

=== ORAL PRESENTATION ABSTRACTS ===

”PLANTING FOR FUTURE”, THE EASAC DOCUMENT
PREPARED BY AN EASAC EXPERT PANEL

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Agriculture faces major challenges to deliver food security at a time of increasing pressures from climate change, social and economic inequity and instability, and the continuing need to avoid further loss in ecosystem biodiversity. The introduction of new EU legislation requiring farmers to reduce reliance on crop protection chemicals creates additional challenges for maintaining levels of crop productivity.

Previous EU agricultural policy had focused on constraining food production but there is a new realisation that the EU should now increase its biomass production for food, livestock feed and other uses, including renewable materials to support the bioeconomy. The production of more food, more sustainably, requires the development of crops that can make better use of limited resources. Agricultural innovation can capitalise on the rapid pace of advance in functional genomics research and it is unwise to exclude any technology a priori for ideological reasons. Sustainable agricultural production and food security must harness the potential of biotechnology in all its facets.

In previous work, EASAC has described the opportunities and challenges in using plant genetic resources in improved breeding approaches, for example by using marker-assisted selection of desired traits. In the presented report, EASAC explores some of the issues associated with the genetic modification of crops, where the EU has fallen behind in its adoption of the technology, compared to many other regions of the world. Concerns have been expressed that a time-consuming and expensive regulatory framework in the EU, compounded by politicisation of decision-making by Member States and coupled with other policy inconsistencies, has tended to act as an impediment to agricultural innovation. Controversies about the impact of genetically modified (GM) crops have too often been based on contested science or have confounded effects of the technology with the impact of agriculture per se or changes in agronomic practice. It is vital to address the policy disconnects because there is a wide range

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of opportunities in prospect for improving agricultural productivity and efficiency, environmental quality and human health, by using all available technologies where appropriate.

Previous work by member academies of EASAC has documented where there is excellent, relevant science to be nurtured and utilised, and where problems have arisen because of the failure to use science to inform the modernisation of regulatory approaches to benefit-risk assessment. The goal of the presented report is to clarify the implications for policy-makers of alternative strategic choices in utilising the tools, collectively termed crop genetic improvement technologies, for delivering sustainable agriculture.

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ALLELE DISCOVERY SUPPORTED BY PHENOTYPING OF
DROUGHT RESPONSE OF CEREAL PLANTS

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Genetic improvement of complex traits, such as drought adaptation can be advanced by the combination of genomic and phenomic approaches. Semi-robotic phenotyping platform was used for computer-controlled watering, digital and thermal imaging of barley plants grown in greenhouse. In soil with 20% water capacity the reduction in green pixel-based shoot surface area of tested barley variants ranged from 0% to 76% as compared to control plants grown with 60% water capacity. The EcoTILLING and the subsequent DNA sequencing have defined four (A-D) haplotypes of the HvA1 gene encoding the group 3 LEA protein. The green pixel mean value of genotypes with haplotype D was higher than the mean value of the remaining haplotypes under drought. Thermal images indicated genotype-dependent variation in elevation of the canopy temperature of drought-exposed plants. The drought-induced changes in leaf temperature showed low correlation with the water use efficiency ($r_2 = 0.431$). The haplotype/trait association analysis based on the t-test has revealed a positive effect of the haplotype B of the gene encoding the barley fungal pathogen induced mRNA for pathogen-related protein (HvPPRPX) on harvest index, thousand grain weight, water use efficiency and grain yield. The presented pilot study establishes basic methodology for the integrated use of phenotyping and haplotyping data in characterization of genotype-dependent drought responses in barley.

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=== ORAL PRESENTATION ABSTRACTS ===

COMBINING DIFFERENT BIOTECHNOLOGICAL TOOLS
FOR BETTER INTROGRESSION OF RESISTANCE
TRAITS IN POTATO

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The new tools of plant genetic enhancement, molecular biology, marker assisted selection (MAS), haploidization or other *in vitro* techniques, such as stress selection as well as the data from functional genomics and metabolomics should be used together towards a common goal, the transfer of resistance genes into crops. Today challenges such as climate change and exponential increase of human population impose new strategies to increase crop yield. 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 motifs like nucleotide binding sites, leucine rich repeats (NBS-LRR). Although it was thought that resistance could be transferred into crops by one gene, it occurred soon that one R gene could only confer a short-lived resistance. In order to achieve the goal of durable, sustainable resistance the only way is to transfer more quantitative trait loci (QTLs) and to apply more biotechnological tools for the introgression and expression of resistance traits. 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.

This strategy is going to be exemplified in the case of potato crop. Potato ranks third in the global crop production but suffers great losses because of diseases and pests.

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The goal of this presentation is to discuss the strategy of combining different biotechnological tools with the ambitious aim to deliver 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, the analysis of trichomes and foliar metabolites and cytogenetic analysis. Other examples for multiple resistance traits or resistance genes stalking as for instance the *Rpi-blb1* and *Rpi-blb3* genes from the wild species *S. bulbocastanum* to induce resistance to late blight, or the combination of gene transfer for PVY resistance with stress selection for drought will be also presented.

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BIOTECHNOLOGY OF BIOMASS SUPPLY CHAIN:
CENTRAL EUROPEAN PERSPECTIVE

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By applying advanced plant breeding and biotechnology to dedicated energy crops, we should deliver sustainable energy solutions that (1) displace vast amounts of fossil fuels and provide greater energy security; (2) create new economic opportunities for farmers and rural communities; (3) protect the land, air and water; (4) meet our commitments to stakeholders. Due to the increased demand for growing dedicated biomass crops some previous and new molecular breeding methods as well as *in vitro* somatic techniques have to be integrated into the creation of specially designed „bioregeneration” crops. In order to elaborate new biological solutions and growing techniques, our Bioregeneration Crops Working Group is conducting some R&D activities since 2010 on perennial rhizomatous grasses (PRG, *Miscanthus giganteus* sp., giant reed, Hungarian powergrass), as well as on interesting perennial herbaceous subshrubs (PHS, Virginia mallow, giant energy mallows, hemp mallow) which can probably be grown under marginal field condition. As a new candidate of the bioregeneration energy plants, energy mallow species are able to serve considerably the Biomass Supply Chain (BSC) strategies in our region. Biotechnological and environmental research of these crops is necessary before we take them into cultivation for industrial biomass production chain. The aim of our biotech-assisted breeding program is to develop new methods for energy mallows propagation and industrial-scale nursery operations. There is substantial variation in water use efficiency (WUE) both within and across biomass crops. Therefore we investigate the role of different watering regimes on growth dynamics and biomass production of PRG and PHS crops. We also investigate the ability of different PRG and PSS ecotypes and/or cultivars for

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tolerate and removal of heavy metals under *in vitro* and hydroponic culture conditions. Our team conducts basic research on induced polyembryony, vegetative embryoidogeny, hemmorhizogeny in order to develop industrial-scale somatic seedling technology (SST) for the future biogeneration crops. To prognosticate the effect of climate changes on growing techniques, we are studying natural habitats as well as biotechnologically propagated growing fields of SST populations (giant reed and *Miscanthus* sp.). We believe in creating new opportunities for growers, sharing the value of our innovations, and collaborating broadly with scientists and industry groups in Pannonian regions.

Acknowledgements. This work is partly supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0041 project and co-financed by the European Union and the European Social Fund. Additional financial support is also gratefully acknowledged for the MOP Biotech Co Ltd. (Nyíregyháza, Hungary) and Ereky Foundation (Debrecen, Hungary).

=== ORAL PRESENTATION ABSTRACTS ===

COMPARATIVE ANALYSIS OF THE SEQUENCES OF
GENE FAMILY *psbA* IN CYANOBACTERIA

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The D1 protein of Photosystem II (PSII), encoded by the *psbA* genes, is an indispensable component of oxygenic photosynthesis. Due to strongly oxidative chemistry of PSII water splitting, the D1 protein is prone to constant damage and requires its replacement by a new copy every 5h under low light conditions and every 20 minutes under intense illumination, whereas most of the other PSII subunits remain ordinarily undamaged. In Cyanobacteria the D1 protein is encoded by a *psbA* gene family, ranging from 1 to 6 members. The presence of multiple *psbA* genes encoding different D1 isoforms is an indication of their importance in regulatory mechanisms responsible for maintaining a functional PSII upon changing environmental conditions in natural habitats of cyanobacteria.

Here we present a comparative analysis of the sequences of gene family *psbA* in cyanobacteria, with characteristic features of all *psbA* genes and the D1 protein isoforms that they encode.

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=== ORAL PRESENTATION ABSTRACTS ===

ROOT ARCHITECTURE OF *BRACHYPODIUM* – ROOT
ARCHITECTURE OF *BRACHYPODIUM* – MORPHOLOGICAL,
MOLECULAR AND GENETIC APPROACHES

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Root is the main plant organ that is responsible for water and nutrient uptake. Hence, its architecture determines strongly how it can fulfill the above role. On the other hand, the characteristics of root architecture are affected significantly by the underground environment, such as water and nutrient availability in the soil.

In our group, characterization of various *Brachypodium distachyon* (L.) P. Beauv. ecotypes grown in pots or rhizotrons under either well-watered or reduced watering conditions is conducted. Comparison of plants grown in pots focuses mainly on the length and thickness of primary and nodal roots; however their photosynthetic and other shoot parameters are measured, too. Thus, the evaluation of different groups involving distinct ecotypes with similar shoot or root parameters can inform us about the importance of the presence/absence, thickness or length of different root types as well as their correlation with shoot parameters under optimal and adverse circumstances. Moreover, growing plants in rhizotrons allow us to follow the daily growth rate of primary, nodal and even lateral roots. Hence, the ecotypes can be distinguished on the basis of their root morphological plasticity in response to water-deficit stress.

The plant-specific LBD (Lateral Organ Boundaries Domain) gene family is essential in the regulation of plant lateral organ development. Several LBD genes are related to almost all aspects of plant development, including embryo, root, leaf, and inflorescence development. Our group studies the transcript pattern of various LBD genes in different plant parts, selecting especially the root-specific ones that are probably involved in the lateral root formation in *Brachypodium*.

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Reverse genetic approaches of the above-selected LBD genes can inform us about their spatial and temporal expression. Therefore, transformants containing constructs with self-promoter and reporter gene together with transformants with ectopic over-expression of the selected LBD genes are being analyzed. Silencing of the selected LBD genes is also planned in the future.

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THE INVOLVEMENT OF CHROMATIN PROTEINS
IN *AGROBACTERIUM*-MEDIATED PLANT
GENETIC TRANSFORMATION

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Agrobacterium-mediated genetic transformation is the dominant technology used for many years in production of genetically modified transgenic plants. Recent study has demonstrated the implication of plant genes and proteins in *Agrobacterium* mediated transformation. We investigated the role of several histone proteins and an anti-silencing factor A (*SGAI*) in plant transformation. Transgenic *Arabidopsis* plants containing additional copies of cDNAs encoding histone H2A (*HTA*), histone H4 (*HFO*), or *SGAI* displayed increased susceptibility to transformation. Over-expression of all tested histone H2B (*HTB*) and most histone H3 (*HTR*) cDNAs did not increase transformation. A parallel increase in transient gene expression was observed when the histone *HTA* or *HFO* cDNAs were co-transfected, together with a plant active *gusA* gene, into tobacco protoplasts. An increase in *gusA* transcripts when the histone *HTAI* cDNA was over-expressed in protoplasts was also detected. No such increase in *gusA* activity was seen when a *SGAI* cDNA was co-transfected with a *gusA* gene into BY-2 protoplasts. Over-expression of histone or *SGAI* cDNAs does not increase expression of a previously integrated transgene, nor could *HTAI* reverse silencing. These data suggests that histones may increase transgene expression by working directly on the promoter of incoming DNA, or that histones may play a role in stabilizing transgene DNA (and thereby transgene expression) during the initial stages of transformation and *SGAI* might lead to enhanced plant transformation by allowing T-DNA and complexed proteins greater access to plant target DNA, thus facilitating T-DNA integration.

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APPLICATION OF TISSUE SPECIFIC GENE EXPRESSION TO
IMPROVE FUNGUS RESISTANCE IN WHEAT

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The rust disease (*Puccinia* spp.) belongs to the group of most important fungal diseases in wheat (*Triticum aestivum* L.). The leaf rust (*Puccinia recondita* f. sp. *tritici*) and the stem rust (*Puccinia graminis* f. sp. *tritici*) cause – depending on the year effect – very severe loss of quality and quantity in cultivated wheat year by year in Hungary. Wheat is one of the major agricultural crops in Hungary as well as in many countries all over the world. The conventional breeding methods were not able to produce an outbreak in providing considerably tolerant genotypes. Based on this observation it seems to be useful to take new approaches to develop the resistance of wheat against rust diseases and to combine the genetic transformation and the classical plant breeding. We expect stabilization of yield quality and yield safety by the increased resistance against upper mentioned rust fungus diseases with the use of available tools and processes of biotechnology.

When plants are attacked by fungi part of their response is producing PR (pathogenesis related) proteins as chitinases, glucanases. But the induced self-defense mechanism does not provide enough protection in most of the cases, because either they are not effective or they are activated too late because there are many different processes of metabolic pathways.

Our goal was to build the *cmg1* gene, coding for a 83.2 kDa exoglucanase enzyme of *Coniothyrium minitans* into wheat with direct genetransfer.

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Considering the constitutive gene regulation, the mentioned resistant plants produce the protein of hydrolase activity in all parts of the plants, what means a certain risk of GM technology in today's public opinion. According to the upper mentioned facts we inserted the transgene under tissue specific regulation, so that the protecting recombinant protein is present only in the green tissues of the transgenic plants and we cannot detect it in the starchy endosperm of the wheat kernel.

To achieve this goal *cmg1* gene had to be inserted into a gene construction where it was under regulation of the wheat's own ribulose-1,5-bisphosphate carboxylate-oxygenase gene's (*rubisco*) promoter.

We developed a modified biotest system, after the standard methods, so that we could be able to test the biological efficiency of this „general“ resistance. After the molecular genetics proofs – PCR, RT-PCR, Western Blot, etc. – the transgenic plants underwent a biotest, where all the available races of leaf rust in Hungary was used for provoked infection. The results of biotests indicated that many of the GM wheat lines showed considerably high-level resistance against *P. recondita*.

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SMALL RNA BASED VIRAL METAGENOMICS IN
CULTIVATED GRAPEVINE

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In plants, microRNAs and siRNAs are small RNAs regulating gene expression at the post-transcriptional level. Here, we use deep-sequencing, computational and molecular methods to identify, profile, and describe conserved and non-conserved miRNAs. We also identified and characterized vsRNAs derived from grapevine field plants naturally infected with different viruses belonging to the genera Foveavirus, Maculavirus, Maravirus and Nepovirus. These vsRNAs were mainly of 21 and 22 nucleotides (nt) in size and were discontinuously distributed throughout viral genome. In addition we have identified host and viral genes targeted by small RNAs.

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IN VITRO MICROPROPAGATION OF
LYCIUM BARBARUM

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In vitro culture was successfully initiated by using seeds from cultivar Ningxia 1 as inocula cultured on Murashige and Skoog (1962) hormone-free medium, solidified with agar. The highest proliferation rates were obtained on the MS media with either 0.3 or 0.5 mg/l benzyl adenine, gelled with wheat starch as an agar alternative. The experimental treatments with 0.5 mg/l benzyl adenine ensured proliferation rates superior to the ones with 0.3 mg/l benzyl adenine, but the shoots obtained on MS + 0.3 mg/l benzyl adenine were longer and more robust. Also, the inoculation of large microcuttings onto the multiplication media ensured superior results regarding *in vitro* survival rates, the number of shoots regenerated / plantlet and the vigor of the plantlets. The microcuttings inserted vertically into the media yielded superior growth and multiplication as compared to the microcuttings placed horizontally on the surface of the media. The shoots regenerated in the multiplication stage could be used for cyclic *in vitro* multiplication. Explants either two centimetres or four centimetres in length proved to be effective. The non-rooted shoots resulting from the treatment with 0.3 mg/l benzyl adenine were either rooted *in vitro* on hormone-free MS medium gelled with starch or used for tests of *ex vitro* rooting and acclimatization. The optimal number of microcuttings in the *in vitro* rooting stage proved to be 40 explants/jar and the rooted plantlets were efficiently acclimatized *ex vitro* by three methods: float hydroculture in floating cell trays, floating perlite as well as Jiffy7 pellets. A successful alternative to *in vitro* rooting and subsequent *ex vitro* acclimatization was the direct *ex vitro* rooting and acclimatization in Jiffy7 pellets, in the same stage, using shoots excised from plantlets cultured in the multiplication stage. For direct *ex vitro* rooting and acclimatization, all other substrates we tested except for Jiffy7

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pellets failed. *Ex vitro* rooting in floating perlite was stimulated by IBA but the technique was difficult and the results were not conclusive. After *ex vitro* acclimatization the resulting plants grew rapidly and vigorously.

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CHARACTERIZATION OF TWO SER/THR TYPE *ARABIDOPSIS THALIANA* PROTEIN KINASES: THEIR POSSIBLE ROLES IN REGULATION OF ABIOTIC STRESS RESPONSES

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Due to global warming of overall climate, it is an increasing demand to breed plant cultivars tolerant to high salt concentration in order to improve their chance to survive deleterious effects of abiotic stress conditions. Our aim is - within the frame of a Hungarian-Romanian TET (TET_12_RO_1-2013-0010) - to characterize and compare abiotic stress response regulatory genes arisen from *Arabidopsis* and extremely high salt tolerant picoalgae. Identification of a set of cDNAs conferring dominant stress tolerance phenotypes was described in *Arabidopsis* (Papdi *et al.*, 2008). Two Ser/Thr protein kinases (from this set line N022 and the CRK5) were chosen for abiotic stress response investigations. Preliminarily, we functionally characterized the CRK5 protein kinase which is involved in regulation of gravitropic responses of *A. thaliana* roots (Rigo *et al.*, 2013). CRK5 is a PM-associated kinase that forms U-shaped patterns facing outer lateral walls of root epidermis cells. CRK5 phosphorylates hydrophilic loop of PIN2 *in vitro*, and PIN2 shows accelerated accumulation in brefeldin bodies in *crk5* mutant. Delayed gravitropic response of *crk5* mutant thus likely reflects defective phosphorylation of PIN2 and deceleration of its brefeldin-sensitive membrane recycling. In future, we would like to investigate role of CRK5

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protein kinase in NO signaling also. We expect to find new information about role of NO molecule in auxin signaling and oxidative stress. Additionally, for comparative studies, we plan to investigate the osmotic and oxidative responses of the other protein kinase, line N022, which has unknown function yet.

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APPLICATION OF RECOMBINASE TECHNOLOGY TO
PRODUCE MARKER FREE TRANSGENIC CROPS

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One of the main concerns regarding GM plants is the presence of the selection marker gene in their genome. Although several non-antibiotic and non-herbicide based selection systems exist, but they are the most frequently used, which raised the fear in the public. The biggest problems addressed by the green movements are the so called “escape of these selection marker genes into the environment causing negative effects on it. The other problem with the most widely used methods modifying the plant genome is the random insertion of the “foreign” gene into it and as a consequence it may generate unpredictable expression patterns. To reduce some of the concerns and make the gene technology approach acceptable by the society scientists have to address all these issues. Selection marker genes have to be chosen carefully or have to be removed when they are no longer necessary in the genome. The genes for modifying the physiological characteristics of the plant or the property of the products made of plant tissue have to be chosen prudentially introducing genes from the same species or from the same family. To avoid random insertion for example site-specific genome editing is powerful and highly desirable methodology. Both site-specific recombination and restriction enzyme mediated gene targeting are feasible approaches in genome modification of plant cells.

A method based on the widely used Cre/lox recombination system has been developed in our laboratory to produce selection marker free transgenic barley plants. The inducible recombination system is under the control of a cold responsive promoter. It has been demonstrated that the system works properly cutting of the unnecessary DNA fragment from the barley genome. The remaining mutated loxP site provide an option for site specific insertion of any desirable DNA fragments to engineer specific properties of either the plants or plant based products.

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EFFECT OF UV-B STRESS ON THE PHOTOSYNTHETIC
FUNCTION OF *ERUCA SATIVA* MILLER

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The global warming can determine the alteration of normal functionality of the ecosystems, threatening with extinction a significant number of species. Most important environmental factors inducing different types of stresses on plant photosynthesis are: temperature, excessive light, CO₂ concentration. Destruction of the ozone layer that absorbing short wavelength UV radiation, increases the amount of UV-B radiation reaching the Earth's surface. These radiations can cause damage of photosystem I and II, carbon fixation, low levels of chlorophyll and sucrose in plants.

The study of photosynthesis utilizing chlorophyll fluorescence measurements is one of the most modern techniques used by plant physiologists and ecophysiologicalists. The modern fluorometers can measure the chlorophyll fluorescence in the laboratory and in the field. For the study of UV-B stress on *Eruca sativa* we have utilized the MONI PAM fluorometer that monitors the efficiency of PS II in greenhouse conditions under normal and enhanced UV-B irradiation. The monitored parameters are: PAR (Photosynthetic Active Radiation), basic fluorescence level (F) maximal fluorescence level (FM'), ETR (Electron Transport Rate), YII (efficiency of Photosystem II). Measurements were done on control samples, plants treated with low UV-B radiation and high UV-B radiation producing a wide range of adaptative responses including irreversible damages of photosynthetic processes.

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THE EFFECT OF THE P19 SILENCING SUPPRESSOR
ON ANTIVIRAL siRNA BIOGENESIS

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Here we showed that in CymRSV – *Nicotiana benthamiana* interaction DCL2 dependent 22nt small RNAs are generated in the greatest number. However this is masked by the 1 nucleotide shortening of the siRNA induced by P19.

DCL2 and DCL4 driven small RNAs are crucial in mediating RNA silencing against RNA viruses. DCL2 and DCL 4 are capable of cleaving double stranded viral RNA into 22 and 21 nucleotide (nt) long viral small interfering RNAs (vsiRNA) respectively. And both can program RISC complexes to cleave the complementary viral RNA. P19, a viral protein capable of suppressing silencing, can invert the size ratio of vsiRNA in Cymbidium Ring Spot Virus (CymRSV) infected *Nicotiana benthamiana* (N.b.) plants. We know that a wild type CymRSV infected N.b. the amount of 21 nt long vsiRNAs is greater than 22nt vsiRNA's, however in N.b. infected with P19 deficient CymRSV (Cym19S) it alters and 22 nt vsiRNA becomes the most common. We set our goal to shed some light on the underlying mechanism of the ratio change.

We have proved that this can not be explained by the CymRSV's ability to replicate in tissues where the Cym19S becomes silenced. By showing that DCL2 and DCL4 are both present in the assessed tissues and there is no significant difference in expression levels even when the viruses are present. We also investigated the accumulation of small RNAs in these tissues, with next generation sequencing and find that there is a small divergence of 21 and 22 nt vsiRNA ratio but the presence or absence of P19 distinguishes the samples the most.

We discarded the possibility that the ratio shift is due to the P19's putative ability of conserving the 21 nt based on the fact that P19 binds 21 and 22 nt siRNA in vitro with roughly the same affinity.

We hypothesized that P19 is capable of inducing the shortening of the bound siRNAs by one nt. We had reported that when P19 was co-agroinfiltrated with GFP and

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its hairpin into N.b. we found that 22 nt siRNS could not be detected, and a great number of 20 nt appeared on the northern blot, though none of the P19 independent small RNA (24nt siRNA, miRNA) shown any change after interaction with plant endogenous RNase. This hypothesis can also explain the enrichment of 20nt siRNA in our and in previously published small RNA sequencing. *In silico* mapping of vsiRNA reads also supports this theory.

==== POSTER ABSTRACTS ====

GROWTH DYNAMICS AND BIOMASS PRODUCTION OF
GIANT REED UNDER DIFFERENT WATERING REGIMES

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In recent years many national and international initiatives have started in order to identify new sources of renewable energy. This growing interest in renewable energy is driven by two main reasons. Firstly, fossil fuels such as oil, coal, and natural gas are limited resources on our planet and if the level of our consumption does not change, the estimated times of depletion of these energy sources will be approximately 50 years for oil, 70 years for natural gas, and 170 years for coal (International Energy Outlook, 2006). Secondly, the combustion of fossil fuels emits large amounts of gas into the atmosphere, increasing the natural greenhouse effect. Carbon dioxide (CO₂) and methane (CH₄) are the main components of greenhouse gases (GHGs). The new policies for sustainable energy production in recent years have endorsed for the use of non-food, perennial grasses for biomass plantation for the next generations of bio-fuels production. To maximize the limit of carbon dioxide emissions (recognized as the main source of the greenhouse effect), in January of 2007, the European Commission has to recommend an obligatory minimum biomass participation in the energy balance of 20% by 2020. So, the generation of biomass for energy production is becoming a real business opportunity for farmers all over the world, even though the use of grains and other food is giving rise to ethical issues. Tall perennial grasses, such as giant reed (*Arundo donax* L.), have been evaluated as potential lignocellulosic bioenergy crops. C3 grasses like giant reed have been evaluated for bioenergy use

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in the region. High dry matter yields of about 30 t/ha⁻¹ have been reported in temperate climates for giant reed. Thus, there is an ever increasing need to achieve greater crop production with less water use and/or more efficient water use. This is important for all crops, but it is especially needed for bioenergy crops to allow for production on marginal lands and to minimize competition with food crops. There is substantial variation in water use efficiency (WUE; g biomass produced kg⁻¹ of water transpired) both within and across crops. The objective of our study is to investigate three different watering regimes (75, 50 and 30 % of saturation percent) on growth dynamics and biomass production of three different ecotypes (S: Hungarian ecotype, B: North-American ecotype, E: Spanish ecotype). The preliminary data for number of new buds, plant height and number of leaves after one month from transplanting showed no big differences among the ecotypes and among treatments indicating that giant reed could consider as drought tolerant plant; not only to stay survive under limited water supply circumstances but also produce significant biomass feedstock for energy and paper purposes. As well as Fv/Fm ratio for ecotypes under different watering regime after one month also showed no big differences among ecotypes.

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==== POSTER ABSTRACTS ====

COMPARATIVE PERFORMANCE OF COPPER UPTAKE BY
TWO ECOTYPES OF GIANT REED (*ARUNDO DONAX* L.)
GROWN ON HYDROPONIC CULTURE

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Contamination of water bodies such as streams, drains and ground water resources with trace elements represents a potential threat to humans, animals and plants, and thus removal of these metals from contaminated waters has received increasing attention. Increase of man's needs requires development in life activities, progress industrialization, transportation tools, enhancement of agriculture and exploitation of natural resources. Soil and water resources are extremely exposed to pollution from different aspects. Agrochemicals in particular, have created severe problems, since they release thousands of chemicals to the environment. Studies on the effect of environmental pollutants on agro-ecosystem have been carried out. On the other hand, the importance of heavy metals as environmental pollutants is well known and well documented in literature. Copper toxicity is a problem of both agricultural and environmental significance. Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. Despite its environmental and agricultural importance, the concentration, distribution and fractionation of anthropogenic, and naturally occurring Cu in soils is poorly known. Although the total Cu content in soils is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact. Copper availability to biota (as a nutrient or toxin) and its mobility are the most

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important factors to be considered when assessing its effect on the soil environment. Copper must be absorbed in small amounts on a daily basis to maintain good health. A daily dietary intake of 1–2 milligrams is required. However, high levels of copper can be harmful to health. Inhaling high levels can cause irritation to the nasal passages, mouth, eyes and throat, and ingesting high copper concentrations can lead to nausea, vomiting and diarrhoea. Exposure to very high levels can damage the liver and kidneys and may lead to death. Copper is classified as a hazardous substance. Giant reed (*Arundo donax* L.) is widely employed as raw material for the production of paper. It is also a good candidate for soil phytoremediation as it is a high plant with deep roots and fast growth. Giant reed's ability to tolerate and accumulate heavy metals such as nickel, cadmium, zinc and lead has been well documented. The aim of this study is to investigate the ability of two different ecotypes ("ESP" and "08") of giant reed for tolerance and removal of copper under hydroponic culture using different concentrations of copper as follow; 0, 1, 2, 3, 5, 10 and 26.8 mgL⁻¹. To determine the effects of copper on giant reed chlorophyll a and b, activities of catalase (CAT), peroxidase (POD), superoxidase dismutase (SOD), and photosynthetic activities were measured. Our result confirmed that giant reed showed a potential of phytoremediation of contaminated soil charged with low concentrations of Cu.

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==== POSTER ABSTRACTS ====

BREEDING STRATEGIES OF *SIDA HERMAPHRODITA*
FOR BIOENERGY

E. KURUCZ^{1,✉}, P. SZARVAS¹ and M.G. FÁRI¹

Virginia mallow (*Sida*, *Sida hermaphrodita* Rushby) is a perspective perennial semishrub plant species originating from North-America. This endemic and endangered species will probably receive much more attention in Central Europe as one of the best dedicated candidate among temperate climate biomass crops. Based on former investigations, *Sida* can cultivate under marginal land conditions and it can tolerate the oscillations of climate changes and drought with a biomass output around 10-20 t/ha. Besides this one other important economical character is that the water-content of *Sida* stems decrease below 40% until November, preceded the perennial grasses and woody energy species. As a new candidate of the second generation energy plants, *Sida* is able to serve considerably the Biomass Supply Chain (BSC) strategies in our region. The breeding of biomass plants can be economical and sustainable, if the cultivated plants are adaptable, and it possible to grow calculable and CO₂-positive field cultivation way. Biotechnological and environmental research of *Sida* is necessary before we take it into cultivation for industrial biomass purposes. The aim of our biotech-assisted breeding program is to develop new methods for *Sida* propagation and industrial-scale nursery operations. The directions of our investigations are the followings:

Improvement of seed germination: *Sida* is a semi-domesticated species therefore it has some field propagation difficulties. Freshly collected unimproved matured seeds have a very low germination rate which is varied between 5 to 15%. Therefore, one of our main seed physiological researches was to increase the seed germination percentage of our *Sida* populations up to 70-80%. By means of special seed-priming methods such high germination capacity now is possible and we are able to manage large-scale propagation in industrial plantlet factories.

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Polyploidization program: Another important area of mallow breeding and research is neodomestication and crossing of mallow species with $2n=28$ chromosomes. In 2010 the *Sida* polyploidization program has been initiated, both from the seeds originated from our former collection (under the name of *Napaea dioica*) as well as from seeds originated from wild populations (USA).

Investigation of *Sida* stress-tolerance: We started to study the *Sida* dry-stress tolerance in the point of view of the functional changes / disorders of the photosynthetic system.

Discover of *Sida* plant pathological background: Because of deficient knowledge of *Sida* phytopathological background, the farmers who plant *Sida* from root cuttings confronted considerable risks. We think that it has to give preference to propagation from seeds that from root cuttings. The most serious disease of *Sida* was *Sclerotinia* blight, moreover we observed other soilborne stem and root infections as well as airborne seed infections which molecular identifications and characterizations are in progress.

Breeding of ornamental *Sida*: In 2011 different stem colour variations have been identified in our populations. The discovered stem colours are linked closely to the colour of the pistillum, indicating genetic marker of clonal stability of the colour variations. Due to their lovely purple, yellowish and other stem colour characters, *Sida* can also be appropriate species for ornamental purposes.

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==== POSTER ABSTRACTS ====

SPONTANEOUS VIVIPARIA AND NODAL SHOOT
FORMATION AFTER WINTER SEASON
IN GIANT REED (*ARUNDO DONAX* L.)

G. ANTAL^{1,✉} and M.G. FÁRI¹

Due to the increased demand for growing dedicated biomass crops some previous and new molecular breeding methods as well as *in vitro* somatic techniques have to be integrated into the creation of specially designed „biogeneration” crops. Such as perennial rhizomatous grasses (PRG), like bamboo species, *Miscanthus* sp., giant reed, switchgrass, sugarcane and energy cane hybrids etc. can be cultivated under marginal fields. In order to prognosticate the effect of climate changes on biological and growing techniques, the Working Group of Biogeneration Crops conducted some R&D activities about natural habitats as well as on biotechnologically propagated synplant populations of giant reed and miscanthus since 2010. In the case of giant reed there were observed some surprising phenomenon closely linked to the climate changes. We observed spontaneously formed viviparia and sprouting of secondary nodal shoots from two-year-old stems. Among the monocotyledonous plants species it can be found some good examples for viviparia. One of the known examples is *Poa bulbosa*, however similar phenomenon has already described in *Festuca viviparum* and *Deschampsia alpina*. In 2010 we conducted field research in southern-east part of Portugal where asexual viviparia was also observed in some wild (naturalized) giant reed populations. We measured that there were about 2.500 pieces of well developed shoot-buds and micro-shoots of 1-10 cm lengths per fully developed panicle. Thirty shoot-buds were removed and separated randomly by hand and they were placed into tap water where all of them rooted successfully. We think that such modification of generative panicles to vegetative propagules is considered as strong physiological alterations of which exact background is till now unknown. We think that they were probably formed under extreme heat stress conditions. In spring of 2013, we observed another vegetative reproduction ability of giant reed in Hungary. From the nodes of two-year-old stems there were observed spontaneous

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secondary nodal sprouting; this is well known phenomenon across the Mediterranean, subtropical and tropical countries. In June of 2013 we find that this phenomenon was quite usual in some location at Lake Balaton mostly next to the roads and railways, gardens and parks too. In middle of July, 2013 we measured that a medium-size cane of 1 m diameter and 2.5 m high contained in average 20 pieces of secondary sprouting stems and 200 nodal (secondary) shoots of 10 to 40 cm lengths. There were isolated one-node cuttings and they were planted to soil for rooting. From these lateral shoots there were successfully isolated shoot-meristems under *in vitro* conditions.

Based on our observations carried out on giant reed, the following conclusions can be summarised:

- 1.) To prevent potential invasiveness in our climate it has to be avoiding establishing farms at surrounding areas of water banks and lakes;
- 2.) The undeveloped young secondary nodal buds of the second-year-old stems are highly useful pathogen-free source for initiation of *in vitro* propagules.

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==== POSTER ABSTRACTS ====

FOLIAR APPLICATION OF PLANT PROTEIN HYDROLYSATES
ON VEGETABLE CROPS

**É. DOMOKOS-SZABOLCSY¹✉, B. BLIZMANN¹, A. SZTRIK²,
J. PROKISCH² and M.G. FÁRI¹**

Nowadays plant derived protein hydrolysates using as foliar application in crop production is an intention especially in the organic agriculture. Uptake mechanisms are not well described however presumably it is got into primarily through stomas. The main advantage of hydrolysed protein as organic nitrogen fertilizer is that it can provide readymade building blocks for protein synthesis. At the same time some protein-building amino acids are fundamental metabolites in the process of vegetable tissue formation and chlorophyll biosynthesis. Hence the increased chlorophyll concentration can contribute more intensive photosynthesis. In our case the objective was to investigate the effect of different plant (soybean, lupine and pea) derived protein hydrolysates in 2 mg L⁻¹ concentration as foliar application for some quantitative, qualitative and physiological parameters of red pepper and tomato in field experiment. As expected on the basis previous experiment no significant difference was shown in the average weight of tomato nor green pepper. However the soy protein hydrolysates increased the photosynthetic pigment content both in tomato and green pepper leaves. The photosynthetic efficiency were higher applied lupin and lupin+pea hydrolysates in the same vegetables however all the treatments were in the normal concentration range (0.75-0.85 mg L⁻¹). As concern the qualitative parameters significant increasing were measured in the ascorbic acid content of pepper samples using all treatment and the total carotene also was significantly higher in case of soy hydrolysate.

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==== POSTER ABSTRACTS ====

IN VITRO ROOTING AND GROWTH OF ANANAS USING
DIFFERENT INORGANIC SELENIUM FORMS

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A. SZTRIK², J. PROKISCH² and M.G. FÁRI¹

After bananas and mangoes the pineapple is the third most economically important tropical fruits in the world. The pineapple is routinely propagated vegetatively however *in vitro* micropropagation of it has many advantages over conventional methods. For instance, this technique allows an efficient and rapid increase of selected elite pineapple varieties. At the same time *in vitro* propagation can provide opportunity for controlled biofortification. This is important whereas pineapple is excellent source of several vitamins however it contains small amount microelements especially, selenium. Based on these knowledge, the goal of our study was to see if the sodium-selenate and the red elemental selenium nanoparticles (nanoSe) as inorganic selenium forms are able to influence the rooting and growth of pineapple *in vitro* culture combining with plant hormones (NAA or TIBA). The experimental data clearly indicated that the nanoSe is better tolerated selenium form than the selenate. The selenate in 10 mg L⁻¹ concentration inhibited the root developing and shoot growth, and the plantlets completely died by 100 mg L⁻¹ of it. However, no toxic symptoms were shown in case of nanoSe in the applied concentration range (1-100 mg L⁻¹) regardless of rooting media composition. Both of two selenium forms could uptake in pineapple *in vitro* culture however selenate accumulated in higher amount than nanoSe applied the same concentration in the media.

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==== POSTER ABSTRACTS ====

ESTABLISHING A MULTIFUNCTIONAL SYSTEM
FOR FUNCTIONAL EPITOPE SEARCH AND EDIBLE
VACCINE EXPRESSION IN PLANT

SÁRA PÓLYA¹, EDINA POCZKODI¹, ANNA POZSONYI¹,
GÁBOR TÓTH¹ and LÁSZLÓ TAMÁS^{1,✉}

The main aim of the project we have been working on is to produce edible vaccines in selection marker gene free transgenic barley endosperm. To reach these goals a system has to be established to generate proper transgenic barley lines. The most suitable epitopes of a particular antigen can be quickly selected through the expression of several molecules in plant tissue culture, followed by the appropriate functional studies. To build up the above mentioned system transformation cassettes have been assembled and tested. Results are presented here on the development of the barley heterologous protein expression system.

Antigenic proteins which trigger an effective mucosal immune response can also be produced in transgenic plants. This plant derived edible vaccines are not only cheaper and safer than recently used subcutaneous vaccines but they are considered to be more effective. Edible vaccines are particularly effective against those pathogens which enter through the mucosal membrane.

The cholera toxin B subunit (CTB) as a strong mucosal adjuvant was used to elicit the mucosal immune response, because CTB has an effective adjuvant activity as a carrier protein for genetically fused unrelated proteins. Genes of potential immunogenic proteins or epitopes are able to fuse easily in this cloning system with the gene of the adjuvant protein.

Our intention is to develop a high throughput transient expression system to test and investigate these potential antigenic proteins. Both *Agrobacterium* mediated and biolistic methods are able to use for barley cells transformation. To achieve high expression level in short time the maize ubiquitin promoter, which is a strong constitutive promoter in cereal was used to drive the protein expression in the

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barley tissue. The produced proteins can be used for small scale functional studies in immunology assays because they have desirable biochemical and immunological properties.

For animal feeding experiments and biopharmaceutical industrial applications endosperm specific expression system has to be established. The aim is to create selection marker gene free transgenic barley plant. The vaccine genes are driven by a wheat HMW glutenin promoter, which is strictly endosperm specific. The genes were chosen for preliminary studies can be cloned into the stable transformation vector in one single step.

==== POSTER ABSTRACTS ====

EX SITU CONSERVATION AND MICROPROPAGATION
ON *FRITILLARIA MELEAGRIS* L. AT BIOLOGICAL RESEARCH
CENTRE JIBOU

**ENIKO SELEK¹, LIA MLADIN^{1,2}, OANA SICORA¹ and
COSMIN SICORA^{1,✉}**

One of the main activities of the Botanical Gardens is *ex situ* conservation of threatened and endangered species. Among the species from the Romanian Red List of Endangered Species is *Fritillaria meleagris* L. known under the popular name “lalea peștriță”, “bibilică”. At Biological Research Centre this species is conserved and multiplied *in vitro* for potential repopulation of habitats where *Fritillaria* existed, but now has disappeared (Valea Sălajului, near Cehu Silvaniei). In Sălaj county the species exists, on small habitats, near Chiesd, on the place named Coronzel and in “Poiana Mică” from Poarta Sălajului. Because of the anthropic activities, mainly excessive grazing, the population of *Fritillaria* decreases every year. For this reason, we set up an *in vitro* propagation protocol from bulb scales. After a few passages on MS medium with different concentrations of hormones, we obtained plantlets for going for the next step. After the acclimation the plantlets will be cultivated in the Botanical Garden “Vasile Fati” for *ex situ* conservation. Our goal is to produce enough biological material for possible repopulation of the areas where *Fritillaria* doesn’t exist anymore.

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==== POSTER ABSTRACTS ====

PHOTOSYNTHETIC ANALYSIS OF MOUNTAIN FLORA
WITH MONI PAM FLUORIMETER

LIA MLADIN^{1,✉}, OANA SICORA¹ and COSMIN SICORA¹

Due to a higher exposure of the mountain plants to UV radiation, there is a greater vulnerability of those plants regarding the climate changes. The degradation of the ozone layer causes a specific increasing of the UVB radiation reaching the Earth. The mainly effects of UVB stress in plants are: CO₂ deficiency, stomata closure, diminishing of chlorophyll level, damage of PSI and PSII, sugar decline.

We have studied 3 species from the mountain area: *Vaccinium* sp., *Juniperus* sp. and *Carex* sp. with a MONI PAM fluorimeter. The technique of Pulse Amplitude Modulation (PAM) offers, besides images of chlorophyll fluorescence, images of all relevant photosynthetic parameters: chlorophyll fluorescence, photosynthetic photons flux density (PPFD), PSII efficiency (YII), photosynthetic active radiation (PAR), electron transport rate (ETR) and others. In this way can be detected the photosynthetic activity and its spatio-temporal variations.

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==== POSTER ABSTRACTS ====

EFFECTS OF SALINITY STRESS ON POTATO
(*SOLANUM TUBEROSUM* L.) MICROPROPAGATION

**ANDREEA NISTOR^{1,✉}, MIHAELA CIOLOCA¹,
NICOLETA CHIRU¹, MONICA POPA¹ and CARMEN BADARAU¹**

The effect of salinity on plantlets growth was determined under saline medium and non-saline with five varieties of potato (Christian, Roclas, Marfona, Riviera, Tresor). Plantlets belonging to those varieties, were propagated through single nodal culture. To study the effects of salinity (NaCl) on the growth of single nodal explants, they were cultured on MS media with different concentrations of NaCl including 0, 25, 50, 75 and 100 mmol l⁻¹. Growth of single nodal explants on the media with NaCl indicated that all the characters differed significantly among salinity levels. By increasing salinity level the value for all the parameters decreased.

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==== POSTER ABSTRACTS ====

PRELIMINARY RESULTS REGARDING NBS PROFILING OF
POTATO + *SOLANUM BULBOCASTANUM* SOMATIC HYBRIDS

ABDELMOUMEN TAOUTAOU^{1,✉}, TÜNDE-ÉVA DÉNES¹,
CONSTANTIN BOTEZ² and ELENA RAKOSY-TICAN¹

Potato, after cereals, is the most important crop with a production of more than 373 MT and 368 MT in 2011 and 2012, respectively. Late blight caused by the oomycete *Phytophthora infestans* is the most devastating disease of potato. Due to the concerns of consumers about possible adverse effects of GMOs and pathogen resistance to fungicides, resistance breeding is an optimal alternative. Conventional resistance breeding of potato against *P. infestans* was based on introgression of resistance (R) genes from the wild species *S. demissum*. However, this kind of resistance, based on *S. demissum* R genes, was easily overcome by the pathogen.

Since genetic manipulation by gene transfer is still not accepted by the public and single gene resistance is short lived, somatic hybridization is a good option to overcome the sexual incompatibility. There are 5 families of R genes. All the R genes against *P. infestans* are members of the Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) family. NBS domain is a highly conserved domain in the resistance genes toward pathogens. Primers constructed based on NBS domain sequence tag target the R genes and their homologues.

In this study we used NBS primers to reveal the polymorphism between the somatic hybrids of potato in comparison with parental lines. Somatic hybrids between potato cultivars Delikat and Rasant with *S. bulbocastanum*, their backcross progenies (BC1) and parental lines, which carry two known resistance genes *Rpi-blb1* and *Rpi-blb3*, one of those genes or none of them have been comparatively analyzed. The polymorphism of NBS domain will be also evaluated in relation with late blight resistance assays done with detached leaf assay and in the field.

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==== POSTER ABSTRACTS ====

CYTOGENETIC ANALYSIS OF SOMATIC HYBRIDS
AND BACKCROSS PROGENIES BETWEEN
POTATO AND *SOLANUM BULBOCASTANUM*

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Potato late blight is the most devastating disease of potato caused by the oomycete pathogen, *Phytophthora infestans* a very virulent and adaptable pathogen. The wild potato species, *Solanum bulbocastanum* (2n, 2x24), is highly resistant to all known races of *Phytophthora infestans*, even under intense disease pressure. Oomycete infects foliage and tubers alike, resulting in 16% annual yield loss. Because potato varieties and *S. bulbocastanum* are sexually incompatible somatic hybrids were produced by protoplast electrofusion. Putative somatic hybrid shoots were selected through flow cytometry, only the hexaploid plants being regenerated. The BC1 and BC2 progenies were obtained by crossing hybrids with different potato cultivars. The ploidy level of somatic hybrids and their derived BC progenies maintained *in vitro* is presented here after chromosome counts and analysis. The chromosomes were stained with 4'6 diamidino-2-phenylindole (DAPI) and numbered under epifluorescent microscopy (Olympus BX-60). Micromesure 3.3 software was used to measure chromosomal parameters from electronically captured images and assemble ideograms of parents, somatic hybrid clones and BC progenies.

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==== POSTER ABSTRACTS ====

AN INVESTIGATION INTO THE GROWTH POTENTIAL OF
DIFFERENT STRAIN OF *RHIZOBIUM* ON DIFFERENT MEDIA

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RODICA POP¹**

Rhizobium are a group of bacteria that have the ability to nodulate legumes. In agricultural systems the symbiotic associations between legumes and soil bacteria designated as rhizobia is the most important nitrogen-fixing agents. These bacteria infect the root nodules of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. Different environmental factors like (water deficit, salinity, extreme temperature and mineral nutrients) they affect not only the formation of nodules but also affects the symbiosis. The main objective of the experiment was to identify which medium is more suitable for the development of different strains of rhizobia. In the present study, strains of *Rhizobium* were isolated from root nodules. *Rhizobium* strains are rod shaped to pleomorphic, forming random colonies and mucus producing. The *Rhizobium* strains were isolated using different media Yeast extract mannitol agar (YEMA) with Congo red, a medium with yeast extract, mannitol and soil extract and a medium for N₂ fixing organisms. The Petri plates were incubated at 28 °C in the dark. Further characterizations were done by performing morphological traits and also determine what media is more suitable for the growth of rhizobia. Regarding to number of colonies formed on all media, *Rhizobium trifolii* wild type showed a larger number of CFU compared to *Rhizobium trifolii* which has a lower number of colonies. The optimum medium for the growth of *Rhizobium* species was seen to be the one with mannitol, yeast extract and agar (YEMA), also appeared to work well medium for N₂ fixing organisms for the wild type. In conclusion rhizobia can grow best on glucose and mannitol and in general are easy to culture.

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==== POSTER ABSTRACTS ====

EFFECTS OF SALINITY ON THE GERMINATION CAPACITY
OF SOME *ARABIDOPSIS THALIANA* MUTANT LINES

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Arabidopsis thaliana, a small spring annual plant belonging to the Brassicaceae family, is used since the 1940s as a model in plant genetics and biotechnology due to some advantages, like small genome, large production of seeds and short life cycle. From the three major ecotypes found in the spontaneous flora (Columbia, Landsberg and Wassilewskija) a series of loss of function and gain of function mutants were created since the 1960s in order to answer questions related to several genes and proteins involved in the physiological mechanisms of plants. The most attention was laid on the genes and proteins involved in plant adaptation to abiotic and biotic stress conditions. The glycine-rich RNA-binding proteins are a major class of proteins found in superior plants that are known to have a big implication in plant adaptation to low temperatures and other osmotic and oxidative stresses. The main focus of this research was to assess the involvement of two glycine-rich RNA-binding proteins (GRP2 and GRP7) in seed germination capacity under salt stress conditions. The plant material was composed out of seed batches obtained from the T-DNA insertion *Arabidopsis thaliana* mutant lines labelled *grp2* (knock-out mutant for the glycine-rich RNA-binding protein 2), *grp7-1* (knock-out mutant for the glycine-rich RNA-binding protein 7), *WS7ox* (overexpressor for the GRP7 protein) and the Col0 ecotype, which was used as control. For the germination assay, three different concentrations (100 mM, 150 mM and 200 mM) of NaCl were used, with whom the control medium was supplemented. The control medium chosen consisted of ½ MS (Murashige & Skoog) vitamin medium without sugar. Fifty seeds were placed on each petri dish and they were assessed for seven days. After this period, the germination rate was estimated. Three independent replicates were used and the statistical analysis was obtained with the help of the trial version from GraphPad Prism software. The results obtained showed that the seeds belonging to the *grp2* mutant were not affected by the high salt concentration, in contrast to the ones obtained from the *grp7-1*

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mutant and *WS7ox* line. Also, in comparison to the wild-type, the germination rate of these seeds was higher. As a conclusion, we can assume that even though the two proteins taken under observation belong to the same class and family, they have different roles regarding the seed germination capacity.

=== POSTER ABSTRACTS ===

MODIFICATION OF THE PHENYLPROPANOID PATHWAY
AFTER DIFFERENT SALICYLIC ACID TREATMENTS
IN WHEAT

**ORSOLYA KINGA GONDOR¹, TIBOR JANDA¹ and
GABRIELLA SZALAI¹**

Salicylic acid (SA) was isolated first from bark of the willow tree (*Salix*) and used as a medicine. Its biosynthesis is part of the phenylpropanoid pathway where its first precursor is the phenylalanine. Two routes from phenylalanine to salicylic acid have been described that differ at the step involving hydroxylation of the aromatic ring. Phenylalanine is converted into cinnamic acid (CA) by phenylalanine ammonia lyase. Cinnamic acid can be hydroxylated to form ortho-hydroxycinnamic acid (oHCA) followed by oxidation of the side chain. Alternatively, the side chain of cinnamic acid is initially oxidized to give benzoic acid (BA), which is then hydroxylated in the ortho position. Flavonoids are important protectants during stresses and their biosynthesis derived from the cinnamic acid via trans-hydroxycinnamic acid. The aim of the work was to investigate physiological/biochemical processes induced by the different exogenous SA treatments.

Mv Emese winter wheat variety was used for the experiments. Plants were grown in hydroponic solution. SA treatment was carried out either by soaking seeds in 0.5 mM SA for overnight before sowing or by addition of 0.5 mM SA to the hydroponics of seven-day-old plants for a day. Leaf and root samples were collected after 1 and 7 days of the hydroponic SA treatment. For detection of the oxidative stress the lipid peroxidation was measured via malondialdehyde (MDA) content spectrophotometrically. 0.5 g plant material was used for determination of SA, BA, CA, oHCA and flavonoids. Methanol soluble free, methanol soluble bound and methanol insoluble bound fractions were measured. The analysis was carried out using an HPLC equipped with a UV-VIS and fluorescence detector.

It can be seen from the results that the level of SA and its precursors changed after the treatments. The level of BA, CA did not change while the SA and oHCA content increased after 1 day of SA treatment in the leaves. The MDA concentration

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also increased compared to the control which alludes to the increased oxidative stress. oHCA can serve as an antioxidant so its elevated level can be a consequence of the stress. The BA content decreased, SA content increased while the oHCA content did not change in the methanol soluble bound fraction. The level of SA, BA, CA and oHCA did not change in the methanol insoluble bound fraction. Seed soaking did not have any effect on the content of the endogenous SA and its precursors in either of fractions. Free oHCA increased after seven days but there was no change in the bound fractions. The SA level increased in the case of hydroponic SA treated plants in all the fractions while the BA and CA content remained at the initial level. The CA content decreased while the oHCA content increased in the free fraction in the roots of the hydroponic SA treated plants after one day. The MDA concentration also increased as a marker of the oxidative stress. The SA content increased in all the fractions. The level of free SA increased after the seventh day of hydroponic SA treatment while the CA and BA did not change.

Some of the flavonoids were also analysed. Myricetin (M), kaempferol (K), quercetin (Q) and rutin (R) were measured. The M, Q and R level increased in the leaves after the SA treatments. R and K level decreased in the free fraction in the roots after one day and it still remained at this level for seven days compared to the control values. The content of the methanol soluble bound M and R decreased after one day but increased after seven days. The Q concentration increased only in the free fraction of the leaves after one day and a decrease could be observed in the roots in all the fractions. The level of free rutin slightly increased but it decreased in the roots after one day. An increase could be seen in the free rutin content in the roots after seven days.

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==== POSTER ABSTRACTS ====

EFFECTS OF UV-B RADIATION UNDER DIFFERENT ABIOTIC
STRESS CONDITIONS IN WHEAT

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At the continental climate high UV-B radiation, especially in combination with aridity, causes several morphological and physiological changes in plants, leading to the decrease in crops quality and quantity. In the background of diverse plant responses to UV-B there are different processes such as the changes in the enzymatic antioxidant system and numerous other defence mechanisms, which can be linked to the salicylic acid-dependent signal transduction. Salicylic acid is known as a signal molecule which has role in the induction of acclimation processes under abiotic stresses conditions in plants. The aim of the present work is to better understanding of the effects of UV-B radiation, cadmium and drought stress and the cross-talk between these abiotic stresses in wheat. So we studied the effects of these stresses individually and in combination. Seedlings of winter wheat (*Triticum aestivum* L. Mv Emese) variety from Martonvásár were grown in growth chamber under normal light conditions or at normal light combined with UV-B radiation at the same time. One part of the two weeks old plants was the control, second part of the plants was treated with 50 μM $\text{Cd}(\text{NO}_3)_2$ for 7 days and the third part of the plants was treated with 15 % polyethylene glycol (PEG-6000) for 5 days. The three different stresses induced changes in the antioxidant system, salicylic acid metabolism and biosynthesis were characterised in leaf and root samples using spectrophotometric methods and HPLC. Seedlings showed reduced growth and induced anthocyanin production under supplemental UV-B radiation. PEG+UV-B treated seedlings showed smaller degree of shrivelling, increased content of bound oHCA and free SA, and induced activity of glutathione reductase and catalase, compared to the seedlings treated only with PEG. Cd+UV-B treatment enhanced the effects of Cd. The leaves of these plants became yellowish, the bound oHCA and SA levels of them significantly increased while the activities of the antioxidant enzymes

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decreased. However, in the roots, the first organ exposed to Cd, the antioxidant system induced. Although UV-B decreased the plant development, it was found that under these conditions UV-B radiation caused successfully hardening against to drought stress, but resulted in stronger damages Cd-treated wheat seedlings.

==== POSTER ABSTRACTS ====

“GLOBALIZATION” IN THE SEXUAL REPRODUCTION OF
CEREALS: WIDER CHOICE, BETTER HYBRIDS

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Ultrawide hybridization (UWH), i.e. non-GM trait transfer by sexual crossing between distant species or genera, has long been applied to introduce novel genes into crop plants. Major hurdles prohibiting more widespread and routine use of UWH are the low frequency and viability of hybrid embryos, which makes embryo rescue and nursing plants by tissue culture compulsory steps in the process. Beyond its agronomical importance UWH, however, offers a fascinating model to study early phases of reproductive development. Here we asked whether low success rates of UWH in cereals are caused by a low frequency of fertilization (including egg activation and pollen tube formation) and/or by incomplete or blocked endosperm development.

By using an optimized wheat (♀) x barley (♂) hybridization system and via a comprehensive microscopical study we have found that pollen tube formation and fertilization can successfully be accomplished. Endosperm development, however, was essentially blocked at or before the onset of the cellular phase, which can cause early embryo abortion. Via suppressing this endosperm block we were able to produce normal, endosperm-containing wheat-barley hybrids. As a result, harvested hybrid seeds germinated readily without the aid of tissue culture. The majority of these plants contained the full, 7-chromosome complement of barley.

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