 2010, the pond dam of an aluminum manufacturing plant to the west of Hungary suddenly broke and flooded many towns in toxic red sludge. 600,000 - 700,000 m^3 of sludge was estimated to have affected seven resident areas near the Ajkai Timföldgyár plant (Ajka), 160 km southwest of the capital Budapest**.** Two people died while seven were missing and hundreds of people were injured. In Devecser, 400 houses were flooded and 40 people had to evacuate to Somlvóvásárhely. In Kolontár, the mud level rose up to 2 meters.

According to the National Disaster Management Directorate, the sludge or waste of aluminum production consists of heavy metals, which makes people toxic if ingested. Red mud has great potential to immobilize heavy metals and reduce plant uptake. More and more attention is being paid to the red mud which is a fine-textured residue, derived from the digestion of bauxite for aluminum manufacturing, with alkaline property and enrichment in iron oxide. Applying red mud to treat harbor dredging contaminated with Cd and Zn could reduce metal mobility and availability. Also red mud could significantly increase metal sorption and decrease soluble metal concentrations in heavy metal contaminated soils. Furthermore, the laboratory column leaching results demonstrated that adding red mud in severely mining-contaminated soils and disused mine tailings could drastically reduce heavy metal contents in effluents. Red mud could also reduce the intake of heavy metals by plants. Adding 2% of red mud (w/w) in two contaminated soils could reduce intake of Cd, Zn, Cu, and Ni by oilseed rape, pea, wheat, and lettuce. The main problems with red mud are high salinity, pH and heavy metals contents. The red mud reduced the shoot yield of barley seedlings by 25 % when red mud applied to normal soil with 5%. In addition, red mud reduced

the germination percent for Radish and Tobacco in compare with normal soil, so it is dangerous to introduce edible plants to red mud contaminated soil. For these reasons, our aim is how we can avoid these problems and help to decontaminate this region. Biotechnologically propagated *Arundo donax* L. "syn-plants" (AD) plantlets were used as phytoremediation plant to uptake heavy metals and decrease salinity and pH. The plant toxicity, trace metal availability and biomass production for AD was tested with pure red mud and mixture of this red mud, a local non-contaminated soil by 50% dry weight fraction, local red mud contaminated soil and local uncontaminated soil as a reference. After pot experiments, our results showed that, electrical conductivity for both, pure red mud and red mud contaminated soil was decreased by 37.2% and 4.1% respectively. Pure red mud pH was also decreased by 1.0%. The available concentrations of Cd, Pb, Co, Ni and Fe were decreased after AD cultivation. Red mud increased plant Fe and Ni concentrations; however, none of these exceeds toxic limits reported elsewhere. Biomass yield of AD seedlings in pure red mud and mixture was increased by 40.4% and 47.2% respectively, in regardless with local uncontaminated soil. From these data, we could say that AD syn-plants has significant effect to decontaminate and remediate red mud contaminated soils and, in the same time gives higher biomass production under such abiotic stress conditions.

Acknowledgment

This work was supported by Interest-Trade Co, Pro-Team Co, MOP-Biotech Co, (Nyíregyháza),Kristály 88 Co. (Budapest), Ereky Károly Biotechnológy Foundation and several R & D projects in the US.

RECOVERY AND CONSERVATION OF THE GRAPEVINE AUTOCHTONOUS GERMPLASM IN ITALY

M. BOSELLI

Department of Biotechnology, University of Verone, ITALY

Grapevine cultivation is very important for Italian wine farming and economy.

The great international competition, also sustained by the diffusion of "new wines" produced in countries recently entered in the wine world, pushed us to focus on our peculiarities that can represent the only chance to be competitive in the international trade. We can summarize these important peculiarities in the presence

of many autochthonous grapevine varieties that allow us to produce a number of typical and different wines.

In the last 20-30 years the Italian grapevine cultivation underwent profound transformations. We are looking at a considerable reduction in the number of varieties and clones used for wine production. There are a lot of reasons that can explain this fact: the necessity to satisfy the demand of national and international markets for standardized products; the high adaptability to lots of different environments of some superior genotypes. Furthermore, the necessity to respect the Italian production disciplinary (D.O.C.) has increased the risk of losing the genetic variability obtained by centuries of natural and human selection.

KEY FACTORS LEADING TO 14 YEARS SATISFACTORY CULTIVATION OF BT-MAIZE IN SPAIN

DR. JAIME COSTA[1] AND IR. IVO BRANTS[2]

[1]*Regulatory Sciences Manager, Monsanto Agricultura España, Madrid, SPAIN*

[2] *Regulatory Sciences Lead EMEA, Monsanto, Europe SA, Brussels, BELGIUM*

The European Union has adopted the most complex system in the world to regulate the cultivation and use of genetically modified (GM) varieties of domesticated plants. The currently implemented regulations of these new varieties are more strict than those regulating the introduction and use of new species. GM cultivation requires thorough risk evaluations by Member State institutions and the European Food Safety Authority (EFSA) before and after approval for commercialisation. The use of GM labeling and traceability, in seeds or fractions generated from it for use as food or feed, are additional requirements. Since the use of GM labels is often misinterpreted as a safety warning, the cultivation of GM varieties has faced many challenges or bans in different EU countries. Many Bt maize varieties derived from MON810, a Cry1Ab protein containing event, are currently being grown as a standard in some areas of Spain where maize suffers from regular attacks of corn borer pests (*Ostrinia* and *Sesamia*).

The positive EFSA Scientific opinions on MON810 (www.efsa.europa.eu/en/panels/ gmo.htm) are shared among all EU countries. Spanish cultivation of Bt-maize for 14 years reached 26,5% adoption but represents 80 % of the potential Spanish area where the target pests *Ostrinia* and *Sesamia* are identified as an agronomic problem. The key factors explaining the success of Bt-maize in Spain are:

- Every year Spain needs to import 8-10 million tons of maize or cereal products at affordable prices to elaborate 20 million tons of feed which is the base of a potent transformation industry and supports around 200.000 direct jobs. If the feed industry would choose non GM products, the job losses from likely delocalization of the livestock farms would have serious consequences.
- The genetic improved event MON810, which protects the crop against target pests *Ostrinia* and *Sesamia* through confined expression in the crop tissues of a very safe and specific Bt-protein, has been shared with 10 different seed companies. These companies have included event MON810 in a range of over 120 maize varieties with a diverse genetic background and well adapted to the different soil and climatic conditions in the corn borer areas. This deployment has facilitated overall market penetration of this trait close to 80% in some regions with a total surface of 97.326 ha in 2011.
- Excellent performance against corn borers, with reduced contamination from mycotoxins (improved food and feed quality).
- An economic attractive alternative for farmers coupled with the elimination of insecticide applications for control of corn borers. Generalized GM labeling of feed products, but no need for segregation of GM grain, which would mean an additional discrimination versus grain imports.
- Pragmatic self regulation of traceability and coexistence, through Good Agricultural Practices developed and disseminated by industry. Following 14 years of growing adoption of Bt-maize requiring GM labelling, no single case of litigation among farmers for coexistence reasons has been reported.
- After 14 years of Bt-maize adoption and through rigorous implementation and monitoring of insect resistance management there has been no reporting of resistance being developed in the target pests *Ostrinia* and *Sesamia.*

PHOTOSYNTHETIC ACTIVITY CHANGES IN TOBACCO CULTURES TREATED BY RED ELEMENTAL SELENIUM NANOPARTICLES

É. DOMOKOS-SZABOLCSY[1], M. KATÓ[1], O. ZSÍROS[2], GY. GARAB[2], J. PROKISCH[3], M. FÁRI[1]

[1]Department of Plant Biotechnology, University of Debrecen, HUNGARY; [2]University of Debrecen, Institute of Bio and Enviromental Energetics, Debrecen, HUNGARY
[3]Hungarian Academy of Sciences,Institute of Plant Biology, Szeged, HUNGARY

Basic on previous experiments the red elemental nanoselenium (nanoSe) proved to be better tolerated selenium form than the selenate *in vitro* tissue culture. The elemental nanoSe in higher concentration (265-532 μM) stimulated the organogenesis (callus initiation and root regeneration) at the same time inhibited the vitrification of tobacco tissue. However the selenate in 265-532 μM concentration range inhibited the callus growth and the root formation, the microshoots on regenerating medium had been vegetated for a while and died.

To better understand the difference between biological effect of nanoSe and selenate we studied in detail the efficiency of photosynthetic pigment system on dark adapted tobacco plantlets measured by chlorophyll A fluorescence transient with Handy PEA. The chlorophyll A and B content and rate were determined by spectrophotometric method. The molecular organization of pigment system was observed by circular dichroism.

No significant difference in the chlorophyll A and B content was observed compared with the 532 μM nanoSe treatment and the control plants. However the 53 μM selenate already decreased both chlorophyll A and B content. The highest chlorophyll A/B ratio (5,06) was measured in case of 53 μM selenate which refers to some problems in the chlorophyll B synthesis. Reduction in the variable to maximum fluorescence (Fv/Fm) was only monitored in leaves of 53 μM selenate treated plants. The nanoSe didn't cause any significant difference in the Fv/Fm ratio.

The shape of the psi-type CD spectrum of 532 μM nanoSe treated tobacco leaves was very similar to the control but 53 μM selenate treated plants' leaves were differed both in blue and red-light range. These results showed that chloroplast structure of selenate (53 μM) treated plants might be disorganized however nanoSe

had no such strong effect even when it was applied in higher concentration (532 µM).

Acknowledgment

This work was supported by Interest-Trade Co, Pro-Team Co, MOP-Biotech Co, (Nyíregyháza),Kristály 88 Co. (Budapest), Ereky Károly Biotechnológy Foundation and several R & D projects in the US.

BRIDGING GENOMIC AND PHENOMIC LEVELS IN SUPPORT OF CEREAL BREEDING FOR DROUGHT TOLERANCE

ANDRÁS CSERI[1], RÓBERT MIHÁLY[2], LÁSZLÓ SASS[1], JÁNOS PAUK[2], IMRE VASS[1] AND DÉNES DUDITS[1]

[1]Institute of Plant Biology, Biological Research Center, Szeged, HUNGARY
[2] Cereal Non- Profit Company, Szeged, HUNGARY

Genetic improvement of complex traits such as drought adaptation can be advanced by integration of genomic and phenomic approaches. Semi-robotic work station serving as a Complex Stress Diagnostic System was used for computer controlled watering and digital or thermal imaging. The stress responses of individual plants from 23 genotypes were recorded by monitoring green mass production, leaf temperature and seed yield parameters. In soil with 20% water content the reduction in green pixel-based biomass ranged from 0% to 80%. As a reverse genetic approach allele mining was conducted on a panel of drought related genes in a set of barley genotypes using EcoTILLING. In the group of tolerant genotypes with less than 45% reduction in biomass a characteristic haplotype of the *HvA1* gene could be identified.

Thermal images showed warmer canopy temperature of drought exposed plants, in selected cases the actual leaf temperature could be related to green mass production. The grain yield data provided a basis for ranking genotypes by identification of tolerant and sensitive categories. EcoTILLING and DNA sequencing were used for detection of single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) in candidate genes for drought tolerance such as the Dehydration-Responsive Factor-1(*HvDRF1*) and Sodium/proton Antiporter (*HvNHX1*). Potential links between stress-responsive phenotypes and allelic variants are discussed. The Complex Stress Diagnostic System includes a root

phenotyping platform. We grow barley seedlings in plexiglass rhizoboxes filled with soil. By using digital camera root-specific traits, including a number of roots, length, growth rate were measured. After identification of genotype with drought stimulated root growth we detected higher frequency of S-phase cells by fluorescent labelling technology based on incorporation of 5-ethynyl-2'-deoxyuridine (EdU) than in roots of sensitive plants.

INDUSTRIAL-SCALE HIGH-TECH CLONAL PROPAGATION PLATFORMS FROM SUSTAINABLE SOMATIC EMBRYOGENESIS IN HIGHER PLANTS

MIKLÓS G. FÁRI[1], MIHÁLY CZAKÓ[2] AND LÁSZLÓ MÁRTON[2]

[1]*Department of Plant Biotechnology, University of Debrecen, Debrecen, HUNGARY*
[2]*Department of Biology, University of South Carolina, Columbia-SC, USA*

Somatic embryogenesis (SE) research is an important area today, however already in 1902 *Gottlieb Haberlandt,* the founding father of plant tissue culture wrote "Without permitting myself to pose further questions, I believe, in conclusion, that I am not making too bold a prediction if I point to the possibility that, in this way, one could successfully cultivate *artificial embryos* from vegetative cells" Since the first major publication of SE and subsequent regeneration of plants from carrot (*Reinert J.* & *Steward F.C.*, 1958, Naturwiss., 45: 344-345), large volume of scientific knowledge has accumulated by hundreds of scientists on this field, we can confess that we know more and more about finer details and, in spite of the intensive research the SE based industrial scale plant propagation has marginal impact in the practice. Routine introduction of SE based industrial technologies, in spite of the great expectations, has only been taken place in *Arundo* propagation and in few more near practical applications models, such as coffee, cacao, and sugarcane. In order to utilize the achievements of the 21th century biotechnological revolution in the area of clonal agriculture as well as scientific and technological gape should be filled up as soon as possible. On just the 110th anniversary of the delivery of Haberlandt's basic contribution, we can report a major scientific and technological breakthrough in the field of SE-based plant propagation and breading.

Here we report that after more than ten years of USA-Hungarian joined research the 1.000.000th *Arundo* syn-plants (SE derived, elite, virus and other pathogen free) has been produced commercially and, sold in international trade by plantlet stage. Syn-plant is our terminology for propagules produced from synchronized sustained embryogenic callus cultures by our multi-operational culture processing method (APO-system). The new approach consists of the properly synchronized cultivation and conversion of somatic embryos into functional plantlets in some steps, time efficiently with minimal loss, and without encapsulation of the SE. The obtained acclimatized syn-plants can be treated just like other field-ready propagules used in the horticulture or forestry. This presentation is going to summarise the most important scientific and technological limitations of recalcitrant SE systems, as well as new possibilities which may help their further development based on our experience with the syn-plant system. Challenging the limitations of the SE system applications, special attention has also to be paid to macro scale solutions. Questions will be raised if our knowledge about the biology of SE systems is enough and if our technological level and experience is satisfactory. During the development of the industrial scale *Arundo* syn-plant technology we learned that the accumulation of scientific knowledge regarding SE systems is not enough for the industrial-scale propagations novel technical solutions and tools are also required. *The above work has been supported by the MOP-Biotech Kft (Nyíregyháza), Kristály 88 Kft (Budapest) and by the Károly Ereky Biotechnologcal Foundation (Debrecen).*

REDOX REGULATION OF DEVELOPMENT AND GENE EXPRESSION IN MAIZE

ZSOLT GULYÁS[1], ÁKOS BOLDIZSÁR[1], DÁNIEL CARRERA[1], GABRIELLA SZALAI[1], GÁBOR GALIBA[1,4], GÁBOR KOCSY[1]

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[2]Faculty of Information Technology, University of Pannonia, Veszprém, HUNGARY

Redox regulation is important during plant growth and development, and in the response to various environmental effects. The fine tuning of this process is

ensured by the interaction of reactive oxygen species (ROS) and the antioxidants. Seedlings of a chilling-tolerant maize line were treated with different concentrations of various reductants (glutathione, ascorbate, mercaptoethanol, dithiotreitol) and an oxidant (H_2O_2) in nutrient solution for 6 days. The induced redox changes were monitored by the detection of ROS, thiols and disulphides. The effect of these changes on the development of plants and on the expression of redox-sensitive genes was studied.

The applied chemicals induced an elevation in the amount of ROS, except for mercaptoethanol, in root apex. The concentration of thiols increased in the first days which affected the redox potential, too. The treatments reduced the length and fresh weight of roots, especially in the case of the higher concentrations. The development of shoots was significantly affected only by the higher concentration of reductants. Among the studied redox-sensitive (thioredoxin5, 2-cysteine peroxiredoxin-A, glucose-6-phosphate dehydrogenase) and antioxidant system-related genes (ascorbate peroxidase and glutathione reductase) the expression of thioredoxin5 and ascorbate peroxidase gene was reduced by H_2O_2 and the higher concentration of ascorbate, dithiotreitol and mercaptoethanol. The glutathione reduced the expression of genes encoding gamma-glutamylcystein synthetase and glutathione reductase.

The redox changes affected both the development of roots and shoots and the expression of several genes.

This work was supported by the European Union, the National Development Agency and the Hungarian Scientific Research Fund (OTKA) (TÁMOP-4.2.2/B-10/1-2010-0025, CNK80781 and K83642).

INVESTIGATION OF TISSUE SPECIFIC TRANSGENE EXPRESSION IN WHEAT

MILÁN IVANICS[1], ANDRÁS KIS[1], GÁBOR TÓTH[1], ANDREA BALOGH[1], KRISZTINA TAKÁCS[2], BALÁZS BARNA[3], KLÁRA MANNINGER[3], JÓZSEF FODOR[3], BARNABÁS JENES[1]

[1]Agricultural Biotechnology Center, Gödöllő, HUNGARY
[2]Central Food Research Institute, Budapest, HUNGARY
[3]MTA Plant Protection Institute, Budapest, HUNGARY

In case of the attack of plant pathogen fungus the plants produce so called pathogenesis related (PR) proteins, as chitinases and gulcanases. These enzymes bear cell-wall degrading activity and degrade chitin and glucan (with 1,3 glucosid bonds), the main cell-wall components in the majority of fungus. In the lecture we describe here how the rust resistance in wheat was improved in Agricultural Biotechnology Center with the available tools and methods using the cmg1 gene derived from *Coniothyrium minitans* fungus which codes for a 83,2 kD 1,3-exo-glucanase enzyme.

We prepared a gene construct for specific gene expression in wheat leaves (p*RBCsCmgNOS*). In the construct the useful gene (*Cmg1*) is under the control of the promoter derived from the small subunit of wheat *Ribulose-1,5-bisphosphate-carboxilase* (RuBisCO) gene.

At the same time we grew up wheat seedlings as donor plants to provide inoculi for the gene transfer experiments. Gene delivery was carried out by biolistic method using a selectable marker gene (*bar*) beside the p*RBCsCmgNOS* construct in co-transformation. During the transformation experiments the bombarded wheat tissues underwent strickt pre-selection on culture medium complemented with *phosphynotricine* (PPT). We further selected the regenerated wheat plants applying the total herbicide (Finalé/Basta) at the doubled field dosage. The surviving plants were screend py PCR for the presence of the transgene.

The PCR+ plants were grown up greenhouse and we made biotest on them. Tolerance against the Hungarian *Puccinia* pathotypes were carried out with mixed spores provocation tests. Refynary of the biotest was made by specific staining of gungal tissues and their observation under the microscope.

Our preliminary results ar as follows:

After the tests of first 30 transgenic plants 12 plants carried also the useful gene (*cmg1*). Screening the progenies of 2 transgenic lines we observed considerable level of leaf rust resistance.

We also focus on the presence of protein encoded by the transgene *cmg1*, so we produced specific IgG antibody in rabbits and after purificaton we started the inscreening for the tissue specific occurance of CMG1 enzyme by Western blot as well as competitive ELISA.

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RECENT PROGRESS IN FRUIT TREE SELF-INCOMPATIBILITY STUDIES

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The molecular basis of fruit tree self-incompatibility was realized two decades ago when pistil expressed ribonuclease enzymes have been suggested as cytotoxic agents for self-pollen tubes. The past two decades have witnessed a sharp increase in our knowledge regarding the pollen component gene, self/non-self discrimination, transition from self-incompatible to self-compatible phenotype etc. Besides *S*-genotype determination, which is of crucial importance in commercial orchards, our analyses are mainly focused on the evolutionary history of and phylogenetic relationships among and within fruit tree species. In apricot (*Prunus armeniaca*), we clarified that self-compatibility might have occurred in Eastern Anatolia and by spreading to the West it has resulted in a serious loss of genetic diversity. In sweet cherry (*P. avium*), we declared overlapping allele pools of sweet and sour cherries in the gene centre of these species. The comparison of almond (*P. dulcis*) and peach (*P. persica*) indicates the genome shaping effects of selective forces under different ecological conditions. Self-incompatibility analysis confirmed the genome of old apple (*Malus* × *domestica*) cultivars from the Carpathian basin was enriched by several *Malus* taxa and hence differs from the modern commercial cultivars. Our studies on apricot, sweet cherry, almond, peach and apple helped to understand the effects of self-(in)compatible phenotype on the genetic constitution of a fruit tree species.

This work was funded by the OTKA PD78124 project and also supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

TRANSRIPTIONAL AND METABOLIC CHANGES ELICITED BY DROUGHT STRESS IN *TPS1*-EXPRESSING POTATO LEAVES

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Drought stress severely reduces the yield of major crops world-wide. To decrease the effect of drought, several plants accumulate low molecular weight water-soluble compounds such as trehalose to provide stress tolerance to their cells without disturbing cellular machinery. Previously, we demonstrated that lines of the potato cultivar White Lady expressing the trehalose-6-phosphate synthase (*TPS1*) gene of yeast exhibit improved drought tolerance. The aim of our work was to investigate the responses of the drought-sensitive potato cultivar White Lady and its drought-tolerant *TPS1* transgenic variant to prolonged drought stress at both the transcript and metabolite levels.

The mRNA expression profiles of leaves were compared using a potato microarray containing 42,034 potato unigene probes. 379 genes were identified whose expression depended on genotype, drought stress or their interaction and had at least 2-fold change in their expression. Twice as much gene with altered expression was found in wild-type than in *TPS1* transgenic leaves, and 112 of these were common. Expression of 57 genes was changed only in *TPS1* transgenic leaves. Majority of the genes with altered expression implicated in photosynthesis and carbohydrate metabolism were down-regulated in both the wild-type and *TPS1* transgenic plants. In parallel with this, starch concentration of the leaves was very low. We found four transcription factor genes uniquely up-regulated in *TPS1* transgenic leaves which are good candidates for future functional analysis in order to understand the regulation of the 57 genes whose expression was changed only in *TPS1* transgenic leaves.

Metabolites were analysed by GC-MS. At the metabolite level, the amounts of fructose, galactose and glucose were increased and decreased in the wild-type and *TPS1* transgenic leaves, respectively, while the amounts of proline, inositol and raffinose were highly increased in both the wild-type and *TPS1* transgenic leaves under drought condition that appears to be a general response of potato to drought stress. *This work was supported by the Hungarian grant OTKA F68318.*

IDENTIFICATION OF SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN COLD STRESS BY GENE EXPRESSION STUDIES IN CEREALS

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Cold signal transduction is a process, which starts with the perception of cold stimulus and leads to the expression of different cold induced genes resulting cold acclimation and finally increased freezing tolerance. So cold acclimation is accompanied by changes in many physiological and biochemical processes. These processes are controlled by the expression of hundreds of genes may be altered when plants are exposed to low temperatures. The main cold regulated genes are *Cbf* (C-repeat Binding Factors) transcription factors, which are known regulators of the cold-regulated (*Cor*) genes.

In the present study we identified signal transduction components involved in cold acclimation and frost tolerance of einkorn wheat (*Triticum monococcum)* genotype *'G3116'* using pharmacological approach. Our main target pathways were the phospholipase C and D (PLC and PLD) pathways and we analyzed the role of calcium in plant abiotic stress responses also. For the examination we used certain inhibitors, signal transduction pathways were blocked: we decreased the calcium response by blocking calcium channels using lanthanium and by chelating calcium using EGTA. Phospholipase C signaling pathway was inhibited by neomycin and the activity of phospholipase D was blocked by buthanol. We used control and cold treated plants. We analyzed the expression level of *CBF*s and we found that *Cbf12* and *Cbf14* genes are cold induced, so we focused on these genes and in addition *Cor14b* showed high expression level also. Real-time PCR technique was used to analyze the expression level of these cold regulated genes.

We observed that cold induction of *Cbf14* did not change after block of calcium response, but cold induction of *Cbf12* and *Cor14b* genes were decreased. In conclusion *Cbf12* and *Cor14b* affected by calcium response in plant, but the cold

induction of *Cbf14* is calcium independent. In order to investigate the role of PLC and PLD pathways in the induction of *CBF-COR* system, inhibition of PLD using buthanol, leaded to decrease of the cold induction of all the tested genes. The block of PLC pathway by neomycin resulted in decreased *Cbf12* expression suggesting its dependence from the PLC pathway, but *Cbf1*4 and *Cor14b* appeared to be independent.

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ARUNDO DONAX: A NATURAL BIOREACTOR SYSTEM FOR ENVIRONMENTAL CLEAN UP AND CROP FOR BIOMASS PRODUCTION

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Monoculture-forming monocots are important components of different ecosystems and are often able to produce huge biomass, more than most cultivated plants. Arundo donax is not only one of the most promising biomass energy crops, but it has at the same time great remediation potential. We developed a system where biomass/energy production is combined with environmental remediation and wastewater treatment. Since Arundo is by far the largest biomass producer plant > 100 dt/ha/y) a large amount of contamination can be removed by harvesting the biomass. We have developed and patented a universal cell culture initiation medium and procedure which allowed us to establish embryogenic cell cultures, micropropagation and genetic engineering protocols, which have successfully been used for a great number of species from a diverse group of monocot genera (Cyperaceae, Juncaceae, Poaceae, and Typhaceae). This technology allows us to produce millions of plantlets for large (>1000 ha) projects and at the same time somaclonal breeding procedures can also be used in order to generate and select elite lines with hyper remediation ability for different contaminations. Elite lines for TCP dehaloperoxidation and for salt tolerance have been selected.

Novel plant/bacterial consortiums can also be constructed by colonizing the sterile plantlets with bacterial strains isolated from polluted environments, and/or with selected bacterial strains with special features such as certain chemical decomposition abilities to treat specific soil and surface water contaminations (such as halogenated organics, hydrocarbons and oil) After colonization, the acclimatized plants can be introduced into the polluted environment where the gradual clean up will take place.. Simultaneously, with the clean up of the area and/or the waste water, valuable biomass is produced for direct burning for heat and electricity (as pellet or chips), for pulp, charcoal/biochar, cellulosic alcohol, other liquid fuel, biogas or even for hydrogen production.

TISSUE CULTURE OF RED LIST SPECIES FROM THE CARPATHIAN BASIN AND THEIR POTENTIAL USE IN THE PRODUCTION OF PHARMACOLOGICALLY IMPORTANT COMPOUNDS

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We have established a significant number of embryogenic and organogenic cultures from explants of protected/ endangered species of the Carpathian Basin. These cultures included relatives of saffron (*Crocus sativus*), namely *C. heuffelianus* (Demeter et al., Plant Cell Tiss. Org. Cult. 100:349-353, 2010), *C. scepusiensis*, *C. tommasinianus* belonging to the *C. vernus* aggregate as well as *C. banaticus*. To our best knowledge, tissue cultures of these species have been performed in our laboratory for the first time. In addition, we possess cultures of *C.sativus*. Except *C. scepusiensis*, that are organogenic callus cultures, all other systems are embryogenic and all cultures were capable of plant regeneration. *In vitro* cultures of *C. sativus* are known to produce carotenoid derivatives or their metabolites that are of culinary and medicinal use (crocin, crocetin, picrocrocin and safranal, class I of compounds). In addition, they are able of producing considerable amounts of antioxidant compounds including enzymes playing a role in oxygen radical scavenging (class II). Although cultures of wild saffron are not likely to contain

significant amounts of class I compounds, they are potential producers of antioxidants of class II. Another system of our laboratory consists of embryogenic cultures of snowdrop (*Galanthus nivalis*) capable of plant regeneration with efficient bulblet regeneration. Snowdrop belongs to *Amaryllidaceae*, a family known to produce peculiar alkaloids of medicinal use. Besides the red list species mentioned, *Plantago lanceolata* cultures producing significant amounts of pharmacologically important products are part of our collection. Our aim is to screen and optimize all *in vitro* systems mentioned for well-known and potential novel biologically active compounds.

METAL TRANSPORTERS AS A MEAN FOR BIOFORTIFICATION

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Transition elements such as zinc, iron, cobalt, nickel, manganese, and copper play essential roles in the biological systems as enzyme cofactors, or as part of regulatory and structural proteins. The most abundant ion in the metal binding proteins is Zn^{2+}. Bioinformatics approaches suggest that 10% of the human proteome comprises of zinc-binding proteins. With such a large number of proteins requiring Zn^{2+} adequate intake is essential for human health. It is estimated that worldwide, two billion people are suffering of mild to severe Zn^{2+} deficiency. For humans, the main source of Zn^{2+} is through diet, therefore through agricultural products, which in many parts of the world are represented by plant-based foods. Consequently, plants play a significant role in defining humans' Zn^{2+} nutrition and in general humans' health. The low content of Zn in the endosperm of cereal kernels is one of the main causes of Zn deficiency in humans.

Among the transporters involved in the deposition of Zn and other metal cations in the grains are the ubiquitous CDFs (Cation Diffusion Facilitators). Much interest in this transporter family is now focusing on the implication for human health. Potentially, plant CDFs could be used to give raise to cereals with an

increased nutritional value, by the production of plants with elevated micronutrient content within seeds. We are currently addressing this issue by over-expressing barley CDF (HvMTP1) in the endosperm under promoters of genes for storage proteins deposited in the developing endosperm (*e.g*. D-Hordein). As a control we have expressed the HvMTP1 under the control of an aleurone specific promoter Ltp2.

Key words: zinc, metals, Cation Diffusion Facilitators (CDFs), Metal Tolerance Proteins (MTPs), endosperm, vacuole.

PRODUCTION OF SELENIUM NANOPARTICLES BY BACTERIA AND ITS APPLICATION IN BIOLOGICAL SYSTEMS

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Bioavailability and toxicity of selenium (Se) are strongly related to the chemical form Organic Se forms are less toxic but highly bioavailable forms, therefore they have significant importance in functional foods, food and feed supplements. Yeast is applied for the industrial production of selenium-methionine from inorganic Se. Recent interest in the field of nanotechnology has stimulated research into the chemical synthesis of selenium nanoparticles that are composed of elemental selenium [Se(0)]. Reports on the reduction of selenite to elemental Se by sulphate reducing, selenium respiring bacteria has been published first time in the 1990s. A novel technology for producing Se nanospheres in homogeneous in form and size within a short period of time (4-24 hours) has been developed in our laboratories. The technology developed is a manufacturing process which enables forming of a suspension as well as a powder containing valuable Se spheres having unique characteristics. Material prepared in such a way can be used in the food industry as food or feed additive. The relative simplicity of the technology developed allows for significant decline in prices which can further broaden the range of useful high

quality raw materials available. We applied yogurt bacteria for the production of nanospheres. The technique is the first to use lactic acid bacteria and other probiotic bacteria (Species of *Lactobacillus and Bifidobacteria, Streptococcus thermophilus*) to form the product, Se nanospheres. The invention enables the production of red and grey elemental Se nanospheres in high purity by using micro-organisms applied in the food industry. These bacteria are commonly used, non-toxic and harmless. The main advantage of the process makes possible the production of uniform nanospheres in a specific size of 50-500 nanometers (with 5-20% standard deviation) in diameter depending on the species used for the fermentation. Compared to the conventional used chemical synthesis it is better regulated technology. The process of the technology is suitable for producing elemental Se nanospheres sized 50-500 nm, wherein the size distribution of nanospheres is generally characterized by a percentage deviation from the mean size of 5-20%. The microorganism used in the process of the technology may be selected from the group consisting of the following species: *Lactobacillus bulgaricus, Lactobacillus acidophillus, Bifidobacterium bifidum, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus rhamnosus and Bifidobacterium longum*. By the use of microorganism belonging to the genus *Bifidobacterium*, grey Se comprising 400-500 nm sized nanospheres can be produced. By the use of microorganism belonging to the genus *Lactobacillus*, red Se comprising 100-300 nm sized nanospheres can be produced. Red Se is produced comprising 50-100 nm sized nanospheres by the use of microorganism belonging to the species *Streptococcus thermophilus*. The process of the technology is advantageously performed by using a liquid medium containing carbon- and/or nitrogen-sources, inorganic ions and other organic materials as necessary. Skimmed milk can be used very well as a medium. The liquid medium applied has to contain selenium in the form of a selenite salt, in the form of sodium-selenite, in 20-400 mg L^{-1} Se concentration. The fermentation time is between 4 and 48 hours. Drying the produced yogurt we obtained the Lactomicrosel, what is a dried, Se enriched yogurt. Purified elemental Se nanospheres can be obtained by digesting the bacteria. Different methods (enzymatic, chemical, and physical) were tested for this. The most effective method was the digestion with concentrated HCl. The effect of different Se forms (inorganic, organic and nanosize selenium) on mice were compared in a toxicological experiment. The feed concentration was 0, 0.5, 5.0 and 50 mg Se kg^{-1}. The feeding period was 2 weeks. The lowest toxicity was obtained in the case of nanoselenium while the bioavailability was the best in case of low

concentrations. In the highest concentration (50 mg Se kg^{-1} in the feed) lethality was 90 % in the inorganic Se treatment and 0 % in the nanosize Se group.
We have found that several species of probiotic bacteria are capable of producing spherical elemental Se nanospheres having an average diameter in the range of 50-500 nm when 10-10 000 mg Se L^{-1} was added to the medium in the form of selenite ions. In these bacteria, if the selenite concentrations above 1-2 mg L^{-1} seemingly induce detoxification processes, whereby the bacterium reduces selenite and excretes Se intracellularly in elemental form. This selenium form has a very low toxicity and a very good bioavailability. According to the toxicological and the feed testing experiments this Se form is a promising food and feed additive.

PLANT COMBINATORIAL BIOTECHNOLOGY – THE CASE OF POTATO

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The new tools of plant genetic engineering, molecular biology, cellular engineering, marker assisted selection (MAS) and haploidization or other *in vitro* techniques, such as stress selection as well as the data from functional genomics can be used together towards a common goal, the transfer of resistance genes into crop gene pool.

Classical breeding although successful in obtaining good quality crops failed to improve crop resistance to biotic and abiotic stress. The majority of the crops have got wild relatives, which developed during a co-evolutionary race between the host plant and disease or pest, different mechanisms of resistances, some controlled by resistance genes (R genes). It appears that many of those genes are clustered on few chromosomes and have some common structures like leucine rich repeats (LRR). Although it was thought that resistance could be transferred into crops by one gene, it occurred soon that one R gene transfer is conferring a short-lived resistance. Moreover, the causing agent of the disease develops new strains resistant to that gene. In order to achieve the goal of durable, sustainable resistance the only way is to transfer quantitative trait loci (QTLs) and to apply

more biotechnological tools for the introgression and expression of resistance traits for as long as possible. Creating diversity by using more wild genetic resources and mechanisms and combining more biotechnological tools i.e. combinatorial biotechnology would eventually allow us to obtain a new generation of crops with both good quality and resistance to biotic or abiotic stress.

Potato ranks third in the global crop production and represents not only an important vegetable rich in carbohydrates, minerals and amino acids, but also an important source of animal feed, starch and ethanol – biofuel, and recently of human health promoting compounds discovered in the tuber skin.

We are going to discuss a strategy of combinatorial biotechnology where different tools should work together to deliver the resistance genes into potato gene pool. In the case of potato such a combination includes: genetic transformation of Solanum chacoense (chc resistant to Colorado potato beetle – CPB), for MMR deficiency to increase homeologous recombination, somatic hybridization of potato cultivars with chc by protoplast electrofusion, selection of resistant hybrids by using molecular markers (RAPD) linked to leptines biosynthesis, repellents for CPB, testing of resistance by the use of laboratory bioassay and choice test and finally integration into breeding new varieties resistant to CPB. Other examples for multiple resistance traits or resistance genes pyramiding as for instance the Rpi-blb1 and Rpi-blb3 genes from the wild species *S. bulbocastanum* to induce resistance to late blight will be also presented.

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SEGREGATION OF *GFP* AND *NPT*II TRANSGENES IN T1 AND SELF-POLLINATING GENERATIONS OF TOBACCO

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The use of GFP has proved to be very useful in many studies such as monitoring transgene presence and expression, visualizing young organs and viability. Also, the use of GFP as a genetic marker has enabled the researchers to

study cellular functions from a different point of view. The green fluorescent protein (GFP) was extracted from jellyfish *Aequorea victoria* and since the discovery it has been an important tool used in biology. This small protein (27 kDa) emits green fluorescence when excited by UV or blue light. The studies have shown that GFP is an important tool for real-time monitoring of plants grown in the field, because once GFP is driven by a promoter, its expression will be reliable and predictable. This characteristic of being able to predict the pattern of GFP fluorescence, in all plant organs of various ages, is an important application in the field of agriculture and ecology.

The advantages of using GFP instead of other marker genes that allow visual detection of transgene expression, such as *uidA, luc* and *GUS*, reside in the fact that this protein does not require the addition of a substrate or cofactors for fluorescence to be detected and it does not present destructive characteristics for the plant tissue, as shown by Molinier *et al.* (2000). Its expression was detectable from all parts tested from plants grown in the laboratory and in the field. These transgenic plants containing the *gfp* gene were modified with *Agrobacterium tumefaciens* strain *LBA 4404*, which was transformed by electroporation with plasmid **pHB2892**. This plasmid contains the *gfp* gene controlled by the 35S CaMV promoter and the *nptII* gene isolated from the vector pRT99. According to Molinier *et al.* (2000), the seedlings in T1 were divided into three classes by their level of GFP fluorescence (high, low and no fluorescence), all possessing the T-DNA in a single locus. The segregation for their experiment was 25% high-level fluorescence, 50% low-level fluorescence and 25% of the seedlings showed no fluorescence (1:2:1). The absence of GFP fluorescence was correlated with kanamycin sensitivity and absence of T-DNA, while high and low levels of GFP fluorescence corresponded to kanamycin resistance (*nptII*) and presence of T-DNA, which concluded that low-level fluorescence is indicative of hemizygous plants. The quantity of GFP fluorescence can distinguish, by screening, between homozygous and hemizygous seeds and seedlings.

The purpose of this study is to identify and observe the segregation of *gfp* in transgenic plants of tobacco (*Nicotiana tabacum L. cv. Samsun NN*) in the context of self-pollinating generations but also the segregation of *nptII* gene in T1. All the plants used for this study were T2 generation, grown on MS medium from seeds provided from *in vitro* transgenic plants received in 1998, the same source of the tobacco plants that Molinier *et al.* (2000) used in their experiments. Since then, the T1 plants were regenerated and transferred until they grew floral buds for self-

pollinating and produced the necessary seeds which conducted to this present study. The experiment for identifying the segregation type of *gfp* and *nptII* transgenes was repeated three times in the past two years and the data was collected. The expression of *gfp* was monitored at macroscopic level by using an appropriate UV lamp. Both homozygous and hemizygous plantlets could be easily identified and after taking digital photos a ratio between different states of gfp expression could be calculated and compared by applying χ^2 test. After the analysis based on the experimental data the segregation of the *gfp* gene does not match the expected values for Mendelian segregation.

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EXTENDED TIME-LAPSE IMAGING IN BIOTECHNOLOGY

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Most living organism share a common attribute, namely the dinamics of constant changes of parameters describing their actual state. Most methods used in life sciences provides data on actual, momentary parameters. This so-called snapshot-studies leaves out of scope one of the most important feature of living things, as their dinamics remain unrevealed. Time-lapse sudies of biologycal structures has a great importance in any experiment related to a timescale parameter. Time-lapse imaging is a powerfull method for nondestructive observation. Low time resolution sequecnes can be used in plant related investigation under phisiologycal and pathologycal conditions. For example one of our recent developments, called IS-MOS compares images acquired in visible green, near infrared and thermal spectrum offering a unique possibility for plant water distribution, early plant disease detection and many other parameters on large scale and in circadian manner, with a runtime of 7/24. We also developed time-lapse videomicroscopy for monitoring growth properties of Arundo donax primordial embrios. Cell culture of mammalian cells can be also investigated by this method, offering a chance to

learn more about the biologycal effects of natural products in vitro. We are following the growing cells under standard physiological conditions using time-lapse microscopy using one minute time resolutuion from the attachment of monolayer cells to complete confluency of senescence. The methodology is based on the establishment of four inverse incubator microscopes and the persfusion of fresh cell culture medium during the incubation period without touching the cell culture flasks. Our in situ staining and perfusion system played a key role in our recent papers published in international journals (video: http://www.youtube.com/watch?v=QDRC0JOjkc0). These investigations included quantitative parameters (confluency, cell number, cell size, motility, generation time, morphology, cell-cell interactions, apoptosis, necrosis) and will be extended to characterize the impact of eg. nanoproducts exerted on cell populations, with particular attention to cell-cell and cell surface interactions based on the experience accumulated during the analysis of 150 thousand images.

POSTERS

A NEW ELEMENT IN THE FIGHT BETWEEN PLANTS AND VIRUSES: THE HC-PRO PROTEIN OF PVY INHIBITS THE STUBSNF1 KINASE COMPLEX IN POTATO

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Potato virus Y (PVY) is one of the most important pathogens of potato. The viral infection is a strong biotic stress and can cause an extreme loss in tuber yield. In infected plants, significant biochemical and molecular changes occur. A part of these changes serves the synthesis of virus particles, while the other part of changes is necessary for plant protection. In plants, the SNF1-related protein kinases (SnRKs) are the central integrators of transcription networks in stress and energy signalling. SnRKs are heterotrimeric enzymes consisting of the serine/threonine kinase, along with two subunits, SNF4 and GAL83.

In potato there are at least two SnRKs: PKIN1 and StubSNF1. We found that the StubGAL83 subunit of the StubSNF1 kinase complex can interact with the helper component proteinase (HC-Pro) protein of PVY in yeast two-hybrid system. HC-Pro is a multifunctional protein. It is a known gene silencing suppressor, plays an important role in the movement of the virus within the host plant and is responsible for the spreading of the pathogen through aphids. In yeast two-hybrid system, we localized the regions responsible for the interaction on both molecules with nested deletions and by cloning PCR-amplified fragments. We found that StubGAL83 binds to the N-terminal region of HC-Pro that is not essential for replication or movement of the virus but is involved in aphid transmission. The HC-Pro binding site on StubGAL83 overlaps with the binding site of the StubSNF1 kinase. Since StubGAL83 is a positive regulator of the kinase activity, interaction of HC-Pro with StubGAL83 may reduce the activity of StubSNF1 and facilitate the multiplication and spreading of the virus.

To verify the interaction between StubGAL83 and HC-Pro in a plant system, we are expressing the two proteins in *Nicotiana benthamiana*. We constructed antisense

StubGAL83 potato plants to investigate their susceptibility against the PVY infection. Preliminary results show that the StubGAL83-repressed plants are more susceptible for viral infection than wild-type plants.

THE POTENTIAL OF ARTIFICIAL PLANT OVARY (APO) CONCEPT IN PLANT BIOTECHNOLOGY

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In the past ten years, the industrial scale micropropagation technology development focused on the utilization of new technical achievements and molecular biological technologies. Major efforts have been made for the development of liquid culture based bioreactor technologies where large surface of the explant is in direct contact with the culture medium, resulting in faster and better growth conditions than the on media solidified with agar or other subtances.
After several years of research with different bioreactor models our conclusion was that a multifunctional programmable set up is the most promising system for the programmed control of the complex morphogenetic events in the bioreactor chamber. Two versions of this multifunctional programmable plant micropropagation equipment have been developed, one large, multi-shelfed for large scale programmed plant propagation, the Artificial Plant Ovary or APO, and another, a small version for laboratory research the LAB-APO. which have been used the Phyto Bio Reactor Laboratory in the Department of Plant Biotechnology of UD since 2011. The LAB-APO can be placed on any culture room shelf, every part is autoclavable allowing safe sterile working conditions. The sytem is protected by a patent application "*Berendezés és eljárás a növények tömeges, programozható mikroszaporítására*" c. szabadalom által védett (P 09 00018, 2009. 01. 20., HPO, Budapest)
The advantage of the APO system is that in the same chamber several subsequent changes in the culture conditions can be administered from stationer culture to different horizontal or vertical motions, programmed medium exchanges for the induction developmental changes, medium level regulation, and regulation of the

acclimatization process (humidity, temperature, gas composition of headspace such a way that the APO chamber remains in the culture room, closed.

Presently, the main goal of the LAB-APO based research is to develop cost efficient micropropagation processes for important ornamental and other important plant species. *Iresine* sp., *Hosta* sp., *Chryzanthemum* sp., *Nephrolepis* sp., *Gerbera* sp., *Musa sp., Ananas sp., Eucalyptus sp.*) In the case of Hosta sp Blue Angel 50% , in Iresine 75% decrease in labor cost was achieved. With the help of the LAB-APO the biological background of the different sustained somatic embryogenesis based technologies can also be studied, such as nutrient uptake utilization, toxicity, metabolism, and growth regulation by endogenous and exogenous factors. These studies are focused, and directly applied on the propagation of 3rd generation biomass plants , Arundo donax and Miscanthus sp.

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THE EARLY WORKS OF KARL EREKY (1898-1901)

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The name of Karl (Károly) Ereky may be known by scientists as well by those, who are interested in the biotechnology. It is not by chance that his name sounds familiar, because he was that Hungarian mechanical engineer, who created the term *biotechnology* (*Biotechnologie*) in 1919. But how was Ereky able to create to concept of this important, autonomous but still multidisciplinary science of the 20th and 21st century, since the biotechnology is one of the most advanced and most developing discipline of our age.

Károly Ereky was born on October 20, 1878, in Esztergom. After the secondary school in Sümeg and Székesfehérvár, Ereky studied at the Budapest University of Technology, where he graduated as mechanical engineer in 1900. During his academic years and before, Ereky showed an interest in the contemporary development of the sciences; he was interested not just in the

engineering, but also in the biology, chemistry, economics and agriculture. He published in the column of the Hungarian scientific journal *Hungarian Industry* (Magyar Ipar), called „Industrial innovations". Ereky's journal articles appeared also in the weekly *Sümeg and its sorroundings* (*Sümeg és Vidéke*) and other journals, in addition he had founded an own journal with the name *Latifundium* (*Nagybirtok*). Ereky followed both the Hungarian and the international scientific literature. He read a good number of international journal articles, which were also translated by him. Ereky wrote reviews about the most recent scientific achievements and inventions of his time, since he spoke many languages and he was familiar with the modern research.

In my research I used mostly the „Industrial innovations" column of the *Hungarian Industry*, concerning the early scientific activity of Karl Ereky. During his academic life he published a number of articles, which presented his scientific views and methods determining his later attitude towards the science, politics and economics. I have analyzed 265 of Ereky's journal articles between 1898 and 1901 published in the *Hungarian Industry*, which demonstrate that Ereky was well apprised of the industrial development of his age. In these publications Ereky dealt with the researches carried out in the agriculture, chemical industry, mechanical engineering and electronics. There is an other issue, however, the so called complex industry, which combines the four different disciplines; the 36% of Ereky's short bulletins deals with this complex industry.

Among these publications I have found the first mention of many industrial and technical innovations in Hungary. Some of them, like the 'Spirit made of wood' (1898. XIX (48)) or the 'Human waste-matter and the bacteria' (1900. XXI. (12)), which are already concerned with the 'use of the life'. On my opinion, the so called 'grey literature' of the science, like the early publications of Karl Ereky, should be analyzed more intensive than today. Ereky was an extraordinarily premature and innovative expert compared to his age, but his multidisciplinary approach would make him a leading scientist even today as well.

EFFECT OF CADMIUM ON SOMATIC EMBRYOGENESIS OF HYBRID FIRS TYPES IN *IN VITRO* CONDITIONS

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Somatic embryogenesis is the process of development of embryo (or embryo-like structures), without fusion of gametes from somatic cells or from plant tissues in *in vitro* conditions. Somatic embryos undergo similar morphological, structural and physiological stages of their development as embryos in the seeds.

The aim of our work is to give a picture of the impact of cadmium on the growth of fresh weight and dry weight in two lines of fir´s hybrids: *Abies alba* x *Abies cephalonica* (line AC 78) and *Abies alba* x *Abies numidica* (line AN 72) during the period of cultivation (0^{th}-20^{th} day). We used two lines: AN 72 and AC 78 induced from immature zygotic embryos and long kept in Petri dishes in a nutrient medium DCR (BAP - 0.5 mg.l-1). Tissue was in the exponential growth phase (8^{th} day) transferred to basic medium (DCR) enriched with different concentrations of $Cd(NO_3)_2.4H_2O$ (250 μM and 100 μM). Samples for analysis of the growth (increase in fresh weight and dry weight) were performed at 5 days (0^{th}-20^{th}) five repeats.

Effect of cadmium was manifested in the morphology of tissue - in the control medium (DCR without cadmium) was tissue white to yellowish white in color, sometimes translucent with prominent somatic embryos. Cadmium in both concentrations after 10 days caused tissue browning due to the accumulation of toxic products (mainly phenols) and visual loss of tissue. Cadmium in both lines had stimulatory effect during the first days of cultivation: DCR + 100 μM stimulated growth of both lines more intense as the tissue in the control medium; but after about 10 days culture growth was inhibited. Cadmium has a negative effect on the micromorphology of somatic embryos, which is reflected incoherence, disintegration and weaker organization of bipolar somatic embryos.

In conclusion, the effects of heavy metals lead to slower growth, or to stop it after some time (depending on the concentration of metal compounds and the exposed plant), which was confirmed in other experimental models (Godbold and Huttermann, 1985; Masarovičová *et al.,* 2004).

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CELLULAR EFFECTS OF NANOSELENIUM PARTICLES

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The biological effect of nanostructures came into the research focus in the last decade. In agreement with the conclusions of the EU on the „Impact of Engineered Nanomaterials on Health: Considerations for Benefit-Risk Assesment „ (Chapter 5, p. 37) that was released in September 2011 and serves as a research guide, our intention is to develop a methodology that takes into consideration the double nature of nanoparticles related to their physical and chemical interactions. The investigation of nanostructures beyond their micromechanical, biophysical properties was made possible by the image forming system developed by our team. Adherent monolayers of human keratinocyte cell line (HaCat) was used to investigate the dinamic parameters of cell breeding in the presence of chemically and biologically porduced nanoselenium particles. 1,35 $X10^6$ HaCat cells were placed in two T-25 flask using RPMI 1640 (PAA E15-842) + 10% FBS (PAA A15-101) + 1% antibioticum-antimycoticum (GIBCO 15240-062). Flasks were then placed under four custom built inverted microscope located inside a Sanyo MCO-18AIC CO_2 incubator. Cell breeding was carried out under standard conditions at 37°C and 5% CO_2. Transmission light microscopic images were taken in every minute using our eTox LTS extended time-lapse imaging system. Image acquisition parameters were tuned for maximal greyscale dinamic range resolution (averaging

of 5 auto-intensity histogram equalized images) Near-infrared illumination was applied and exposure times were minimalized to avoid phototoxicity. Time difference between images taken from each flask were not more than 6 seconds ± 8%. Quantitative analysis of the image sequences was carried out using NHI ImageJ software pack and plugins. Time dependeny of basic quantitative parameters were analyzed: Cell monolayer surface/ flask surface ratio, Cell number, Number of divisions, Apoptotic event rate, Necrotic event rate, Monolayer motility, Dynamic enthropy. Analysis of custom parameters also possible.

EXTRACTION AND SEPARATION METHOD OF NEUTRAL LIPIDS FROM OIL PRODUCING ALGAE

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Continued use of petroleum sourced fuels is widely recognized as unsustainable because the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable fuels are necessary for environmental and economic sustainability. Microalgae appear to be the source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. Depending on species, microalgae produce many different kinds of lipids and other complex oils. Using microalgae to produce biodiesel will not compromise production of food and other products derived from crops.

In this project we examined the lipid fraction of an oil producing green algae. We developed a microextraction method to obtain the total lipid fraction of the algae avoiding degradation and autooxidation of lipids. Only the neutral lipid fraction can be used for biodiesel production. Therefore we adopted and optimised a method for separation of neutral lipids from total lipid fraction purifying it from other contaminants such as polar lipids, chlorophyll and other pigment molecules.

The amount of neutral lipids were measured by a spectrophotometric method based on the color formation of glycerine from enzimatic digestion of neutral lipids.

MORPHOLOGICAL TRAITS AND PHYSIOLOGICAL RESPONSES OF FOUR WHEAT GENOTYPES WITH CONTRASTING DROUGHT SUSCEPTIBILITY

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Inadequate water supply may impose severe abiotic stress on plants. Its consequences impair the growth of crop plants and decrease yield. The extent of damage depends on both severity and duration of drought stress.

Tolerance to this environmental condition is complex and may be achieved by several different strategies. We set off to uncover possible determinants of tolerance by studying four wheat cultivars with contrasting drought susceptibility. Drought sensitive 'Cappelle Desprez', 'GK Élet' and tolerant 'Plainsman V' and 'Mv Emese' winter wheat (*Triticum aestivum*) genotypes were investigated. Harvest parameters were measured under cyclic drought stressed and control growth conditions, which supported the presumed characteristics of the cultivars.

Morphological, physiological as well as some genetic traits were followed in order to find relevant information on the tolerance mechanisms behind the phenotypes. A detailed morphological analysis of leaf epidermal structures potentially relevant to water loss was performed in all four cultivars. We found that tolerant genotypes had lower stomatal density and smaller leaves thus altogether possessed significantly less stomata per leaves and per plants than sensitive ones. These traits could contribute to more efficient withholding of water through stomata during a drought period. The total number of stomata per leaf was the lowest in the drought tolerant 'Plainmsman V' and the highest in drought sensitive 'Cappelle Desprez' cultivars.

We performed osmotic stress treatment by supplementing polyethylene glycol (PEG) to hydroponic growth media of all four wheat genotypes. PEG concentration was increased stepwise, and relative water content of leaves was measured under osmotically stressed and control conditions. Surprisingly, the two drought tolerant genotypes displayed contrasting behaviour under osmotic stress, 'Plainsman V' and 'Mv Emese' showing the lowest and highest tolerance respectively.

Gene expression analysis was also performed by targeting a soluble starch synthase gene by RT-PCR. Sustained expression of this gene under drought stress displayed correlation of yield parameters among the cultivars investigated.

These features of leaf morphology, genetic as well as physiological responses may contribute to different strategies leading to drought hardiness of the tolerant wheat cultivars investigated, in comparison with the sensitive ones.

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FACTORS AFFECTING *IN VITRO* ROOTING OF *FAGOPYRUM ESCULENTUM* CV. HAJNALKA

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In our previous experiments the optimal cytokinin content of shoot multiplication media using different explant types, i.e. shoot tip or nodal segment was determined. In present study the post-effects of shoot multiplication conditions, the effects of the vitamin and indole-butyric acid (IBA) content of rooting media on *in vitro* rooting of *Fagopyrum esculentum* cv. Hajnalka were studied. Before root induction shoot tip cultures were multiplied on medium contained 10 µM meta-topolin while nodal segment cultures were multiplied on medium contained 5 µM benzyl-adenine. Rooting media contained 0.5 x MS salts, 3% saccharose, 0.5% agar-agar and MS (Murashige-Skoog) or B5 (Gamborg) vitamins were applied in combination with four level of IBA (0, 1, 3 and 5 µM). The rooting experiments were carried out in 400 ml Kilner jars; in each jar four explants were placed on 20 ml of rooting medium and they were grown at 22°C with a 16-h photoperiod at PPF

of 105 µmol s^{-1} m^{-2}. Each treatment consisted of 15 jars, i.e. 60 explants in total. After four weeks the rooting percentages, the number of roots per shoot and the average root length (mm) were counted. The experiments were repeated in triplicate. Data were analysed statistically using multi-, and univariate analysis of variance followed by Tukey's test. High (97-100%) rooting percentage was observed in each treatment. However, root number and the length of roots were significantly different depending on the vitamin and IBA content of the rooting medium and on the conditions of previous shoot multiplication. Moreover, significant interactions were proved between these factors. If rooting was induced after shoot multiplication using shoot tip explants on medium with 10 µM meta-topolin, the highest root number per *in vitro* shoot (7.8) was achieved by application of either MS vitamin and 5 µM IBA or B5 vitamin and 3 µM IBA. The root length was satisfactorily high (34.4 mm) using B5 vitamin and 3 µM IBA. If rooting was induced after shoot multiplication using nodal segments with 5 µM benzyl-adenine, the highest root number per *in vitro* shoot (7.5) and the highest root length (34.7 mm) was obtained at application of MS vitamin and 3 µM IBA. Results can contribute to the development of efficacious micropropagation method.

EVALUATING GROWTH INHIBITORY EFFECTS OF *ROBINIA PSEUDOACACIA L.* WOOD EXTRACTS AGAINST VARIOUS MICROORGANISMS

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The need for more efficient use of natural resources recently became apparent. Lowering the amount of harmful chemicals released into the environment is a crucial task. Besides it is essential to seek for novel resources closer to natural origin.

The aggressive growth of certain plant species is attributed to their metabolic features as well. Several plant secondary metabolites have been identified recently that are crucial for their intensive spread. These metabolites are only present in particular taxonomic groups. The most important attribute they share is that they

regulate the interactions between the plant and the environment. These metabolites often possess traits that inhibit the growth of pathogen microbes. Due to their antimicrobial effects these bioactive substances may be utilized as novel pesticide agents in the future.

In our project we assessed the inhibitory effects of various black locust (*Robinia pseudoacacia* L.) wood extracts on the growth of different microorganisms using agar plate disc diffusion method. Our results suggest that black locust wood extracts evolves comprehensive inhibition against both gram-negative and gram-positive bacteria and *Fusarium proliferatum*. The highest bioactivity was carried by the hexane and methanolic fraction. Further purification using HPLC revealed some particular components that show significant correlation with the inhibitory effects of the extracts. Some extracts were able to lower the infection ability of *Fusarium proliferatum* in *in-vivo* infection tests.

DEVELOPMENT OF CULTURED MICROSPORES OF MAIZE IN THE PRESENCE OF *N*-BUTANOL AND 2-AMINOETHANOL

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Double haploid (DH) technology is an important breeding tool in plant breeding because DH plants reduce time to develop new cultivars. Plant regeneration from maize microspore cultures has been reported in a limited number of genotypes. This is because maize microspore culture is highly genotype-dependent therefore this system is not enough efficient to producing DH plants yet.

The present study focused on the effects of two biogenic alcohols, *n*-butanol and 2-aminoethanol on the androgenic induction of maize microspores. These two alcohols have been reported as triggers for microspore division in wheat anther culture and embryogenesis by disrupting cortical microtubules and detaching them from the plasma membrane inducing symmetric nuclear division (Soriano *et a*l. 2008; Rajaeijan *et al*. 2011). Similar data could not be found in the literature related to maize.

Microspore donor plants (A18 hybrid) were grown in a phytotron chamber (Conviron, PGV 96). Tassels were harvested when most of the microspores were in the late uninuclear stage of development and stored at 7 ºC for 10 days. After the cold pre-treatment anthers were placed in modified YP media (Genovesi *et al.* 1982) in the presence or absence of *n*-butanol (0.2-0.8%) or 2-aminoethanol (2-8 mM) for 6 hours, or *n*-butanol (0.2-0.4%) or 2 mM 2-aminoethanol for 18 hours. Then microspores dehisced from the anthers were cultured in liquid modified YP medium at 29 ºC. The 3-4-week-old microspore-derived structures (embryos and calli) were transferred individually onto a regeneration medium (N_6O_1). Viable doubled haploid (DH) plantlets were potted into soil and grown up to maturity in the phytotron chamber.

Present results demonstrated that both ***n*-butanol and 2-AE increased the number of induced microspores** as compared to control (0.02%). It seemed that 0.2% *n*-butanol for 6 hours (0.04%) and for 18 hours (0.03%) treatment resulted in the highest microspore induction. **The embryo yield was also slightly increased** by the treatments with *n*-butanol and 2-AE as compared to the control. The results show that the ***n*-butanol and 2-AE treatments affected the green plant regeneration frequency** too. Plant regeneration of microspore-derived structures were higher in the cases of 0.2% *n*-butanol (6 hours) and 0.2% *n*-butanol and 2 mM 2-AE (18 hours) treatments than in the control treatments. **The percentage of fertile plants also increased after** 0.2% *n*-butanol (34.6%) and 0.2% *n*-butanol (27%) and 2 mM 2-AE (31%) treatments for 18 hours as compared to the control (21%) treatments.

In summary we found that 0.2% *n*-butanol treatment for 6 hours and for 18 hours and 2-aminoethanol treatment for 18 hours had positive effect on the induction of maize microspores. At the same time the embryo yield was slightly elevated. The plant regeneration and the plant fertility was significantly increased using these treatments. However, further biochemical and structural investigations are necessary to discover the exact mechanism of the cellular and subcellular effects of the two compounds on maize androgenesis.

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EFFECT OF PLANT GROWTH REGULATORS ON BIOSYNTHESIS OF HYPERICIN AND FORMATION OF LEAF DARK GLANDS IN SEVERAL *HYPERICUM* SPECIES

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The aim of this work was to study the effect of selected elicitors (plant growth regulators-PGRs) on accumulation of hypericin and formation of dark glands on leaf in selected *Hypericum* species.

The genus *Hypericum* comprises more than 450 species with different ability to produce hypericins which are accumulated in morphological structures, called nodules or dark glands. The dark glands are multicellular black or dark-red structures which occur on the aerial parts (leaf and flower petals and sepals) of hypericin producing plants. The formation of dark glands is species-dependent. One of the most intensively studied species is hypericin producing *H. perforatum* (St. John´s wort). Today, extracts of plant are utilized in medicine for its antiviral, antimicrobial, antidiuretic activities but especially for treatment of depression.

The representatives of the genus *Hypericum* produce compounds such as naphtodianthrones (hypericin, pseudohypericin, protohypericin, protopseudo-hypericin), phloroglucinols (hyperforin, adhyperforin), flavonoids and essential oils. Hypericin is a taxonomic marker for the genus *Hypericum*, it is a photosensitive dianthrone, dark red, hydrophobic and soluble in organic solution with a fluorescence emission maximum of 590 nm.

In our experiment several exogenously added PGRs were used to influence plant morphogenesis and formation of hypericin in *in vitro* conditions. The shoot cultures of several hypericin producing and hypericin lacking *Hypericum* species were cultivated during 30 days on three different modified liquid MS (Murashige and

Skoog 1962) culture media supplemented with PGRs: medium according to Liu *et al*. (2007) containing half of the normal concentration of nitrate; 0,1 mg/l BA; 0,01 mg/l IBA; media according to Coste *et al*. (2011) as follows: Coste1 (½ the normal concentration of nitrate; 0,4 mg/l 2iP; 0,2 mg/l BA; 0,1 mg/l Kin) and Coste2 (0,4 mg/l BA; 0,05 mg/l NAA) prepared. The number of leaf dark glands and the content of total hypericins (hypericin, protohypericin, pseudohypericin, protopseudo-hypericin) measured by HPLC were determined in the control and PGRs treated plants. It was found that the PGRs positively up-regulated the formation of dark glands in leaf of the hypericin producing species, e.g. in *H. perforatum.* We have recorded more than 2-fold increase of the number of dark glands. On the other hand in the hypericin lacking species, e.g. in *H. canariense* glands were detected neither in controls nor in PGRs treated plants. The stimulatory effect of PGRs supplemented medium was observed also on production of hypericins. In *H. perforatum* approximately 4-fold increase of total hypericin was detected. The formation of hypericins in *H. canariense* was not found.

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EFFECT OF RADIATION MUTAGENESIS ON SOME QUALITATIVE AND QUANTITATIVE TRAITS IN AMARANTH PUTATIVE MUTANT LINES

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Grain amaranth (Amaranthus spp.) is a widely know pseudocereal with interesting nutritional characteristics including proteins, well suited to human nutritional needs.

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. During the years 1998 – 2011, thirteen generations of mutant lines with their untreated counterparts were established. Finally, 4 mutant lines of *A. cruentus* and 3 lines 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 (1).

Detailed analyses of biochemical traits such as soluble oxalate level, protein and amino acid content in the grains of mutant lines showed improved nutritional quality over the control varietes. Comparative estimation looking for amaranth mutants with minimal content of antinutritional soluble oxalic acid was performed by multivariate statistical approach. The C15, C26, C82, D279 and D282 mutant lines were identified as a variants with the significant and long-term lower soluble oxalate concentration in comparison to respective references. Based on analyses of protein content, fractions and electrophoretic profile we can conclude that protein composition of amaranth seeds can be slightly changed by irradiation, although we did not find any significant diference comparing non-treated and mutant seeds. Regarding amino acid composition canonical discriminant analysis revealed line C26 as variant with most significant differences during whole observation period (2, 3).

On the basis of our results we can conclude that this plant material can be considered as valuable matrix useful in further amaranth breeding programme.

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METABOLIC AND HORMONAL CHANGES IN TUBERS OF DROUGHT TOLERANT POTATO PLANTS EXPRESSING THE TPS1 GENE OF YEAST

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Potato (*Solanum tuberosum* L.) is the third most important food crop in the world. Compared to other crops, potato is considered drought sensitive, and even short periods of stress can cause significant reductions in tuber yield. In order to obtain drought tolerant potato plants, we have previously transformed the *S. tuberosum* cv. White Lady with the trehalose-phosphate synthase 1 (*TPS1*) gene of yeast. Although, the transgenic lines became drought tolerant, the plants displayed stunted growth and a lower CO_2 fixation rate. Transcript- and metabolic profiling identified several mRNAs and metabolites with altered amounts in leaves. Since alterations in leaves can influence tuber development and quality we investigated the metabolite and hormone composition of the *TPS1* transgenic tubers.

Plants were grown in greenhouse under optimal and drought stress conditions. The mass of tubers produced by the transgenic plants was 30% less than that of the wild-type (WT) plants under optimal conditions. However, while the stress reduced the yield of WT plants by 50%, the decrease was only 5-10% in case of transgenic plants. The tubers were divided into two groups. One group of tubers were analysed freshly while the other group of tubers was stored at room temperature for three months. The metabolite profile of tubers was analysed by GC-MS. The stress reduced the starch content of tubers, however, increased the concentrations of proteins, fructose, galactose, sucrose and malic acid. Transgenic

tubers were strongly delayed in sprouting. The storage increased the fructose, galactose, mannose, and glucose content in each tuber. In contrast, enhanced concentrations of sorbitol and maltose were detected only in stored WT tubers.

Hormones are known to regulate tuber dormancy. We could measure the concentrations of auxin, abscisic acid (ABA), salycilic acid (SA) and jasmonic acid (JA) by UHPLC. We found that the auxin content was increased during storage in WT but not in transgenic tubers. The stress increased the ABA content of all tubers. Unexpectedly, a further increase in ABA content upon storage was found in WT as well as in transgenic tubers harvested from well-watered plants but not in transgenic tubers harvested from stressed plants. The stress increased the JA and SA concentrations only in WT tubers.

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BIOTECHNOLOGY ASSISTED BREEDING OF SALVIA NEMOROSA L.

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The more and more frequently occurring extreme conditions in the continental weather zone requires the continuous renewal of the market which generates constant challenge for the ornamental plant breeders. Most of the traditionally used decorative ornamental plants are sensitive to these extremes. In 2001, our department initiated a interdisciplinary breeding program in collaborations with Zoltan Kovacs to introduce new or reintroduce forgotten drought tolerant ornamental species into public parks and roadsides.

From the ~900 species the Salvia genus the *Salvia nemorosa L. h*as been known as a medical plant, however, because of it's high adaptation ability and decorative nature it is a highly recommended ornamental plant as well. The *S.n* is a low maintenance, extremely drought tolerant fast growing and generates proper cover outcompeting weeds on roadsides.

Presently 50-60 varieties are available however it is increasing by new hybrids. Great morphological and color variation can be seen within the species from different white to deep violet. The main goal of our research is the production of elite lines with wide color and morphological variation. We have already obtained 31 different clones for further investigation without eliminating the original plants generating an in vitro genebank as it has been done by Italian breeders (*Ruffoni et al, 2004*).

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INTRODUCTION AND BIOTECHNOLOGY ASSISTED BREEDING OF ALYOGYNE SP.

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Majority of ornamental crops used for indoors and landscaping are subtropical or tropical plants, however, for domestic introduction species originated from continental climatic conditions are better fit. There is an increasing demand for new decorative indoor and outdoor ornamental plants . Specially for landscaping stress tolerant, low maintenance but still decorative plants are required. In order to increase the poor winter offering of potted plants, new less demanding indoor plants are required by the market. One new candidate for this purpose is the *Alyogyne* sp. This species has been recenly introduced to our department from Viçosa, Brazil in 2009. We named it „Christmas star mallow" Plants form the *Alyogyne* genus are bushes related to the *Hibiscus genus*. The *A. huegelii* és az *A.* Hakeifolia species are well know ornamentals in Australia and in the USA. The Christmas star mallow is a decorative short day plant with colorful leaves and flowers. For the summer the plants can be transferred outdoors, easily adapts to those conditions. Propagation is also easy, both from cutting and seeds. Cuttings

with few buds can be rooted in 10-12 days in September and form flowering plants by winter time. The breeding potential of the species has not been explored however the *Alyogyne* sp. is a potent new decorative perennial ornamental plant both for outdoors and indoors.

The project has been supported by the *Interest-Trade Kft, the Pro-Team nKft, the MOP-Biotech Kft, (Nyíregyháza),the Kristály 88 Kft. (Budapest),the USC Research Foundation and by the Károly Ereky Biotechnolgy Foundation (Debrecen)*

BIOTECHNOLOGY ASSISTED BREEDING OF SIDA (*SIDA HERMAPHRODITA* L.)

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Our research program is focused on perennial mallow species which are not only ornamentals but the same time they are large biomass producing potential energy plants. The American virginian mallow or sida has been introduced to Europe during the 1930-es (USSR, Ukrain) as a potential fiber plant. It has been studied as a potential ornamental species in Poland from the 1950-es and in Hungary from the 1970-es (by Zoltan Kovacs).Commercial varieties of sida has not been produced, although it may have serious economic impact in the future as biomass plant, animal food and even as a vegetable for human consumption, honey plant, pulp, or phytoremediation plant. From 2003 we have studied sida in our Future Biomass Plants Garden of the Department. The first observations already indicated that the sida population obtained from natural american stands can be an excellent starting material for a complex breeding program in order to improve the germination rate, to increase biomass production by poliploidization as well as to develop in vitro propagation, genetic transformation procedures for further molecular breeding for improved honey production, disease resistance and fiber quality. One of our main area of mallow breeding and research is neodomestication and crossing of mallow species with 2n=28 chromosomes. In 2010, the sida poliploidization program has been initiated both from the sida seeds originated from ZK collection (under the

name of Napaea dioica) as well as from seeds originated from USA wild sida populations (provided by Drs. Czako and Marton). In 2011 different stem color variations have been identified in the population and the propagation the germination rate has been improved by hot water treatment from 5 to ~50% than by low temperature treatment further increased ~70%. From poliploidization experiments 4n = 56 individuals have been obtained and their seeds germinated with a higher rate +70% without frost treatment and resulted in a homogenous population, with less sensitivity to lower light intensities (hypocotyl elongation) under winter greenhouse conditions.. In vegetative propagation experiments, 100% of the 5 cm long root cuttings and 50% of the 5cm cuttings from the transient region developed into plants. The stem color in the somaclones inherited, (it was linked to similar color of the anthers) indicating clonal stability of the color variations in the population.

This work has been supported by the *Interest-Trade Kft, the Pro-Team nKft, the MOP-Biotech Kft, (Nyíregyháza),the Kristály 88 Kft. (Budapest),the USC Research Foundation and by the Károly Ereky Biotechnology Foundation (Debrecen).*

EFFECT OF CRYOPROTECTIVES ON PHASE TRANSITION IN CELL SUSPENSION CULTURES OF *HYPERICUM PERFORATUM* L.: MICROSCOPIC STUDY

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Controlled cooling and vitrification represent currently the most profound methods for long term preservation of plant genetic resources. Special attention is aimed at medicinal plant species such as *Hypericum perforatum* L. that are able to produce secondary metabolites with wide range of pharmacodynamic activities. Successful long term preservation of high producing genotypes is therefore of eminent importance.

There are at least two main factors affecting survival, a vital key determinant of preservation success. We have previously shown that dehydration and cooling rates have significant influence on the outcoming ability to recover an intact plant.

Thermal gradients generated during the phase transition process indicated increased generation and progression of ice front possibly damaging the cell architecture. Therefore, the main aim of this work was to investigate the structural integrity of *Hypericum perforatum* L. cells exposed to different osmotic and cooling rate treatments.

We cooled cell suspension cultures with and without previous treatment with cryoprotective solutions (10% (v/v) DMSO, 10% (v/v) glycerol, 0.58 M sucrose) and PVS3 (1.4 M sucrose, 50% glycerol) by various rates (0,05; 0,1; 0,5; 1,0 a 5,0 °C/min) to -40 °C. In contrast to samples exposed to cryosolutions, we observed progressive dehydration of cells and significant intracellular ice formation during cooling the cells without pretreatment with cryoprotective aditives. This indicates that the freezable water content in non-cryoprotected cells is higher and it seems to be the main cause of the cell damage. It was supported by overall cell morphology difference where cells after exposure to cryoprotective solutions had cytosol localized in the centre of the cell. We also observed that higher cooling rates resulted in faster ice progression with larger ice crystals. Furthermore, dendritic ice was recorded at the highest cooling rate, penetrating the suspension cell clusters. Comparison of the effect of both cryosolutions showed that PVS3 caused more severe plasmolysis as a result of higher osmotic strength than cryoprotective solution, which supposedly leads to detected lower temperature of crystallisation in cells exposed to PVS3. On the basis of this observation we cooled samples exposed to PVS3 by cooling rate 120 °C/min to -120 °C and warmed it by 10,0; 50,0; 100,0; 120,0 °C/min. During warming we observed recrystallisation correlating with the rate of heating. We proposed that it is one of the reasons of severe desintegration of plant tissues after vitrification.

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CYTOGENETIC ANALYSIS OF THE SOMATIC HYBRIDS OF *SOLANUM TUBEROSUM* AND LATE BLIGHT RESISTANT *SOLANUM BULBOCASTANUM*

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The tetraploid (2n=4x=48) potato *Solanum tuberosum* has a large wild gene pool including about 200 wild relatives. Wild species of *Solanum* have numerous genetic characteristics favorable from the agronomical point of view. Typical for these species is their wide geographical distribution and range adaptation. The wide range of habitats of wild potato species is in line with their adaptation to stress environments and resistance to pests and diseases.

Solanum bulbocastanum (2n=2x=24) is an important representative of wild potato species, which is highly resistant to all known races of *Phytophthora infestans* but is sexually incompatible with cultivated potato.

Somatic hybrid plants of various ploidy levels obtained after protoplast electrofusion between *S. tuberosum* cultivars and *S. bulbocastanum*, carrying two known resistance genes Rpi-blb1 and Rpi-blb3, have been analyzed by cytological methods. The introduction of foreign DNA fragments to the host genome may involve structural and numerical chromosome rearrangements.

Primary roots of *Solanum* hybrids, *S. tuberosum* and *S. bulbocastanum* obtained by regenerating nodal sections were used to study somatic metaphase chromosomes. Metaphase stages were stained with DAPI (4'-6-diamidino-2-phenylindole) and observed under UV fluorescent microscope (Olympus BX-60).

At least five cells with metaphase chromosomes of each hybrids and parents were photographed, and MicroMeasure 3.3. software program was used to measure for each chromosome pair the followings: short arm, long arm and total chromosome length. The centromeric index and the arm ratio were then calculated and used to classify the chromosomes as recognized by Levan (1964). In addition, total haploid chromosome length of the karyotype, average chromosome length, and average arm ratio have been calculated.

Karyograms have been constructed by organizing the chromosomes into groups according to their arm ratio, ordering them by decreasing length within each category, and finally numbering consecutively using this same scheme.

Structural chromosome rearrangements are difficult to identify in *Solanum* hybrids, because the chromosomes are small and similar in morphology. Variation in chromosome number has been frequently observed in genomes of somatic hybrids. They can be caused by the fusion process, somatic incompatibility or *in vitro* culture. Further FISH technique will be applied for the identification of resistance genes Rpi-blb1 and Rpi-blb3 integration in the potato genome in relation with resistance to *Phytophthora infestans* in detached leaf or field test and their segregation in BCs generations of the somatic hybrids.

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PLANT BIOTECHNOLOGICAL RESEARCH AT THE FACULTY OF AGRICULTURAL AND FOOD SCIENCES, UNIVERSITY OF WEST HUNGARY

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The research activity of Institute of Plant Biology is focused on two topics of plant biotechnology. Our works in the field of *microalgal biotechnology* are based on the Mosonmagyaróvár Algal Culture Collection (MACC). Recently we have nearly 1000 of self isolated and collected cyanobacterial and microalgal strains from soils. Research studies connected them are: *in vivo* and *in vitro* investigation of their ecological requirements and beneficial compounds for higher plants, as well as their interactions with other beneficial microorganisms and higher plants, which can be used for lucrative plant treatments. Over 300 eukaryotic strains were tested by bioassays, 55% showed slight auxin-like and 43% showed minor cytokinin-like activity. We have observed auxin-like activity in 12 and citokinin-like activity in 23 eukaryotic strains with high biomass production. About 200 cyanobacterial strains

were evaluated according to their plant hormonal activity: 13% of them have auxin-like and 21% of them have cytokinin-like effects. Only one cyanobacterium was found with significant auxin-like effect and two of them with significant cytokinin-like activity among the high biomass producing strains. Microalgal biotechnology has the potential to provide solutions for the unsafe supply of fossil fuels. The lipid content and lipid production of some greean algal strains (*Chlorella*, *Scenedesmus*), which can be useful in biodiesel production, were also evaluated by several cultivation experiments.

Large seeded grain legumes (e.g. pea, faba bean, fenugreek), as recalcitrant plants *in vitro*, were the first objects in our *plant cell and tissue culture (plant biotechnology)* studies. The aim of our first experiments was the evaluation of different parameters in order to establish and maintain of *in vitro* cell and tissue cultures of grain legumes. Afterward we have conducted several experiments on micropropagation properties of some medicinal herbs and ornamental plants (e.g. lavender, foxglove, coneflower, lillies, orchids) isolated from apical meristems and shoot tips. Hereafter we were working on the *in vitro* cultures of pea and *Beta vulgaris* species with the support of several research grants.

Our two fields of plant biotechnological research are connected to each other: we are studying the growth regulator effects of different substances derived from microalgal and cyanobacterial biomass, as natural compounds in the *in vitro* culture media for tobacco, pea, corn, coneflower and orchids.

IN VITRO STRESS SELECTION OF TRANSGENIC POTATO PVYCP - I - PVYCP, INITIAL RESULTS

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Potato, an excellent plant used in alimentation, is the fourth most important crop in the world. It has a number of pathogens, such as: insects *Leptinotarsa decemlineata,* fungi *Phytophthora infestans,* viruses (PVY, PVX etc.) that cause important losses in the annual production. The presence of these pathogens is another reasons for the potato to be one of the main targets for genetic

improvement by gene transfer or other biotechnological tools. In previous experiments transgenic, marker-free, potato was produced using a two-step protocol. In the first step the transfer and expression analysis of reporter gene *gfp* (green fluorescent protein) and marker gene *npt*II (neomycin phosphotransferase) were used to improve transformation of different cultivars of potato (Rakosy-Tican et al., 2007). In the second step *A. tumefaciens* C58C1 pGV2260 with the construct pRGG YCPiPCY (35SCaMV enhancer and promoter, two repeated inverted PVY-CP sequences separated by an intron and pAnos terminator) was used for transforming the best responding potato cvs, Baltica and Desiree. After *Agrobacterium* transformation, regenerated plants were analysed by PCR using different primer combinations and a high percentage of transgenic plants was identified (Rakosy-Tican et al., 2010).

Climate change brought about new challenges for potato production such as lack of water or draught stress. Starting from the premise that a plant already resistant to a particular stress may be resistant to other types of stress too, we try to select variants of transgenic potatoes resistant to PVY, which manifest resistance to drought by using *in vitro* stress selection. Towards this aim callus cultures have been obtained from internodes of cvs. Baltica and Desiree of both control and transgenic potato and potatoes, which have been transformed with agrobacterium but did not integrate the transgene in the genome. The draught stress was simulated *in vitro* on callus cultured three weeks on MS-T media with 5% PEG. Then the callus with first regenerated shoots was transferred on MS-T media, without PEG. The initial results showed that after PEG induced stress, some regenerated shoots suffered necrosis, formed kind of runners or even vitrified. But some of the plants with integrated PVY resistance gene showed also resistance to draught stress. These results have to be further investigated in order to obtain plants resistant to both PVY and draught stress in the two potato cultivars.

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CAN LOW TEMPERATURE STRESS STIMULATE BIOSYNTHESIS OF HYPERICIN IN *HYPERICUM* SPP.?

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Over the last years, human driven environmental alterations caused a significant depletion of plant natural habitats, reducing the phytodiversity. Vitrification method emerges as a procedure that can not only preserve the plant genetic resources, but offers many advantages in long-term storage of plants with agro-economical and pharmaceutical importance. Increased interest is aimed at identification, biotechnology and storage of plant taxons producing bioactive substances applied in diagnostics and therapy of civilization diseases. Hypericin, a photo-sensible molecule produced by several members of *Hypericum* genus, is recently being applied in photodynamic therapy of malignant cancers.

Despite the number of advantages, cryo-storage generates stress environment that can affect the physiological state and genetic constitution of plants. Although the stressor`s influence is mostly considered adverse, our previous results indicate an increase in biosynthetic capacity of hypericin after liquid nitrogen storage. Since hypericin is cumulated in specific anatomic structures of dark nodules, it is expected that stress-induced biosynthesis should be accompanied by increase in number of hypericin storage structures. The hypothesis was tested in selected *Hypericum* genus representatives with different capacity for hypericin biosynthesis, subjected to vitrification and compared to control plants. Overall content of hypericin and its derivatives was determined by HPLC and correlated with number and size of dark nodules. Correlation between the number of morphological structures and hypericin content under influence of cryopreservation treatment-associated stressors is discussed.

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EVALUATION OF SOMATIC EMBRYOGENESIS OF *PINUS NIGRA* AND *ABIES* HYBRIDS

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Somatic embryogenesis in conifers is destined to have an increasingly important role in the study of fundamental aspects of early plant development as well as in genetic engineering studies. The method could also play an important role in forestry as a vegetative technique of rapid plant (trees) propagation. The process can be divided into several steps, as initiation of embryogenic tissues, their long-term maintenance, maturation of somatic embryos and germination leading to plant regeneration. For each step special culture conditions are required.

In *Pinus nigra* Arn. and *Abies* hybrids (*Abies alba* x *A.cephalonica* and *Abies alba* x *A. numidica*) embryogenic cultures have been established by culture of juvenile explants. For *Pinus* the initation frequencies from immature zygotic embryos reached 1.53 to 24.11% and the initiation was dependent on plant growth regulator treatments. Although in *Abies* hybrids the initiation occurred in different explants (immature and mature zygotic embryos, cotyledons dissected from seedlings as well as emblings), the highest intiation frequencies were obtained by culture of immature zygotic embryos (38.1% and 44.6%). For long-term culture the initiated embryogenic tissues were maintained on solid media containing the same plant growth regulators as used for initiation. Microscopic studies revealed the embryogenic tissues were heterogenous in their cell composition and were characterized by the presence of different cell types, as single vacuolised long cells, clumps of meristematic cells and bipolar somatic embryos. Maturation of somatic embryos occurred on medium containing abscisic acid and osmoticum (*Abies* hybrids) or high gelrite content (*Pinus nigra*). Plantlet (emblings or somatic seedlings) regeneration occurred in hybrids as well as *Pinus nigra*.

The initiated embryogenic tissues have also been included in genetic engineering studies.

By using the biolistic method, in *Pinus nigra* transformed tissues were obtained and the presence of foreign genes was proved by PCR method as well as histochemically. In *Abies* hybrids the *Agrobacterium tumefaciens* mediated transformation resulted in transgenic tissues as well as transgenic emblings regeneration. The problems associated with *in vitro* long-term maintenance are aimed to be eliminated by cryopreservation since the slow-freezing metod has successfully been applied to *Pinus nigra* and *Abies* embryogenic tissues.

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SCREENING OF THE SOMATIC HYBRIDS POTATO+SOLANUM CHACOENSE FOR THEIR RESISTANCE TO COLORADO POTATO BEETLE BY USING THE CHOICE TEST

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Colorado potato beetle *(Leptinotarsa decemlineata)* (CPB) represents one of the biggest "plagues" in North-American, Asian as well as European crops/agriculture, causing considerable economic loss in potato cultures on a yearly basis. Fighting this problem is only possible by an intricately clever insecticide management, however recent studies show that these insects are quickly able to develop resistance. Understanding CPB's choice of host plants could lead to a completely new perspective on the area of pest control management.

This study was performed with somatic hybrid plants potato+*Solanum chacoense*, while the testing method subscribes to laboratory-choice experimentation.

Local populations of CPB were collected from the field, which the adults were subjected to several tests, in which they were offered the choice between feeding off CPB-resistant and CPB non-resistant plants. First-phase testing was performed in three batches, each one lasting for 24 hours.

During the second phase, the beetles were induced into hibernation by maintaining them at 4^0C during winter, after which they were given a second choice, according to the same pattern as in phase one.

While the first test delivered positive results with the beetles always choosing the CPB non-resistant plant (cultivar control), the second test ended with beetles eating both species and dying after having been revived from hibernation. The choice-experiment was performed in laboratory, and it is to be regarded as a preliminary test for several others who are yet to follow like for example a more complex laboratory bioassay, field choice testing or the RAPD markers linked to leptines, the deterent glycoalkaloids for CPB.

The laboratory tests point out the fact that in lack of other options, CPB is willing to feed from the resistant plant, in order to survive. This last idea is only to be regarded as a hypothesis - for its validation in further studies will be required.

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NEODOMESTICATION OF ORNAMENTAL AND ENERGY MALLOWES: POLYPLOIDIZATION OF ALYOGYNE HUEGELII

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Marvellous mallow (Alyogyne huegelii) is a flowering plant found in the Southwest part of Western Australia. The species similar to the Hibiscus species and formerly belonged to the Hibiscus genus as Hibiscus huegelii. It is a shrub, which grow up to 4m. It has many alternate branches, although lower ones may be

sparse. Bright green leaves are divided in three to five in outline. Their margins are irregular and their lobates are toothed. The flowers have five luminous petals up to 70 mm long, these are overlapping and have slight ridges. The colour is cream or mauve, or the lilac of the name by which it is traded. The anthers of the flowers are yellow. As with all the Malvales, the flowers last around a day becoming deeply coloured when spent. They are numerous in the long flowering period between June and January. The Alyogyne tolerate many types of soil from the sandy to the stony. It is intolerant of bad drainage. Alyogyne huegelii is moderately frost tolerant but some protection is required from the heaviest frosts. Experience with growing this plant in California has shown that it can survive short periods of minus 3 degrees centigrade without any permanent damage. Propagation can be from seed, which keeps its viability for many years. The seed coat is almost water impermeable, therefore it is helpful to rub the seed against an emery board before planting the seeds, this treatment will break through the hard protective coating and allow moisture to penetrate. Germination will be much faster after this treatment. Seeds can be planted in early autumn.

Our aims to make breeded lines by poliploidization of wild type plant, which are better adapted to our climatic conditions, produces more attractive, biger, longer lasting flowers, and biger green mass for potencial energatical use.

In vitro culture was established from seeds of wild type Alyogyne huegelii. The seeds were surface desinfected and put onto growth regulator free MS medium. The tips of seedling of A. huegelii were treated with 4 antimitotic agent (Colchicine, Oryzalin, Trifluralin and Taxol), paralell with each other, for 1 – 3 days in test tubes. The survived shoots were put onto fresh medium. The best survival rate was experienced, treated with Trifluralin with 3 days.

SELF-INCOMPATIBILITY RNASE ALLELES IN A WILD-GROWING TURKISH APRICOT POPULATION

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In Turkey, besides commercial apricot orchards, several wild-growing populations of apricot are also known. Such natural fruit forests offer the possibility to select some apricot genotypes which may be perspective for breeding. The self- and mutual incompatibility of apricot is an important character for both breeding and cultivation. The *S*-locus controlling this trait is well characterized in *Prunus*. The one and a half century of Ottoman occupation in Hungary has a crucial influence on the Hungarian apricot germplasm. We have recently clarified that this historical connection had great contribution to the formation of Hungarian old landrace cultivars. We have analysed the *S-RNase* (pistil component) and *SFB* (pollen component) gene of 64 trees collected in the region of Erzincan, Turkey. We identified nine previously described alleles in the genome of these trees including: S_2, S_3, S_6, S_7, S_8, S_9, S_{11}, S_{12} and S_{13}. The alleles S_8 and S_9 have been only detected from the Hungarian and Turkish germplasm. S_8 is the a wild type version of S_C, the naturally occurring self-compatibility allele. Since the S_C allele has not been detected in Erzincan, the regions from Central to Eastern Turkey must have an important role in the transition of self-incompatibility to self-compatibility.

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EXAMINATIONS OF PATHOGENS AND PESTS OF PLANT BIOMASS SPECIES

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The new biomass and green energy technology requires technology adapted, environmentally friendly, and healthy species, varieties. An important element of these works is the survey of potential pathogens and pests. Two species of

Gramineae family (*Arundo donax* L. and *Miscanthus giganteus*) and some species and interspecific hybrids of *Malvaceae* family (*Napaea sp., Sida sp., Althaea spp., Kitaibelia vitifolia, Kitaibelia balansae, Kitaibelia* x *Kovatsii., Lavatera sp., Malva spp.*) were investigated.

The most important candidate of the third generation energy crops is arundo (*Arundo donax* L.), which is high yielding, genetically stable and homogeneous, decaploid species, with very few pathogens and pests. Virological tests (ELISA and RT-PCR) were made of *Arundo donax* sporadic plants from Hungary and sterile *in vitro* cultures from our large-scale propagation program. Only one sample originated from Hungarian sporadic plants was positive for *Barley yellow dwarf virus* (MAV–like type). The economically most important American, Italian and Hungarian clones are luteovirus-free. No other pathogens or pests were found by visual monitoring.

Miscanthus giganteus has much more potential pathogens compared to *Arundo*. *Acidovorax avenae ssp. avenae* (bacterial leaf bligh) and *Leifsonia xyli ssp. xyli* (bacterial dwarfing) are quarantine pathogens. More than twenty fungal pathogen of *Miscanthus* were described wordwide, but we could not observe infectious disease between 2006 and 2011. In September of each year mild or medium aphid invasion occurred. In one case (2007 summer) a major damage of *ex vitro* plants of *Miscanthus* were detected caused by mites (*Tetranychus urticae* Koch).

The health status of species belonging to the *Malvaceae* family depends on the weather of the actual year. The most important disease is rust (*Puccinia malvacearum* Bertero ex Mont). Except very dry summers (e.g. 2011) it causes severe disease on *Malva* spp., medium infection on *Althaea* spp. and *Lavatera thuringiaca. Kitaibelia vitifolia, Kitaibelia balansae, Kitaibelia* x *Kovatsii* and *Sida* plants were practically not infected. The most important pests are: flea beetles (*Podagrica fuscicornis* L.; P. *malvae* L.), weevils (*Apion curvirostre* Gyllenhal; *A. longirosre* Olivier) whitefly (*Trialeurodes vaporariorum* Westwood) and aphids (*Aphididae*). Serious sclerotinia wilt were observed on a *Kitaibelia balansae* plantation. The healthiest plants of the family *Malvaceae* were *Kitaibelia vitifolia, Kitaibelia* x *Kovatsii* hybrids *and Sida sp.*

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Pannonian Plant Biotechnology Association and the Ereky Károly Biotechnology Foundation under the auspices of the Agricultural Biotechnology Committee of the Hungarian Academy of Sciences organizing the Pannonian Biotechnology workshop.

This year workshop of the Pannonian Plant Biotechnology Association will be held at Debrecen University, between 4th and 6th of June 2012. We cordially invite scientists and experts from the region to present their latest results on plant breeding and plant biotechnology.

The tentative schedule of the three days workshop

Arrival on Sunday June 3rd

Registration starts at 15.00 p.m. at the Centre of Agricultural Sciences of Debrecen University and the welcome reception will be hosted at 6.00 p.m.

Monday June 4

Workshop starts at 9 th and will be ended at 6 pm

Tuesday June 5

Workshop will continued at 9.00 th till 6 p.m.

June 6th departure day

at 9.00 an excursion will be arranged to “Hortobágy Puszta”

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.

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