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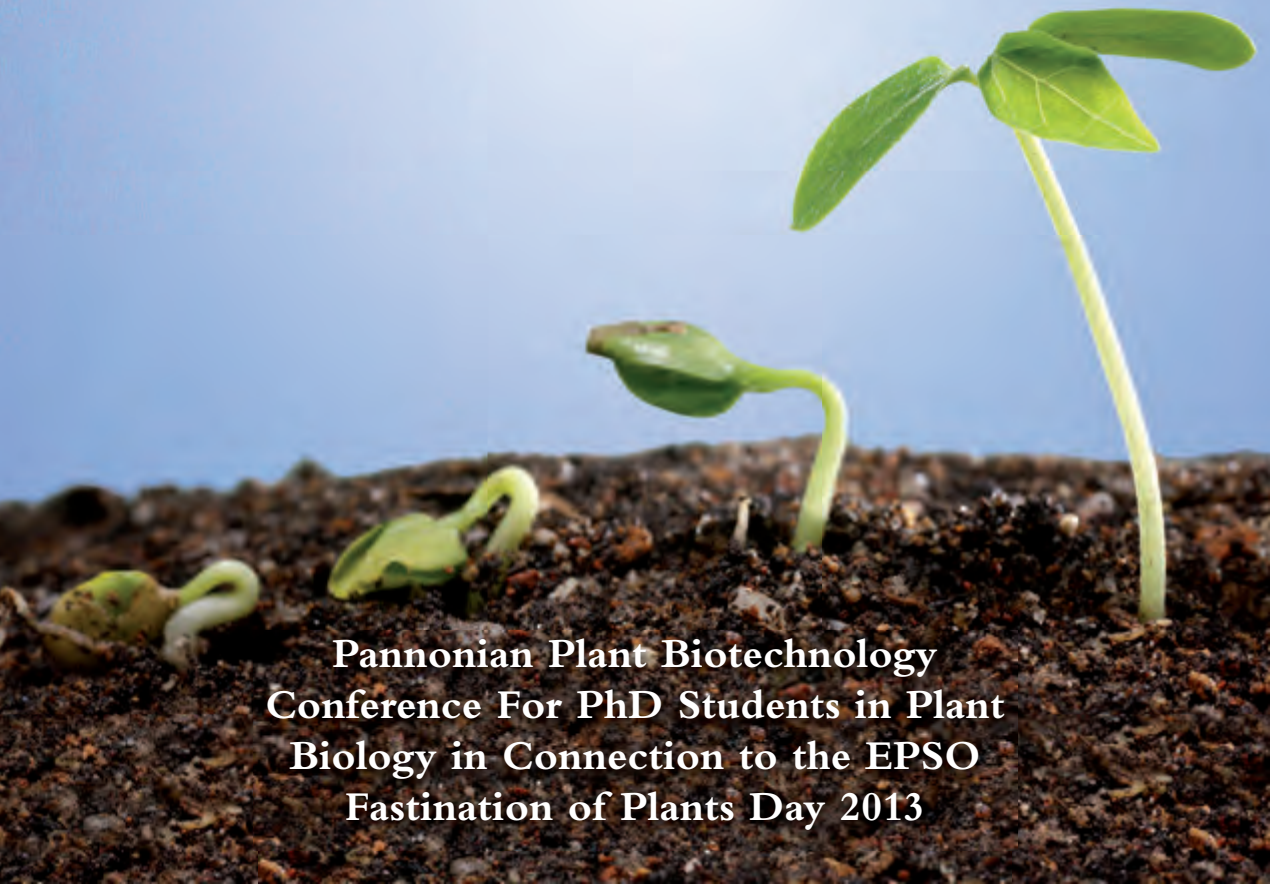

Pannonian Plant Biotechnology Conference For PhD Students in Plant Biology in Connection to the EPSO Fastination of Plants Day 2013

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book of abstracts

Pannonian Plant Biotechnology Conference For PhD Students
in Plant Biology in Connection to the EPSO Fastination of Plants Day

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The Pannonian Plant Biotechnology Association,
in collaboration with the
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The aim of the one day conference for PhD students working in the field of plant biology to exchange their ideas and their research work which is the basis for our future. PhD students are invited from all Doctoral Schools of the Universities for participation and presentations of their studies.

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TRANSCRIPTOME AND FUNCTIONAL MARKER STUDY IN POTATO

Rahim Ahmadvand, Ramin Hajianfar, Zsolt Polgár, and János Taller
Doctoral School of Plant Production and Horticultural Sciences, University of Pannonia, Keszthely

Next Generation Sequencing (NGS) technologies have enabled researchers to sequence complete transcriptome (RNA-seq) from plants in different conditions and time points to generate a large transcript sequence data set for gene discovery, gene functional studies and molecular marker development. Publication of the potato genome sequence has provided the possibility to apply genome sequence as a reference sequence for the transcriptome analysis of this crop. In this study, transcriptome profile of the resistant potato cultivar, White Lady was generated by next generation sequencing (NGS). The plantmaterial was inoculated with PVX, PVY and *Phytophthora infestans* in three independent experiments in three replications under greenhouse conditions. In parallel, control plants were inoculated with phosphate buffer saline (PBS) and distilled water for viruses (PVX and PVY) and *P. infestans*, respectively. After isolation of post inoculation mRNA in different time points, one pooled sample was prepared represented equal volumens of the three experiments and this sample was sequenced with NGS, 5500 XL SOLiD Applied Biosystem. Sequence reads were assembled into contigs, normalized and the fold change, the number of reads per kilobase per million reads (RPKM) were analyzed by CLC Genomics Workbench 4.8 (64 bit) software. The functional annotation of transcript sequences was performed using reference sequences from the Solgenomics database. By comparison of the expression level of treated and control plants, thirty nine thousand transcripts were identified among which 7788 and 4049 genes were upregulated as well down regulated, respectively. About 2600 transcripts of upregulated genes were unknown genes or conserved genes of unknown function. The data set contains 141 NBS-LRR encoding genes with 13 Toll Interleukin-like receptor (TIR) and 50 Coiled- coil (CC) types. The generated data set provides a powerful tool in different aspects of potato genetics such as discovering functional markers and determining the function of important genes in resistance.

In order to develop Intron targeting (IT) markers, which display polymorphism in length or nucleotide variation of the alleles as codominant markers for genetic diversity studies, a number of upregulated transcript sequences were screened to identify intron regions. The transcript sequences were aligned with potato genome sequence (whole genome shotgun contigs) in NCBI database using BLASTn with the E-value 10^{-20} up to June 2012. The program Sim4 was applied to align transcript sequences to the corresponding genes in potato in order to predict the intron regions. The primers were designed based on the exons flanking putative introns. Polymorphism of each locus was evaluated in 24 individuals of a potato F_1 segregating population generated from a cross between the cultivar White Lady and the breeding line S440 and also 24 potato cultivars on different origin were screened with the designed primers. The results of screening indicated that 12 out of 40 designed putative markers showed polymorphism in both the F_1 population and the potato cultivars. Heterozygosity based on the polymorphism of markers ranged from 0.39 to 0.76. The number of amplified alleles in each locus

ranged from 3 to 5 in both analyzed populations. Some of the intron markers could be localized on chromosomes III, IV, V, VIII, XI and XII. These developed intron targeting markers enrich the number of anchor markers of the potato genome, as well as they can be utilized in genetic diversity studies and in marker assisted selection.

DEVELOPMENT OF AN EFFICIENT *AGROBACTERIUM*-MEDIATED TRANSFORMATION METHOD IN *BRACHYPODIUM DISTACHYON* FOR THE CHARACTERIZATION OF LOB TRANSCRIPTION FACTORS

Zoltán Zombori, Mária Szécsényi, Magdolna Gombos, János Györgyey
University of Szeged, Biology Doctoral School
Hungarian Academy of Sciences Biological Research Centre, Szeged

Poaceae is one of the most valuable plant families for the human civilization, big share of the forage and lawn plant species, and all cereal crops grown all around the world come from this family. Beside their fundamental agricultural importance they are also a predominant family of the monocot plants, therefore the establishment of a functioning grass model system – as the *Arabidopsis thaliana* for the dicot plants – is crucial for the plant biology research as well. The requirements of a good model plant are small size, short generation time, self-pollination, simple growth conditions, small, sequenced genome, diploidity and transformability. The *Arabidopsis* possesses these parameters (Bevan et al. 2005), but due to the fundamental differences in growth and development between monocots and dicots, a grass model species that facilitates comparable features to those ones in *Arabidopsis* would gain wide applicability for future crop improvement. The first grass species with available whole sequenced genome was the rice *(Oryza sativa)* (Yu et al. 2002), but its relatively big plant size, long generation time, and its demanding growth conditions make this plant an imperfect model organism. The *Brachypodium* genus is located at the base of the four grass tribes that includes the majority of domesticated cereal and forage crops (Kellogg 2001), and taken together with the grass genome's remarkable colinearity, as well as the other prosperous parameters of the *Brachypodium distachyon* – the single annual species from this genus – such as the small, diploid, sequenced genome (International *Brachypodium* Initiative 2010), and the other traits that fulfill the requirements, this plant seems to be the ideal experimental model plant for the Poaceae.

The development of an efficient transformational method is indispensable in the case of a modern plant model system due to its importance in several research fields. However the most prevalent transformational methods – biolistic and *Agrobacterium*-mediated – were described efficient in *Brachypodium* (Draper et al. 2001), the *Agrobacterium*-mediated technique was chosen for our current experiments owing to its advantages compared to the biolistic transformation, such as lower copy number, and the decreased possibility of the realignment or fracture of the transferred DNA sequences.

The Bd21 inbred line was used as transformation material and experimental genotype, because this accession's genome was sequenced, and tolerates well the conditions of the tissue culturing (Vogel et al. 2006). Two strains of *Agrobacterium tumefaciens* – LBA4404 and AGL-1 – were employed during the improvement of the method. These strains carried different plant expression vectors, with different plant selection markers: Basta or hygromycin phosphotransferase, respectively. For the callus induction 2,4-D was applied on brome embryos

(60 pcs) collected 15-20 days after visible anthesis. After the formation of callus tissues, the calli were subcultured twice to increase the amount of the transformational material. To foster the *Agrobacterium* infection, a 10 min vacuum treatment was performed. The calli and the *Agrobacterium* were co-cultivated for 1, 2 or 3 days to determine the optimal treatment, and then the calli were replaced onto selective media. After 3 weeks and one passage, the surviving calli are grown enough for sampling to verify the success of the transformation using genomic PCR technique specific for the transgene. In the regeneration steps two different hormonal conditions were tested: kinetin or an indole-3-acetic acid (IAA) – 6-benzylaminopurine (BAP) mixture. To provoke strong root development, a 50 μg/l 1-Naphtalaneacetic acid (NAA) treatment was applied to help the plants to survive the stress of the potting the *in vitro* plants in the greenhouse.

In the transformation experiments the callus induction was performed with 90% efficiency. The AGL-1 *Agrobacterium tumefaciens* strain was shown to be the right choice to transform *Brachypodium*; very low transformational efficiency could be observed using the LBA4404 strain. During the transformation the optimal co-cultivation time was determined as 2 days – the shorter co-cultivation decreased the number of the transformed calli, whilst the longer treatment caused the overgrowth of the bacteria, and the elimination of the *Agrobacterium* became more complicated. The plant selection agents were also tested, and the hygromycin B in 50 mg/l concentration appeared to be the better option. In the case of phosphinothricin (PPT) finding the selective concentration was difficult, the best results were obtained using 8 mg/l, but still some non-transformed surviving callus lines could be found. During the regeneration, the root formation was usually weaker than that of shoots. To resolve this problem, an auxin-citokinine mixture (IAA-BAP) replaced the exclusive application of citokinine (kinetin). This modified regeneration media triggered the root formation in the 50% of the rootless lines 2 weeks after the replacement of the original calli. The 50 μg/l NAA treatment was very effective; the plants grew strong root system made them capable to survive the planting in pots in the greenhouse. In the most successful experiment 58 regenerated false brome plants produced seeds.

Since the transformation system was developed, our next aim was to examine the function of LOB domain transcription factors in *Brachypodium* using this tool. During plant development, the separation of meristematic cells from differentiated ones plays pivotal role in formation of plant architecture. The LOB (Lateral Organ Boundaries) transcription factors are plant-specific proteins involved in the above processes, their sequence contains a conserved LOB domain that harbors a conserved 4-Cys motif with $CX_2CX_6CX_3C$ spacing, a Gly-Ala-Ser block, and a predicted coiled-coil motif with $LX_6LX_3LX_6L$ spacing (Lee et al. 2009). They were firstly described in *Arabidopsis*, the LOB domain was found in 42 genes (Shuai et al. 2002). The expressions of many LOB genes are induced by auxin, and they are regulated in the auxin signaling pathway. These genes are expressed in different plant tissues and organs, and they play important role in organ separation and lateral organ development. Understanding their function might have great agronomical importance in lateral root development or biomass production in cereals.

Based on sequence alignment and data mining, several LOB transcription factors were identified in *Brachypodium distachyon*. Two of them were selected for further analysis, similar to the *Arabidopsis* LOB13 and LOB15, respectively. Plant expression vectors were constructed using their cDNA driven by constitutive promoter for overexpression, and their promoters were fused to Green Fluorescent Protein (GFP) in promoter testing vectors. Detailed analysis of transgenic *Brachypodium* lines transformed with these constructs are in progress.

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MOLECULAR CYTOGENETIC CHARACTERIZATION AND PHYSICAL MAPPING OF A 7D.5HS WHEAT/ BARLEY TRANSLOCATION LINE

Klaudia Kruppa[1,2], Éva Szakács[1], Adél Sepsi[1], Márta Molnár-Láng[1]
[1]*Agricultural Institute, Centre for Agricultural Research of HAS, Martonvásár*
[2]*Plant Science PhD School, Szent István University, Gödöllő*

Hordeum vulgare L., the fourth most important crop in the world, carries several genes involved in biotic and abiotic stress tolerance, which can be transferred to wheat via wide hybridization. The transfer of genes of interest into wheat can be achieved by the production of wheat-barley translocation lines. A spontaneous wheat/barley translocation line was detected in the progenies of the Mv9kr1 x 'Igri' hybrid produced in Martonvásár (Molnár-Láng et al. 2000). The translocation and its breakpoint position were visualised using multicolour genomic *in situ* hybridization (GISH) using total DNA from *Aegilops tauschii* (DD) and *Hordeum vulgare* (HH). The rearranged chromosome was then analysed with fluorescence *in situ* hybridization (FISH) using repetitive DNA sequences (Afa-family, pSc119.2 and pTa71) and was identified as a 5HS-7DS.7DL translocation.

Translocation lines are excellent genetic materials for the physical mapping of molecular markers to specific breakpoint intervals (bins). Forty-five SSRs distributed over the 7D chromosome were used to characterize the wheat segment of the translocation and to identify the 7D chromosome segment which had been eliminated by the 5HS barley chromosome arm. The 5HS-7DS.7DL disomic translocation line and its parental wheat and barley cultivars were grown in the field in Martonvásár in 2012. Plant height, tillering (spikes per plant), fertility (seeds per spikelet), length of the main spike, spikelets per main spike, seeds per main spike and seeds per plant were measured after harvest and used for statistical analysis.

With the help of three-colour FISH analysis all the wheat chromosomes were identified. As a control, the Mv9kr1 wheat parent line was used to compare the FISH pattern of the complete 7D chromosome with the translocated 7D chromosome. Strong Afa-family hybridization signals were detected in both terminal regions of the complete Mv9kr1 7D chromosome but the 7D involved in the translocation had weaker signals on the short arm, suggesting the deletion of a short chromosome segment from the terminal region of 7DS.

All the 7DL-specific markers produced PCR products of the expected size on wheat and on the translocation DNA, confirming the presence of the complete 7DL arm. (Fig.1). Ten 7DS-specific markers (Xbarc0184, Xwmc0506, Xgdm0130, Xgwm0735b, Xgwm1258a, Xgwm1123, Xgwm1250, Xgwm1055, Xgwm635a and Xgwm1220) failed to amplify any 7DS-specific fragments, signalling the elimination of a short chromosome segment carrying these markers. The remaining 7DS-specific markers were present on the translocation chromosome. In addition, four markers (Xbarc0126, Xgwm0044, Xgwm0295, Xbarc0214) previously mapped to the terminal bin of 7DS, between fraction lengths (FL) 0.61-1.00, were found to be present on the rearranged 7DS revealing a more precise physical position for these markers which can be located proximally within FL0.61-1.00. As the FISH experiment indicated the absence of a strong Afa-family signal in the terminal 7DS region,

it can be concluded that the missing 7DS-specific markers are located on the deleted, most distal region of 7DS. The present study physically localised ten 7DS-specific SSR markers to FL 0.61-1.00 distally to markers Xbarc0126, Xgwm0044, Xgwm0295 and Xbarc0214. The breakpoint of the 5HS-7DS.7DL translocation appeared to be more distal than that of reported deletion lines, which provides a new physical landmark for future deletion mapping studies.

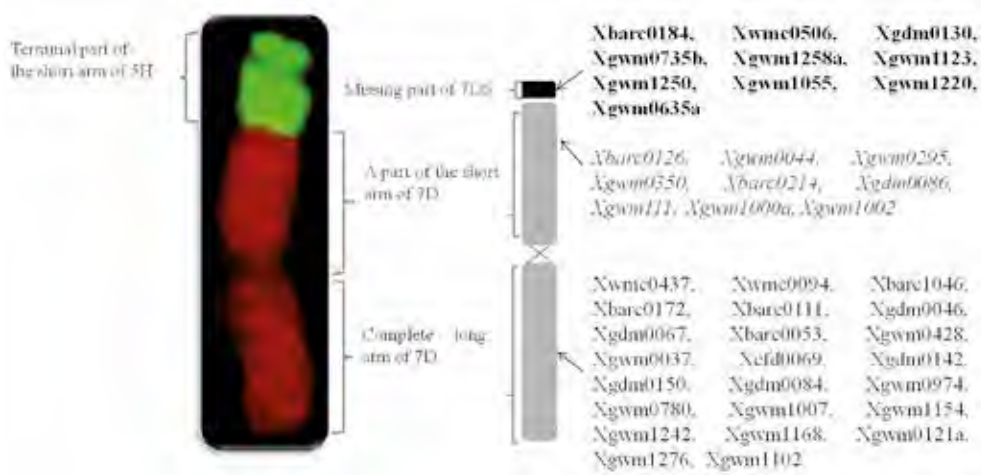

Fig.1. *Left*: mcGISH pattern of the 5HS-7DS.7DL translocation chromosome using *Hordeum vulgare* and *Aegilops tauschii* DNAs as probes.
Right: Physical mapping of 7DS-specific markers on the 5HS-7DS.7DL translocation chromosome. The markers previously mapped physically to the 7DS terminal region are in blue italics. Markers mapped physically in the present study to the deleted 7DS chromosome segment are visualised in orange.

The importance of this line is the maintenance of part of the 5H chromosome in the wheat background, which has previously proved difficult to achieve. In previous wheat × barley crosses, this chromosome was eliminated with high frequency. Another advantage is the possibility of studying how 5HS genes are expressed in a wheat genetic background. The hordoindoline genes are located on chromosome 5HS and play a role in reducing grain hardness in the wheat genetic background (Yanaka et al. 2011). The lower values obtained for agronomic parameters could be caused by the presence of the 5HS chromosome segment, by the small deletion on the 7DS chromosome arm, or most probably by both together. The lower fertility, plant height and yield parameters of the translocation line compared with the wheat parent Mv9kr1 could be interpreted as the effect of non-compensating translocations. Despite the non-compensating translocation, the plants showed good viability together with high genetic stability.

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IDENTIFICATION OF WHEAT – *AE. BIUNCIALIS* INTROGRESSION LINES CONTAINING CHROMOSOME 3M^b AND EVALUATE THEIR POTENTIAL TO IMPROVE MICRONUTRIENT CONTENT OF WHEAT

András Farkas[1,2], István Molnár[1], Sándor Dulai[3], Sándor Rapi[3], Vince Oldal[3], András Cseh[1], Klaudia Kruppa[1], Márta Molnár-Láng[1]

[1]*Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár*

[2]*Szent István University, Plant Science PhD School, Gödöllő*

[3]*Institute of Food Science, Faculty of Science, Eszterházy College, Eger*

Micronutrients are essential in human diet and insufficient daily intake of some of them is one of the alimentary disorders that can provoke serious illnesses. Zinc is among the microelements that have a greatest effect on the health and quality of life as nearly half of the world's population is deficient in Zn. Bread wheat (*Triticum aestivum* L.) is the major staple food crop in many parts of the world, therefore development of wheat varieties with increased grain micronutrient concentrations, known as biofortification, is one of the most promising and cost effective approaches to alleviate micronutrient malnutrition.

Wheat wild related species, such as *Aegilops* sp., constitute a rich reservoir of useful alleles for agronomically important traits such as resistances against biotic and abiotic stresses and yield components. It has also been reported that *Aegilops* species (e.g. *Ae. longissima, Ae. kotshii, Ae. cylindrica, Ae. ventricosa)* have 2- or 3-times higher grain mineral content, including Zn and Fe, than cultivated wheat demonstrating the potential of wild *Aegilops* species for the biofortification of wheat.

Aegilops biuncialis Vis. (2n=4x=28; $U^bU^bM^bM^b$), which is closely related to bread wheat, has good tolerance to several abiotic and biotic stresses such as drought, barley yellow dwarf luteovirus, yellow rust and brown rust. Although the micronutrient content of *Ae biuncialis* haven't been investigated yet, some studies indicated that wild goatgrass species with U and/or M genomes, such as *Ae. geniculata* (UM), *Ae. peregrina* (US) and *Ae. kotchyi* (US) have significantly higher grain micronutrient content than wheat. Other studies have reported that the grain mineral content of wheat-*Aegilops* amphiploids is similar to their *Aegilops* parent, which proved that wild alleles determining the micronutrient content can be expressed in a wheat genetic background.

For the chromosome mediated transfer of useful wild aleles into bread wheat, it would be desirable to produce wheat-*Ae. biuncialis* interspecific translocations. However, the *Aegilops* and wheat chromosomes are unable to pair in the meiosis because of the presence of the *Ph1* locus on the long arm of chromosome 5B in wheat. Sears produced a mutant genotype of Chinese Spring which contains a deletion on the Ph1 locus and consequently it lacks the activity of *Ph1.* Because of the increased frequency of homoeologous chromosome pairing, the Chinese Spring *ph1b* (CS*ph1b*) mutant genotype has been extensively used for the introgression of alien genetic material into cultivated wheat.

The present study reports on the identification of a wheat-*Ae. biuncialis* $3M^b(4B)$ disomic substitution and a $3M^b$.4BS centric fusion originating from a cross between the $3M^b$ wheat-*Ae. biuncialis* disomic addition and the CS*ph1b* mutant and evaluate the potential of *Aegilops* chromosome $3M^b$ to improve grain micronutrient content of wheat. The F_3 generation was characterized by *in situ* hybridization with repeated and genomic DNA probes (FISH and GISH) and by microsatellite markers. The grain iron, zinc, manganese and potassium content were also determined in the $3M^b$ wheat-*Ae. biuncialis* addition, $3M^b(4B)$ substitution and $3M^b$.4BS centric fusion lines and in their parental wheat and Aegilops genotypes.

Sequential FISH and GISH were performed to characterize the genomic constitution of F_3 progenies. All the wheat chromosomes could be identified based on the hybridization pattern of the pSc119.2, Afa family and pTa71 probes. Four F_3 plants lacked the 4B chromosome, which has a characteristic pSc119.2 hybridization pattern (a strong distal band on 4BS and two intercalary and one distal band on 4BL). GISH revealed two intensively labelled $3M^b$ *Ae. biuncialis* chromosomes in genotypes having 42 chromosomes, which were classified as $3M^b(4B)$ disomic substitutions. FISH also detected a terminal deletion on 7BL in the substitution lines, with a deletion breakpoint between the diagnostic bands of the pSc119.2 and Afa family probes in the intercalary region of 7BL. In five F_3 progenies, *in situ* hybridisation demonstrated the presence of two homozygous $3M^b$-wheat centric fusions, with similar FISH patterns. The pSc119.2 patterns of the wheat chromosome arms involved in the centric fusions were similar to that of the 4BS chromosome arm (strong distal band on 4BS), so were classified as $3M^b$.4BS centric fusions. SSR markers specific for the 4BS, 4BL and 7BL chromosome arms were also used to confirm the cytological results.

Grains of the $3M^b$ wheat-*Ae. biuncialis* disomic addition, $3M^b(4B)$ disomic substitution, $3M^b$.4BS centric fusion and the parental lines were evaluated for grain potassium, zinc, iron and manganese concentrations by Atomic Absorption Spectrometry (Varian SpectrAA-50/55). *Ae. biuncialis* MvGB642 had significantly higher micronutrient content than the wheat Mv9kr1(36.8 %, 35.1%, 71.2% and 47.3% over the Mv9kr1 for potassium, zinc, iron and manganese, respectively). The grain zinc (23.2 mg/kg) and manganese (40.9 mg/kg) content of $3M^b$.4BS centric fusion proved a significant increase in zinc (23.4%) and manganese (38.2%) content relative to wheat genotype Mv9kr1. The results of the present study suggests that the 3Mb chromosome arm involved in the wheat-Ae. biuncialis centric fusion contains genomic region responsible for increased Zn and Mn content of the grains. The wheat-Ae. biuncialis introgression lines containing chromosome 3Mb could be used in prebreeding programs aimed to increase the grain micronutrient content of elite wheat cultivars (i.e. 'biofortification').

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CHARACTERIZATION OF STRESS TOLERANCE OF TRANSGENIC BARLEY PLANTS USING CELL-AUTONOMOUS FLUORESCENCE DETECTION METHOD

Bettina Nagy, Gábor V. Horváth
Plant Science Doctoral School, Szent István University
Hungarian Academy of Sciences Biological Research Centre, Szeged

Barley is one of the most important cereal in the world: it is used as a major animal feed crop and as an important element in malt and beer production. In our country, in the 2012 year, barley was planted on 278 000 hectars, and it is ranked third of the total Hungarian cereals production. Due to its smaller genome compared to wheat and better natural tolerance to drought, it became for an ideal genetic model plants of cereals in recent years.

In barley, there is a great potential of breeding genetic resistance against the environmental stresses. Generally, biotic and abiotic stresses produce and accumulate high amount of reactive oxygen species (ROS) in the plant cells *(Moran et al.* 1994, *Price et al.* 1989*),* and can directly modify the lipid membrane which leads to lipid hydroperoxide and lipid peroxide formation that often occurs in response to stress. Those transformation strategies that aim to detoxify these compounds could be used to create stress-tolerant plants. For the first established barley transformation, a thoroughly analyzed candidate gene, the alfalfa *(Medicago sativa L.)* aldo-keto reductase *(MsALR)* was used, which was isolated and analyzed previously in our laboratory *(Horváth et al.* 1999, *Oberschall et al.* 2000, *Hideg et al.* 2003*)*. The MsALR protein accumulated in the cytoplasm of the transgenic plants, but the applied vector system is also provides an opportunity to change the localization of the produced protein and to target the candidate gene into the chloroplasts. Because the main source of reactive oxygen species (ROS) in plants is the chloroplast *(Asada* 2006*),* the ROS react with the polyunsaturated fatty acids resulting in many lipid-peroxidation products, so the amplification of the reactive aldehyde detoxification enzymes in this cell compartment surely increases the tolerance against the environmental stresses. In the regenerated transgenic lines, the transgene encoded-protein levels were identified in different cellular compartments. With the propagation of the progeny, we got enough seed for testing the candidate lines in the transient assay system.

A stable transformed barley lines provide further testing possibility for examining the role of stress resistance-related candidate genes. In barley, a transient expression test system was previously developed to identify the role of Barley powdery mildew resistance genes (Panstruga 2004, Dong et al. 2006). The system suitable not only for transient overproduction of the candidate gene, but also for transient induced gene silencing (TIGS) and for functional pre-screening of larger numbers of drought or dehydration stress related candidate genes in barley (Marzin *et al.* 2008, Nagy *et al.* 2011). Our aim was to develop and improve an effective and easily reproducible analytic system which can be applied in various stress and which is suitable for testing the cellular stress tolerance of the stable transgenic genotypes.

In the transient assay study, the microprojectile bombardment and stress treatments were carried out according to the protocol of Marzin *et al.* (2008). For the stress tolerance experiments of barley leaves, the green fluorescent GFP (*Aequorea victoria*) and the red

fluorescent protein DsRed *(Discosoma sp.)* protein proved to be useful. According to the biochemical features of the DsRed protein, it was susceptible to recognize and characterize the drought stress effect in bombarded barley epidermal cells. DsRed requires several days for maturation into the fluorescent tetramer complex and sensitive to denaturing conditions, so the number of the calculated DsRed expressing epidermal cells – at the end of the applied 4 days long drought stress – is a valuable parameter that can mirror the cellular stress severity caused by dehydration and to estimate the cellular stress tolerance of the candidate transgenic barley lines. The amount of mature, fluorescent DsRed will be reduced according to the level of strictly stressed cells, because the cell death will reduce the amount of fluorescent DsRed *(Gross et al.* 2000, *Baird et al.* 2000*)*. The number of the DsRed expressing cells will increase if the transgenic plants are more tolerant against the applied stress.

Transgenic barley plants producing the MsALR enzyme – targeted to the cytosol or the chloroplast – represented significant differencies both in the fluorescent GFP/DsRed cell ratio and in the measurement of chlorophyll and carotenoid content in the case of the non-stressed and the dehydration stress-treated samples compared to the wild type control leaves, so the transgenic lines proved to be more resistant against the applied dehydration, salt and reactive carbonyl stress.

The main advantage of the transient assay system in our adaptation is the possibility to utilize not only in the case of drought stress, but it could be easily adapted to a variety of other stress conditions may be induced in laboratory. Furthermore it can provide information about the abiotic stress tolerance even when other conditions (e.g., photosynthetic efficiency), yet show no detectable difference. From the results, we can conclude right not only to a better survival rates of the plants against the applied stress, but indirectly to the survival originated higher yield production. The extension of the DsRed/GFP-based transient system to salt and methylglioxal derived oxidative stress tolerance in transgenic barley lines might be a further option for the evaluation of the effect of trangenes on cellular stress tolerance.

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EXPRESSION PROFILING OF POTENTIALLY CUTICLE RELATED GENES OF APPLE SHOWS THEIR PREFERENTIAL ASSOCIATION WITH THE FRUIT SKIN

Attila Molnár[1], Zsolt Albert, András Miskó[1], Magdolna Tóth[2], István Papp[1]
Doctoral Shool of Horticultural Studies
[1] Department of Plant Physiology and Plant Biochemistry, Corvinus University of Budapest
[2] Deparment of Pomology, Corvinus University of Budapest

Based on the *Arabidopsis* model of production and transport of cuticular components *in silico* analysis of apple EST and genomic sequences identified candidate genes potentially involved in these processes. Expression profiling of the selected genes in apple leaves and fruit tissues at the stage of full tree ripeness showed them to be active in fruit skin, in some cases in a tissue-specific manner. Genes with putative functions in the fatty acid elongase complex, wax and cutin modifications, transport and a potential regulator were identified this way. Year-to-year and cultivar specific variations in expression of some of these genes indicate plasticity of lipid biosynthetic pathways in apple.

Cuticle is a spatially continuous lipophilic layer sealing epidermal cells, covering the aerial parts of plants, involved in a range of essential functions. Water loss through the cuticle of fruits is known to influence postharvest processes profoundly. From a marketing point of view this is particularly relevant for horticultural products such as apple fruits, which are often stored for long time before consumption. The typical plant cuticle is composed of a cutin polyester matrix with waxes embedded in and also deposited on the surface, all synthesized by epidermal cells (Nawrath, 2006, Kunst and Samuels 2009). Most molecular studies so far have concentrated on the model species *Arabidopsis*, and the horticultural model plant tomato. Despite the agronomic importance of apple fruit we have no detailed description of

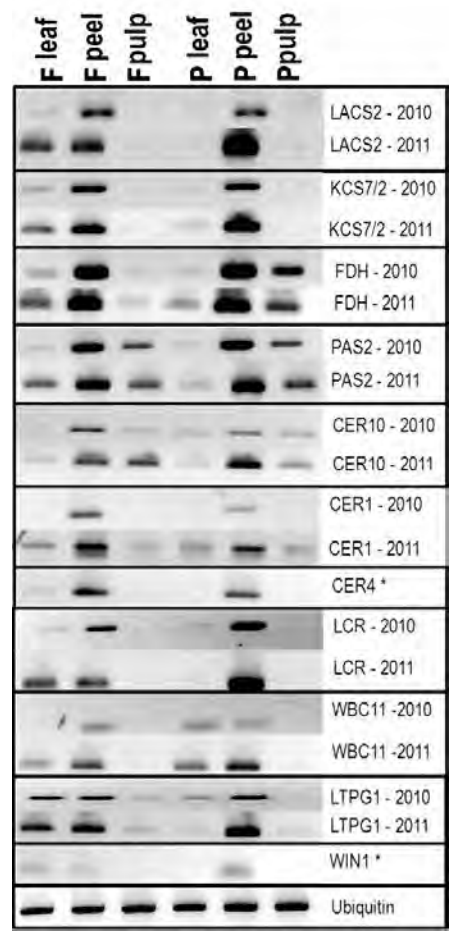

Figure 1. Representative gene specific RT-PCR reactions on apple leaf and fruit tissue specific cDNAs. Abbreviations: F – 'Florina', P – 'Prima' cultivars. For further details see text

genes implicated in the production of its cuticle. Recent publication of the apple genome sequence however makes this type of investigation realistic and feasible (Velasco et al 2010). In our present study apple genes were selected from the genomic sequence based on homology to relevant *Arabidopsis* genes with known functions in cuticular biosynthesis, transport and regulation. The tissue specific expression patterns of the selected genes were then analyzed in apple leaves and fruit tissues. We examined cvs Prima and Florina, which represent summer and winter apple varieties respectively, with contrasting harvest time and postharvest storability.

Total RNA was isolated from the tissues using the protocol of Asif *et al.*, suggested for polyphenol- and polysaccharide-rich tissues. Genomic DNA contamination was eliminated by DNase I treatment, followed by phenol extraction. Total RNA concentration and quality was assessed by using a NanoDrop spectrophotometer, cDNA was synthesized using a First Strand cDNA Synthesis Kit. For RT-PCR GoTaq Flexi DNA Polymerase was used. Experiments with material from the same sample were repeated at least twice with very similar results. Two separate biological samples from each of two consecutive years were analysed. *Arabidopsis* genes were selected from literature in order to search apple ESTs and genomic sequences using the program BLAST. The corresponding full length apple genomic sequences were compared to their *Arabidopsis* counterparts by the program Needle in order to estimate alignment and similarity. Preliminary data identified an apple ubiquitin gene (UBQ11) as useful control for normalization of gene expression levels in the tissues examined in this work. PCR primers specific for the genes above were designed by the Primer3 program. PCR products were purified directly or after separation on agarose gels by using Illustra GFX PCR DNA and Gel Band Purification Kits. Sequencing of the purified samples was done by BIOMI Kft.

The selected *Arabidopsis* and apple genes could be aligned with extensive regions of high similarity at the protein level. The peel samples used for RNA extractions were prepared by a manual dissection method. According to light microscopy, they contained the epidermis and in addition apprx. 5–6 hypodermal cell layers. The apple ubiquitin gene (UBQ11) was used as internal control for normalization of cDNA levels, and EF1 to check presence of genomic DNA in the RT-PCR experiments. The cDNA samples were equally loaded and devoid of genomic DNA contamination. All RT-PCR products showed the predicted sizes after separation on agarose gels. Representative amplification products for each gene were isolated and their primary sequences were determined. The sequences of the fragments corresponded well with the fruit pulp tissues and in the leaves too. The LACS2, KCS7/2, LCR, WBC11, LTPG1and WIN1 homologs showed peel specific expression in some years and cultivars, but in other cases their mRNA also appeared in the leaves.

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CELL WALL INTEGRITY SIGNALING FVMK2 MAPK GENE REGULATES CASPOFUNGIN TOLERANCE ON A β-1,3-GLUCAN SYNTHASE INDEPENDENT MANNER IN *FUSARIUM VERTICILLIOIDES*

Emese Dalma Nagygyörgy, Attila L. Ádám
Szent István University Doctoral School in Biology
Hungarian Academy of Sciences Centre for Agricultrural Research, Plant Protection Institute, Budapest

In fungi, like all other organisms it is indispensable to respond adequately to changes of their environment. This requires a continuous communication, by applying multiple signaling pathways in response to different intra- or extracellular stimuli. In molecular level most processes of signal transduction involve ordered sequences of events including specific receptors. One of the most prominent signal transduction pathways is the mitogen activated protein kinase (MAPK) pathway that plays a basic role in regulation of various cellular activities, such as the regulation of abiotic stress response and cell wall integrity signaling in fungi (Herskowitz, 1995; Ádám et al., 2008), as well as pathogen induced programmed cell death during hypersensitive reaction of plants (Ádám et al., 1997).

Fusarium verticilliodes (Sacc.) *(teleomorph: Gibberella monoliformis)* is a world-wide occurring pathogenic fungi in agriculture causing stalk rots and ear rots of corn, and producing mycotoxins which has significant impacts on human health (Ochiai et al, 2007).

Our experiments were oriented to find out what kind of phenotypic and gene expression changes are associated with CWIS (*C*ell *W*all *I*ntegrity *S*ignaling) MAPK *Fvmk*2 gene after fungicide and drog treatments in *F. verticillioides*. For this reason, we generated the gene-disruption mutants of CWI MAPK *Fvmk2* gene in *F. verticillioides* and than complemented the Δ*Fvmk2* null mutant strains with the functional wild type allele.

One of the tested compounds, caspofungin, used in human healthcare system for treating invasive fungal infections of *Candida* and *Aspergillus* species (Deresinski and Stevens, 2003), belongs to the group of echinocandins, and affects the glucan synthesis of the cell wall. Hot spot 1 region of *Fks1* (β-1,3-glucan synthase) genes in *Saccharomyces cerevisiae* and *Candida* species has a F639Y mutation causing echinocandin resistence. In *Fusarium solani* and in other opportunistic pathogenic (Katiyar and Edlind, 2009) and phytopathogenic *Fusarium* species including *F. verticillioides* the same mutation is stabilized resulting in permanent echinocandin (thus caspofungin) resistance.

The Δ*Fvmk2* mutants had reduced growth rate as compared to the wild type parental strain in solid complex medium (CM) agar plates, hydrophobicity of their surface structures became diminished and they showed increased sensitivity to chemicals interfering with cell wall biosynthesis (calcofluor white (CFW), Congo Red, SDS). Treatments with caspofungin in serial conidial dilution assays and in liquid cultures, respectively reduced the growth of the F*vmk2* CWI MAPK mutants by 50-70%. The complemented strains showed sensitivity values similar to that of the wild type strain.

The role of the CWI MAPK route in caspofungin tolerance was also supported by a 3.5-4-fold increase within 0.5 hour in transcription of the *Fvmk2* MAPK gene measured in the wild type strains exposed to caspofungin. CFW treatment caused lower and delayed induction of *Fvmk2*. As the qrtPCR analyses were performed by using primers upstream of the insertion position of the *hph*-cassette (used in the gene disruption construct), expression levels of the *Fvmk2* gene could be measured also in the *ΔFvmk2* mutants; in these strains reduced expression was observed even in the absence of treatment (control), while after caspofungin treatment the induction of *Fvmk2* was totally prevented. This finding indicates that the functional MAPK protein probably contributes to its own regulation, either under stressed conditions or in the absence of stress. Surprisingly, *Gls2* β-1,3-glucane synthase gene was not regulated on MAPK-dependent manner, its expression after caspofungin treatment was somewhat increased either in the wild type or Δ*Fvmk2* mutants. Results of further qrtPCR assays showed that the chitin synthase gene, *Chs1* was activated by an *Fvmk2* MAPK-dependent manner, while in the case of *Chs6*, another chitin synthase gene no similar effect was observed. On the other hand, CFW treatment increased the *Fvmk2* MAPK-dependent expression of *Chs6*.

These results demonstrated that Fvmk2 CWI MAPK pathway plays an important role in caspofungin tolerance of *F. verticillioides*, similar to other fungal species. But this effect is regulated on a β-1,3-glucane synthase independent manner. This finding also indicates that the formerly described stabilized mutation of hot spot 1 region of G*ls2* β-1,3-glucane sysnthase genes of *Fusarium* species including *F. verticillioides* is only partially responsible for caspofungin tolerance. The mode of action of *Fvmk2* MAPK-dependent caspofungin tolerance in *Fusarium* species could be related to other MAPK-dependent genes, in part to the differences in the expression of one of the chitin synthase genes, namely *Chs1*.

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DEVELOPMENT OF *TRITICUM TIMOPHEEVII* DERIVED SYNTHETIC AMPHIPLOID LINES FOR WHEAT IMPROVEMENT

Péter Mikó, Mária Megyeri, Márta Molnár-Láng
Plant Genetics and Biotechnology Programme, Plant Science PhD School, Szent István University, Gödöllő
Department of Plant Genetic Resources and Organic Breeding, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár

Bread wheat (*Triticum aestivum* L.) is one of the most important crops worldwide. Therefore it is vital to improve the breeding lines continually, adorning them with better resistance that can cope with the main biotic and abiotic stresses. To improve the resistance and adaptability of bread wheat, one possible solution is the utilization of wild species in wheat breeding.

Recent research is focusing on the possible utilization of *Triticum timopheevii* Zhuk. (2n=4x=28, A^tA^tGG) and *Triticum monococcum* L. ssp. *monococcum* (einkorn; 2n=2x=14, A^mA^m). Both plant materials have outstanding resistance to the main fungal diseases, and acceptably tolerate the extreme weather conditions. Tetraploid species, *T. timopheevii* is one of the most promising sources for improving resistance of bread wheat. Most studies on the utilization of the genes responsible for resistance have involved direct crosses between *T. timopheevii* and *T. aestivum*. However, apart from direct crosses between this wild relative and wheat, another possible way of utilization could be the development of new synthetic amphilopid wheat lines using *T. timopheevii* and einkorn as crossing partners.

Before crossing it is necessary to choose the most suitable lines from these species, in order to gain really valuable materials for wheat breeding. Therefore, detailed characterisation was carried out on all of the 56 *T. timopheevii* accessions of the Martonvásár Cereal Gene Bank for the main phenotypic and resistance characters. An analysis of agronomical valuable traits revealed that members of the base species and ssp. *timopheevii* are likely to be more advantageous for wheat breeding than those of the ssp. *armeniacum* group. Although the latter have relatively early heading date, they are more susceptible to fungal and viral diseases, and have a prostrate growth form with lower yielding ability, which could make their utilization in wheat breeding more difficult. After a detailed evaluation of the results, the best 11 accessions from the ssp. *timopheevii* group were selected for a crossability test with an already prebred, semi-dwarf einkorn line, '1T-1'. On the basis of the results of the crossability test one accession (*T. timopheevii* Zhuk. var. *rubiginosum*, Acc. No.: MVGB845) with the best seed set was selected for development of the new synthetic amphiploid, *Triticum timococcum*. Almost 2000 T. timopheevii florets were pollinated with '1T-1', and 255 F_1 hybrid seeds were obtained (seed set: 13%) that had a germination rate of 91%. After crossing, the triploid genome (2n=3x=21) of the selected hybrid seedlings (F_1) was doubled by colchicine-treatment (F_1 -> C_1). Altogether 81% of the plants have survived the colchicine-treatment, resulted in 1497 C_2 seeds (seed set: 4%).

Detailed field assessment on almost 60 spike-rows of C_2 generation was carried out in 2012 under organic growing conditions. Most of the lines were found to be an intermediary type of their parents, but had denser and thicker hairs on the whole plant than their maternal

parent, *T. timopheevii*. Beside the field assessment, an additional trial on artificial leaf rust infection has also proved the high resistance of *T. timococcum*. After the isolation of some spikes, the rest of the spikes were threshed together and drilled in 56 rows to begin selection work from the C_3 population in 2013.

Identification of the hexaploid genome of this synthetic amphiploid using fluorescent *in situ* hybridisation with repetitive DNA probes (FISH) has proved normal doubling with 42 chromosomes in most of the plants of the C_2 and C_3 generations. Detailed identification has revealed that this plant material has 14 chromosomes originated from einkorn, and 28 chromosomes originated from *T. timopheevii*. This result was also proved by genomic *in situ* hybridization (GISH), which was successfully optimised in order to be able to effectively distinguish the A and G genomes. Genomic DNA of the S genome was extracted from the diploid *Aegilops speltoides* Tausch, because this genome is similar to the G genome of *T. timopheevii*. In case of A genome, DNA was extracted from *Triticum urartu* Thumanian ex Gandilyan, the A genome donor of bread wheat, because the A genome of *T. timopheevii* is mostly similar to that of wheat.

New hybrids of *T. timococcum* and *T. aestivum* 'Mv9kr1' (carries the crossability allele kr1) were developed in both combinations, and progenies were back-crossed with 'Mv9kr1' in order to get more useful materials for wheat breeders. Back-crossed plants are being grown in climate chamber under controlled environmental conditions. Based on this new amphiploid, a comparative crossing trial is also in progress, which will hopefully give solid results on the effectiveness of direct cross (*T. aestivum* 'Mv9kr1' × *T. timopheevii*) and bridge-cross (*T. aestivum* 'Mv9kr1' × *T. timococcum*). Study has also begun on the chromosome pairing of C_3 and C_4 plants of *T. timococcum* in metaphase I. of meiosis using optimised GISH and FISH techniques.

In addition, the new synthetic amphiploid has a similar genome composition to that of the naturally occurring species *Triticum zhukovskyi* Men. et Ericz. ($2n=6x=42, A^tA^tGGA^mA^m$), making it possible not only to improve the bread wheat breeding materials, but also to increase the very limited genetic variability of this natural species.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the Grant Agreement n°245058-Solibam and from two National Science and Technology Office projects (*TECH_08-A3/2-2008-0397 – CONFU_08* and *TECH_08-A3/2-2008-0423 – ALKOBEER* – Hungary).

COMPARISON OF THE MINERAL CONTENT OF COMMERCIAL WHEAT (T. AESTIVUM SSP AESTIVUM) FLOUR FROM HUNGARY AND GRAIN OF MV (MARTONVÁSÁR) WHEAT VARIETIES

I. Monostori[1,2], G. Galiba[2], A.F. Bálint[2], F. Szira[2]
[1]PhD School of Plant Sciences, Szent István University, Martonvásár
[2]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár

Healthy nutrition is more and more an issue on the public agenda. Remarkable part of calorie intake covered by cereals and cereal based products. This is essential to know what is the micronutrient content of wheat flours and flour based products.

Wheat grains and flours often contain low amount of microelements. Furthermore, micronutrient content of wheat flour decreases during milling. Insufficient intake of these elements, the mineral malnutrition, is the hidden form of hunger but has global significance. There is not enough information about winter wheat varieties and wheat meals micronutrient content in Hungary. It is important to know, is it possible to satisfy daily human requirements with milling products.

In this study, micronutrient composition of 14 different types of Hungarian wheat flour and 24 Hungarian winter type bread wheat varieties were studied. Cu-, Fe-, Mn-, Zn-, Mo-, and Se- content were analysed in the commercially available wheat meals and in the whole grain and white flour of the winter wheat varieties. The micronutrient content of varieties were analysed for two years, therefore the genetic and environmental (year) variance could be separated.

Significant variation was revealed in the case of all mineral elements of Hungarian wheat flours. The white flour enriched with germ showed higher mineral content than average of normal white flour and the highest Mo concentration. Wholegrain wheat flour has higher micronutrient content except Se and Mo than any other type of white flour. The genetic variation and the environmental effect of wheat varieties were investigated. The micronutrient content of 24 winter wheat varieties is similar to the mineral content of Hungarian wheat flours. Milling reduces the concentration of all elements (e.g. Fe 33%; Mn 88%; Zn 71%; Cu 44%), except Se.

Based on these results, only the daily Mo requirement can be covered by cereal-based products in Hungary. Therefore, agronomic or genetic biofortification is suggested to increase the mineral content of wheat.

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HOW CAN *CIS* ELEMENTS IN THE 3'UTR MODIFY THE STABILITY OF mRNA?

Tünde Nyikó, Farkas Kerényi, Levente Szabadkai, Anna H. Benkovics, Péter Major, Boglárka Sonkoly, Zsuzsanna Mérai, Dániel Silhavy
Classical and Molecular Genetics-Biology Doctorate School, Eötvös Lóránd University
Budapest Agricultural Biotechnologi Center

Nonsense-mediated mRNA Decay (NMD) system is a conserved eukaryotic quality control mechanism that identifies and degrades aberrant mRNAs containing premature termination codons (PTC), thereby preventing the accumulation of truncated and potentially harmful dominant-negative proteins. In addition to its quality-control function, NMD constitutes a post-transcriptional pathway to regulate the expression levels of physiological mRNAs. Although the core trans-acting NMD factors UPF1, UPF2, UPF3 and SMG7 are conserved from yeast to mammals, the *cis*-acting NMD elements are different. In yeast and invertebrates the NMD system identifies a stop codon as a PTC, if the 3'UTR is unusually long (long 3'UTR-based NMD). In mammals, the presence of an intron in the 3'UTR increases the efficiency of NMD. During intron splicing a protein complex, called exon-junction complex (EJC) is deposited onto the mRNA. The EJC consists from four core protein Y14, Mago, Barentsz, eIF4AIII and serves as a binding platform for central NMD factors UPF3, UPF2. Normally the translating ribosome with the help of the associated PYM protein removes EJC from the mRNA unless it locates in the 3'UTR. EJC located in the 3'UTR dramatically intensifies NMD.

Although NMD is well examined in mammals, yeast and Drosophila, little was known about plant NMD. Previously our group has elaborated an efficient agroinfiltration-based transient NMD test system combined with Virus Induced Gene Silencing (VIGS) to define the *cis*-acting elements and *trans* factors of plant NMD. We have found that in plants both long 3'UTR-based NMD and intron-based NMD act efficiently, which appear to be unique among eukaryotes. Interestingly, except vertebrates, plants are the only eukaryotes in which intron-based NMD is active. They have confirmed, that the core NMD factors (UPF1, UPF2, UPF3, SMG7) are required for both types of plant NMD, whereas the plant orthologs of Y14 and Mago the two EJC protein are involved only in intron-based plant NMD.

The main part of our program was to unravel the molecular mechanism, and the regulation of plant intron-based NMD. The eukaryotic NMD evolution model assumes that the long 3'UTR-based NMD is the evolutionary ancient form and intron-based NMD has evolved only in vertebrates. Our previous result that intron-based NMD is active in plants was apparently conflicting with this model. We hypothesised that comparing the mechanism and regulation of plant and vertebrate intron-based NMD systems, would help us to better understand the evolution of the NMD pathway.

In mammals intron-based NMD is mediated by EJC complex containing four core proteins: Y14, Mago, eIF4A3 and Barentsz. Based on our previous results, we hypothesized that plant intron-based NMD could be also mediated by EJC. We have demonstrated that EJC proteins are all necessary for intron-based plant NMD and that they are involved in 3'UTR intron- based plant NMD, perhaps as components of plant EJC. In mammals the ribosome connected PYM protein functions as an EJC disassembly factor to ensure the

efficient translation of wild-type mRNA. We have showed that plant EJC is also disassembled by PYM protein. These data suggest that plant and mammalian intron-based NMD show many similarities in their mechanism and factor requirement, strongly supporting our hypothesis that both types of NMD system functioned in stem eukaryotes.

In addition to eliminating PTC containing aberrant mRNAs, NMD also regulates several wild-type transcripts having NMD features, thus the intensity of NMD might be strictly controlled. The simplest regulatory system would be a feedback control, in which the expression of one or more NMD *trans* factor are regulated by NMD. Our next goal was to identify hypothetical NMD autoregulatory circuits in plants. We have shown that plant SMG7 which is essential for both intron-based and long 3'UTR based NMD activity can sense the efficiency of both types of NMD. I have also demonstrated that plant Barentsz, which is involved only in intron-based NMD, contain intron-based NMD features and is controlled exclusively by intron-based NMD. Importantly, these autoregulatory loops are conserved within Angiosperms. In summary our results suggests that two autoregulatory circuits might allow partially separated homeostatic regulation for the two types of NMD in plants.

THE AUTOREGULATION OF THE KEY FACTOR OF TRANSLATION TERMINATION (ERF1)

Szabadkai L., Nyikó T., Benkovics H. A., Kerenyi Z., Silhavy D.
Classical and Molecular Genetics, Biology Molecular Genetics, Biology Doctorate School Eötvös Lóránd University,Budapest
Agricultural Biotechnology Center, Gödöllő

The gene expression systems recognize and degrade the defective gene products, thereby ensuring that only normal proteins are expressed. The most important quality control mechanism is the Nonsense-mediated mRNA decay (NMD) system, which recognizes and degrades premature stop codon containing mRNAs, thus preventing the formation of truncated dominant-negative-acting proteins. NMD is essential for plants, null alleles of main NMD factors are embryonic lethal. NMD is translation termination coupled process. The NMD system is regulating the expression of many endogenous genes. Previously, we have demonstrated that stop codons are recognized by NMD system if harboring unusually long 3' untranslated regions (3'UTR), or intron in the 3'UTR.

The Eukaryotic Release Factor 1 (eRF1) catalyzes the translation termination on the ribosome. The Arabidopsis gene eRF1 has three copies (eRF1-1, eRF1-2, eRF1-3), but all higher plants have at least two copies. *Arabidopsis* sequence analysis revealed that the eRF1-1 3'UTR is a possible NMD substrate. The stop context of eRF1-1 is very similar to the canonical tobacco mosaic virus (TMV) leaky stop. In addition the next stop codon is located in an NMD irrelevant position in the 3'UTR.

Our group is studying factors involved in NMD, the mechanism, and regulation of this quality control system. At the beginning of the project we wanted to know how NMD and translational readthrough is involved in the regulation of eRF1 protein level.

We have demonstrated that the translational readthrough can rescue mRNAs from degradation if the next stop codon is located in NMD irrelevant position. We have demonstrated using a dual NAN-GUS reporter system that eRF1-1 stop context provides sufficient amount of stop codon readthrough, though to a lesser extent than in the case of TMV, to out rule NMD and could serve as a rescue mechanism. With the same system we have shown that over expression of RF1 protein improves termination efficiency by inhibition of stop codon readthrough.

qRT-PCR analysis demonstrated that NMD-deficient *Arabidopsis* and *N. benthamiana* plants (Upf1-3, 5-Upf1, Smg7-1, amirUpf2) are having elevated eRF1-1 levels, while the other two transcript's (eRF1-2 and eRF1-3) levels are not significantly altered. Using (Green Fluorescent Protein) GFP reporter system we have demonstrated that the *Arabidopsis* eRF1-1 terminator makes the target mRNA an NMD target. When the context of eRF1-1 stop was used instead GFP's stop, it was able to rescue the mRNA. Interestingly, we have shown that overexpression of the eRF1 can amplify NMD activity readthrough independently.

Based on these results we set up a model. In higher plants multiple copies of eRF1 are present. With the help of eRF1s unique structure of NMD cis elements and readthrough, level of eRF1 protein can be fine tuned. The eRF1-1 carries NMD cis elements; however the readthrough of the stop codon can rescue the mRNA. As a result of these two antagonistic features, eRF1-1 transcript could be constitutively but moderately targeted by

NMD. If eRF1 protein level is enhanced, the NMD system will degrade eRF1-1 transcripts. Otherwise eRF1-1 transcript level will be dramatically upregulated, if low eRF1 protein level suppresses NMD.

In accordance with the predictions of the model eRF1-2 and eRF1-3 mutant *Arabidopsis* lines have elevated eRF1-1 levels, while the eRF1-1 mutant did not show significant change in expression of the other two eRF1 copies. In plants overexpressing eRF1 protein level of eRF1-1 is reduced by 2 fold, while the expression of eRF1-2 and eRF1-3 remain unchanged.

Thus, our results indicate the level of eRF1 is regulated in a complex autoregulatory cycle in higher plants. The *Arabidopsis* eRF1-1 mRNA contains strong NMD target 3'UTR and weak, readthrough stop codon capable of inhibiting NMD. These two elements allow the mRNA to be slightly but consecutively targeted by the NMD system, makeing the cell able to quickly adapt to sudden changes in eRF1 protein level.

GLUTARALDEHYDE-SPRAYING: A NOVEL PLANT PHYSIOLOGY TEST FOR ASSESSING REACTIVE ALDEHYDE TOLERANCE *IN VIVO*

Csaba Éva, Gábor Tóth, Mária Oszvald, László Tamás
Doctorate School in Biology, Eötvös Lóránd University, Budapest

Plants are exposed to oxidative damage under various stress conditions. The formed reactive oxygen species (ROS) may at least deplete proteins, DNA and polyunsaturated fatty acid chains of membrane lipids (lipid peroxidation). To prevent this plant cells are equipped with a vide variety of antioxidants and antioxidant systems. The level of these antioxidant systems can be studied by applying specific laboratory ROS treatments (e.g. hydrogen peroxide, paraquat). Besides the direct effects of ROS harmful indirect effects via products of lipid peroxidation should also be considered as part of the oxidative damage. Certain lipid peroxide breakdown products, the reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) contain two double bonds and so are able to cross-link proteins. Against these compounds, aldo-keto reductase (AKR) enzymes present a an effective protective mechanism. Aldo-keto reductases act to reduce different aldehydes and ketones to the appropriate alcohol, using NADPH or NADH cofactors. Besides the above mentioned reactive aldehydes, methylglyoxal, a harmful and high level reactive aldehyde, a by-product of glycolysis can also be reduced by the AKR-enzymes. In addition they may produce osmoprotective polyols from carbohydrates. Reactive aldehyde neutralizing AKRs have been found in certain plant species such as alfalfa, rice or Arabidopsis, but surprisingly no AKRs have been detected in the fully expanded leaves of certain plant species, e.g. wheat and barley. Transgenic plants over-expressing an alfalfa AKR were already produced and they exhibited considerable drought, heavy metal, heat and UV light tolerance. Leaf-disc assays are sometimes used as lab-tests to determine reactive aldehyde tolerance of the plants. We aimed however to establish an *in vivo* reactive aldehyde treatment that could offer more reliable data, about the tolerance of the given species and also about the special effects reactive aldehydes on plant health. At first transgenic barley plants were produced ectopically over-expressing an Arabidopsis AKR enzyme (AKR4C9). In order to study the effect of a reactive aldehyde treatment on both resistant and non-resistant plants, reactive aldehyde tolerance of transgenic and non-transgenic plants were investigated. In the following experiments, tolerance of the transgenic plants against lipid peroxidation generating natural stresses (heavy metal and frost) was studied.

For barley transformation, a transformation cassette was assembled that was based on the pWBVec8 binary vector and contained the rice actin promoter:: *Arabidopsis thaliana At2g37770* gene:: *Agrobacterium tumefaciens nos* terminator construct. A His-tag sequence was fused to 3' end of the transgene. Transgenic barley (*Hordeum vulgare* L. cv. 'Golden Promise') plants were generated by Agrobacterium-mediated transformation. The plants were tested at DNA, RNA and protein levels. Transgene copy number was estimated by qPCR.

His-tag fusion AKR4C9 protein was extracted and purified from transgenic barley plants for enzyme kinetic studies. *In vitro* activity of AKR4C9 was investigated against possible substrates. Additionally ten day-old transgenic and non-transgenic plant seedlings were

glutaraldehyde-treated by spraying 0,1 v/v % glutaraldehyde solution onto the plant shoots, with quantity of 5 ml/plantlet. Plants were also exposed to heavy metal stress by including 10 μM $Cd(NO_3)_2$ in the hydroponic solution. Frost tolerance was investigated by applying -20 °C temperature treatment. After the treatments, the following physiological parameters were determined: chlorophyll-, carotenoide-, malondialdehyde-content, fluorescence induction parameters, respiratory rate and fresh weight.

Transgenic barley plant lines were established, carrying the *Arabidopsis thaliana At2g37770* gene. Copy number of the transgenic plants varied from 1 to 4. The transcription of the transgene and the presence of AKR4C9 enzyme, fused to His-tag at the C terminus was confirmed in all studied lines. Thereafter His-tag fusion enzyme was extracted and purified from the transgenic plants and its enzyme activity was studied. As result, low but detectable activity was found against fructose and moderate activity was found against glutaraldehyde. Based on this, glutaraldehyde solution was sprayed onto the plants aiming a specific *in vivo* reactive aldehyde treatment. This chemical was chosen for the purpose because of its low prize and also because of detected activity of the purified Arabidopsis AKR-enzyme. In general, glutaraldehyde-spraying caused the following symptoms: stopping of growth, chlorosis, decreasing photosynthetic activity, increasing respiratory rate and sometimes wilting. These effects were less pronounced in high AKR-expressing transgenic plants. More precisely the glutaraldehyde treated high AKR-expressing transgenic plants had significantly higher chlorophyll content, and showed higher photosynthetic (F_v/F_m) and lower respiratory activity than non-transgenic plants. Other physiological parameters, namely electrolyte leakage and ascorbate-peroxidase activity were also studied but no effect of glutaraldehyde treatment could be observed in these regards. In the next set of experiments real lipid peroxidation was generated in transgenic and non-transgenic plants by applying heavy metal or frost treatment. As expected, cadmium caused strong lipid peroxidation, and the Arabidopsis enzyme was found to lower the malondialdehyde-content. In addition, the transgenic plants had higher photosynthetic activity and higher chlorophyll content also arguing for higher cadmium tolerance. Freezing stress was also found to cause lipid peroxidation, and the transgene was found to enhance freezing tolerance, based on growth parameters, fresh weight, and regenerative capacity.

Altogether the results presented here argue for the utilisation of glutaraldehyde in specific, but cheap reactive aldehyde assays *in vivo*. This test may be suitable for testing the reactive aldehyde detoxifying capacity (part of the oxidative stress tolerance) of different plant species or varieties which may partially predict their tolerance against various abiotic stress types.

EFFECTS OF EXOGENOUS SALICYLIC ACID ON THE ENDOGEN SALICYLIC ACID LEVEL AND ITS PRECURSORS

Orsolya Kinga Gondor, Tibor Janda, Gabriella Szalai
Doctoral School of Biology, Eötvös Loránd University,
[1]Agricultural Institute, Centre for Agricultural Research of HAS, Martonvásár

Salicylic acid (SA) was isolated first from bark of the willow tree (*Salix*) and used as a medicine. Recently it is known as a phytohormone-like substance and signalling molecule. It has a role in the regulation of many physiological processes such as photosynthesis, transpiration, iontransport, etc. 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 (*o*HCA) 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 lipidperoxidation was measured *via* malondialdehyde (MDA) content spectrophotometricaly. 0.5 g plant material was used for determination of SA, BA, CA, *o*HCA 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 SA, BA, CA did not change while the *o*HCA content increased after 1 day of SA treatment in the leaves. The MDA concentration also increased compared to the control which alludes to the increased oxidative stress. *o*HCA 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 *o*HCA content did not change.in the methanol soluble bound fraction. The level of SA, BA, CA and *o*HCA 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 *o*HCA 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 *o*HCA 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.

An elevated level of *o*HCA was detected in the methanol soluble bound fraction while the content of BA and SA did not change either in methanol soluble or insoluble bound fractions. The level of free SA and *o*HCA increased after the seventh day of hydroponic SA treatment while the CA and BA did not change.

Some of the flavonoids were also analysed. Myrecetin, kaempferol, quercetin and rutin were measured. The myrecetin level did not change in the leaves after the SA treatments. Its level increased in the free fraction in the roots after one day and it still remained at a higher level for seven days compared to the control values. The content of the methanol soluble bound myrecetin increased after one day but decreased after seven days. The kampferol concentration did not change in the free fraction in the leaves but decreased in the bound fractions. Its content diminished after one day in the methanol soluble bound fraction, increased after seven days but did not changed in the methanol insoluble bound fraction in the roots. The quercetin 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.

PRODUCTION EDIBLE VACCINE AGAINST SALMONELLA IN BARLEY ENDOSPERM

Gábor Tóth, Csaba Éva, Sára Polya, Edina Poczkodi, Mária Oszvald, Barnabás Jenes, László Tamás
Doctorate School in Biology, Eötvös Loránd University, Budapest

The concept of plant derived edible vaccines is a recently developed approach in vaccination technology. It utilizes transgenic plants to produce antigenic proteins which are able to elicit an effective mucosal immune response. Production of edible vaccines in cereals endosperm is highly scalable and adaptable for animal feeding experiments and future biopharmaceutical industrial applications.

Edible vaccines could be particularly effective against pathogens which enter trough the mucous membrane such as the gastrointestinal tract. A prominent member of these pathogens is the *Salmonella* genus. *Salmonella enterica* is an important pathogen of animals and humans causing a variety of infectious diseases, therefore it has great public health safety and economical impact. *Salmonell*a in poultry is a world-wide problem and also a major political issue. Control of this pathogen in poultry by immunity is required effective and largely scalable vaccination. The exploitation of adjuvant technology in conjunction with the ongoing developments in identifying key *Salmonella* virulence determinants should form the next generation of *Salmonella* sub-unit vaccines. One of the well known *Salmonella* antigene is the flagellin the principal subunit of bacterial flagellum. Purified flagellin from *Salmonella* serovar Enteritidis elicits strong systemic and mucosal immune response in C+H/HeJ mice administrated orally together with recombinant cholera toxin B subunit (CTB). The CTB is a potent mucosal immunogen and induces systemic and mucosal responses following administration to mucosal surfaces. CTB also has strong adjuvant activity as a carrier protein for genetically fused unrelated antigens.

Our main goal is to produce plant derived edible vaccines against Salmonella in selection marker gene free transgenic barley where antigenic protein is strictly expressed in the endosperm tissue.

To reach this goal the project is divided into two, a transient and a stable transgenic part. The first one is about to develop a high throughput transgenic barley tissue culture system for producing different plant derived recombinant proteins for further studies. In this system not tissue specific but constitutive promoter is used in the transformation cassette. This first approach provides an option to test several recombinant potentially antigenic proteins fused to the Cholera toxin B subunits (CTB) in a short time. These proteins are screened for desirable biochemical and immunological properties. Tested proteins proved to be active antigen are used for stable barley transformation and expressed in the endosperm driven by a tissue specific promoter. For selection marker gene elimination, a novel cold inducible cre-lox system, developed in our laboratory, is used.

A series of transformation cassettes have been assembled carrying either maze ubiquitin promoter for transient or wheat HMW (High Molecular Weight) Glutenin seed storage protein promoter for stable transformation. In the transformation cassettes the gene of interests are the CTB gene (*ctxB*), the *ctxB-uidA* fusion reporter gene and the *ctxB-fliC* fusion antigen, respectively. *UidA* encodes for the b-Glucuronidase (GUS) enzyme and *fliC*

encodes one of the flagellar antigens. For stable transgenic barley production a novel binary transformation vector has been also assembled based on the cold inducible cre-lox selection marker self-excision system for Agrobacterium mediated transformation.

To produce recombinant fusion proteins in transient expression calli derived from barley immature embryo have been utilized as explants and the DNA have been delivered by biolistic method. Three recombinant proteins were expressed the CTB, the CTB-GUS fusion protein and the CTB-fliC fusion antigen. The expression of CTB-GUS protein was studied by b-Glucuronidase histochemical assay and Western-blot, while the transcription of CTB and CTB-fliC genes was studied by RT-PCR while protein expression will be studied by Western-blot. Calli transformed with the CTB-GUS reporter construct showed strong GUS staining. RT-PCR results confirmed the presence of the mRNA transcribed from either the *ctbx* or the *ctbx-fliC* transgenes. The Western-blot studies as well as cloning other potential antigenes are in progress.

Stable transgenic barley lines have already been produced for testing the transformation cassettes carrying the endosperm specific promoter – reporter gene construct using the Agrobacterium-mediated transformation method. Transgenic plants were characterized with PCR and Q-PCR and also with b-Glucuronidase histochemical assay. Strength and expression pattern of the promoter was studied in T0, T1, and T2 generation in different transgenic lines carrying different number of copies of the transgene. The expression pattern proved to be strictly endosperm specific with highly variable expression strength.

In conclusion characterization of CTB-antigen fusion proteins is in progress and our stable transformation system is ready to produce CTB-antigen fusion proteins in barley endosperm.

Posters

IMPROVEMENT OF THE OXIDATIVE STRESS TOLERANCE OF MAIZE *VIA IN VITRO* MICROSPORE SELECTION

Helga Ambrus, Éva Darkó, Beáta Barnabás
Szent István University, Plant Science Doctoral School, Gödöllő
Hungarian Academy of Sciences, Agricultural Institute, Martonvásár

Maize is the one of the most important crop used as animal feed in Hungary. Besides the conventional breeding techniques, the utilisation of up-to-date biotechnological methods to improve the adaptability potential of hybrids is of immerse important.

It is well documented that most of the abiotic and biotic stresses induce the formation of toxic reactive oxygen species leading to oxidative damage of plants (ROS). It has been proven in a number of experiments that the enhanced cold,-drought,-and pathogen tolerance of plants is due to an improved activity of various antioxidant enzymes (Malan et al., 1990; Barna et al., 1992; Pastori and Trippi 1992; Inanneli et al., 1999; Mittler et al., 1999). Oxidative stress tolerant tobacco and potato could be successfully selected *in vitro* from somatic tissue cultures in the presence of paraquat (Shaaltiel et al., 1998; Gullner et al., 1991, 1995; Barna et al., 1993). However, only a few data could have been found on the application of haploid tissue cultures for *in vitro* cell selection. The genetic overlap in the expression of the genes responsible for stress tolerance of plants during the sporophytic and gametophytic life cycles (Acevedo and Scandalios, 1990; Frova, 1990) enables to apply *in vitro* microspore selection to improve oxidative stress tolerance of regenerated DH plants.

The aim of the present experiment was to elaborate a new *in vitro* selection methodology, which is based on the selection of haploid maize microspores *in vitro* by the use of reactive oxygen species (ROS) progenitors.

A single cross maize hybrid A-18 (originating from a paraquat resistant DH line as a female parent and from another DH line with high androgenic capacity as the male parent) were used for *in vitro* selection. Later on, a couple of single cross hybrids of breeding value were also involved in the experiments. The maize anther culture procedure was performed by the method of Barnabás (2003). The ROS progenitors (paraquat, menadione, methionine with riboflavine and t-BHP) were applied at various concentrations and were added to the induction and regeneration media (Ambrus et al., 2006). The effects of these compounds on the androgenic development and on the haploid induction capacity of the microspores have been examined. The fertile DH regenerants have been self pollinated. To detect the efficiency of *in vitro* selection, the oxidative stress tolerance of the regenerated DH progenies has been investigated by various physiological and biochemical tests, including the chlorophyll *a* fluorescence measurements, determination of electrolyte leakage from the cells, chlorophyll (a+b) content and activity of antioxidant enzymes. Detection of toxic ROS *in situ* was also investigated in DH1 plants. In addition, cold germination test was carried out of DH1 plants originated from microspores exposed to paraquat.

The results showed that the ROS progenitors reduced the androgenic response of microspores, the frequency of microspore-derived embryos and calli, and the number

of fertile DH plants. In some cases, (menadion) nuclear degradation and chromosome condensation could be detected.

DH offspring could be obtained not only from A-18 genotype, but from three other F1 hybrids. Physiological investigations demonstrated that 5 DH1 lines originated from microspores exposed to paraquat in A-18 hybrid showed higher paraquat tolerance as compared to the control. Detection of ROS in the leaf discs by *in situ* staining (DAB and NBT) indicated that less toxic oxygen forms were accumulated in the leaves of *in vitro* selected lines. Among the lines derived from hybrids of breeding value, 11 selected DH lines showed elevated paraquat tolerance. They also presented increased cold tolerance.

Our results indicated that *in vitro* selection of microspore exposed to ROS progenitors provide a suitable method to improve the oxidative stress tolerance of DH maize plants. These plants showed elevated adaptation ability to several abiotic stresses.

IDENTIFICATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA ISOLATED FROM DIFFERENT *CAPSICUM ANNUUM* VAR. *GROSSUM* CULTIVARS

Zoltán Füstös, Ágnes Belák, Mónika Kovács, Anna Maráz
PhD School for Food Science, Corvinus University of Budapest, Department of Microbiology and Biotechnology, Budapest

Endophytic bacteria are harmless endosymbiotic microorganisms, which reside inside the plant tissues and it has been proven that they have important roles in plant vitality, seed germination ability and they may confer resistance to abiotic and biotic stress factors. They also improve the plant defence against pathogenic microbes and degradation of xenobiotics. They may have positive influence on the plants' physiology by producing phytohormons, and they might have protection against plant pathogens through biofilm formation and antibiotic production. It is also noteworthy, that they are able to degrade plant produced compounds in soil that would otherwise be allelopathic (Ryan et al, 2008). On the other hand, researches have recently shown that the interactions between endophytic and pathogenic bacteria could result prevention or – in some cases – even promotion of the penetration of human pathogenic bacteria into the plant tissues (Teplitski et al, 2009). If endophytic bacteria prevent the penetration of pathogens, they have a protective role, but otherwise they can cause serious food safety problems.

Our goal was the isolation of endophytic bacteria from different cultivars of *Capsicum annuum* var. *grossum* grown in soil and hydrocultures followed by the analysis of the isolates with traditional and molecular biological methods. Furthermore we wanted to identify them at species level. Additionally, we aimed to determine the potential endophytic nature of these bacteria by using the seed germination test.

During our research work we isolated endophytic bacteria from plant tissues and seeds of *Capsicum annuum* var. *grossum* (cultivars Ho and KPA) grown in soil and hydroculture (Experimental Farm of the Corvinus University of Budapest, Soroksár). Cultivation of bacteria isolated from seeds, vegetative tissues and fruits has been performed after surface disinfection by sodium hypochlorite and ethanol. Samples of the disinfected seeds and organs were placed into TGY (tripton-glucose-yeast extract) broth, supplemented with Nystatin and incubated at 25 °C. The positive samples were subcultured in TGY, differential chromogenic (WL) and coliform selective (VRBL) agar plates to form pure colonies. From the two cultivars we were able to isolate totally 304 bacteria; 9 from seeds, 153 from hydroculture and 142 from soil grown plants.

Thereafter, the isolates were tested for cell, colony morphology, biochemical, physiological and spore forming ability. Using the STATSOFT STATISTICA 10 program, it was possible to cluster the isolates into different phenotypic groups, thereby were able to recognize the clonally identical isolates and to reduce the number of strains selected for molecular biological analyses.

Because endophytic bacteria must not inhibit seed germination, even in higher cell concentration, therefore we tested the impact of the potential endohytic bacteria on the

plants by using the germination test. The surface disinfected seeds were shaken gently in a solution containing 1-2×10^8 cells/ml, supplemented with carboxy-methyl-celullose during 6 hours and evaluated after 7 and 10 days (Niranjan Raj et al, 2003). Based on the results, it can be concluded that most of the isolates were not at all or in lower extent inhibitory for the seed germination, while some isolates had stronger inhibition (near 30%).

Identification and typing of the isolates were realized by molecular analyses. As the first step, we performed molecular typing by using a rep-PCR method with the application of the M13 minisatellite primer. After molecular typing, isolates from different plant tissues and seeds were clustered based on the molecular fingerprints, by using the GelCompar II program (Applied Maths NV).

Identification of the isolates, which were expected belonging to different species, had been performed by sequencing the rDNA and *rpoB* amplicons. First we detected the isolates belonging to the *Pseudomonas* genus, because in contrast to other bacterial genera rDNA-based identification is not reliable for the *Pseudomonas* genus, therefore we used a primer pair developed specifically for detection of *Pseudomonas* spp in our laboratory. Based on the molecular analysis we have identified isolates belonging to *Pseudomonas*, *Stenotrophomonas*, *Acidovorax*, *Thermoleophilum*, *Cupriavidus*, *Ralstonia*, *Xanthomonas*, *Acetobacter*, *Nanobacterium*, *Agrobacterium*, *Rhizobium*, *Bacillus*, *Delftia*, *Enterobacter*, *Comamonas*, *Pantoea*, *Erwinia*, *Leclercia*, *Serratia*, *Mesorhizobium* and *Achromobacter* genera. Among the identified species we could find endophytic bacteria already described.

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R1 LATE BLIGHT *(Phytophthora infestans)* RESISTANCE GENE HOMOLOG IN THE CULTIVAR WHITE LADY

Ramin Hajianfar, Rahim Ahmadvand, Zsolt Polgár, Istvan Wolf, János Taller
Doctoral School of Plant Production and Horticultural Sciences, University of Pannonia, Keszthely

Potato is one of the most important food crops in the World along with rice and wheat. According to the statistics of the Food and Agricultural Organization (FAO) the total production of potato in the world was estimated to be 374 382 274 tons and European contribution to this was 130 200 365 tons which corresponds to 34.7%. Among the diseases which threaten the quantity and quality of potato late blight disease caused by *Phytophthora infestans* is the most destructive all over the world. Late blight is damaging the whole plant including the green parts and the tubers. Up to now many R genes conferring resistance to late blight were identified which originate from different wild potato species. Among them *Solanum demissum* from central Mexico has the most late blight resistance (R) genes. The eleven genes of *S. demissum* designated as R1-R11 have been utilized by potato breeders since the first half of the twentieth century. For classification of these R genes the so called R-gene differential series of Black are used. R1 was the first cloned gene in the late blight resistance gene group which is introgressed into many potato cultivars. The R1 gene is localized on the short arm of the chromosome V in the potato genome where multiple other disease resistance genes can be found in a so called resistance gene hot spot. In the present research program we were interested to identify R genes conferring resistance to late blight in our potato breeding material. The highly late blight tolerant cultivar White Lady was used as plant material. Since some R genes have been already isolated and from the early pedigree it was not clear which R gene is present in White Lady in the first step of this study we tried to detect these genes with the published primers. In a second approach we performed a gene expressional analysis to identify the already isolated R genes among transcriptomes (TC) and to detect R gene homologues. To the gene expressional analysis next generation sequencing was performed on an ABI SOLID platform and the obtained transcriptome database was screened for the known R gene sequences. As a result the presence of some known R genes and several R gene homologues could be revealed in White Lady. Here we want to focus only on the R1 gene for which difference was detected between the published and the White Lady sequence.

Phenotyping was done using the aggressive *P. infestans* isolate H12/10 which is a mixture of different races having the 1, 3, 4, 7, 10 and 11 avirulence genes. Molecular genetic test of R1 was performed with the reported primers which amplified a 1400 bp fragment of this gene in the Black differential R1 line, but no product was obtained in White Lady. This was against our expectations, because infection tests indicated the presence of R1 gene in this cultivar, since no symptoms could be observed after infection with the H12/10 isolate. Based on the published sequence of the R1 gene, 13 different primer pairs were designed on the exons surrounding the intron. Among these one primer pair, R1L333 resulted a 400 bp long band. This band was polymorphic in an

F1 population of 24 genotypes deriving from a cross between White Lady and the late blight susceptible breeding line S440. The TC which contains this intron was identified and the sequence of it was compared to that of the published R1 sequence. The 4737 bp long TC which had the R1L333 IT marker showed 96% similarity at the nucleic acid level and 92% identity at the amino acid level with the 10388 bp long R1 gene. To prove that the gene identified with the R1L333 marker is really a homolog/allele of the R1 gene the complete sequence should be isolated and the nucleotide order of it should be determined. To isolate the complete sequence of the gene we are constructing a cDNA library from the same RNA what was used for RNA-sequencing. After that, functional analysis is planned to prove the real function of this gene with overexpression in tobacco as well with gene silencing (RNAi) in White Lady.

OCCURENCE AND SEQUENCE CONSERVATION OF BEET CRYPTIC VIRUSES IN DIFFERENT TAXONS OF THE *BETA* GENUS

Sándor Hinel, Anita Szegő, Viktória Zs. Győrfi, Noémi Lukács
Doctoral (PhD) School of Horticultural Science, Corvinus University of Budapest, Faculty of Horticultural Science, Department of Plant Physiology and Plant Biochemistry

In the light of recent results the role of plant viruses has been re-evaluated. Viruses are now considered as important players of host's evolution, and it is widely acknowledged that a significant number of viruses is not only non-pathogenic, but mutualistic, i.e. its presence is advantageous to the hosts. An extreme example for such mutualism is the dsRNA-virus-fungus-plant symbiosis described in *Curvularia*, that confers extreme heat tolerance to the roots only if all three partners are present[1].

Our goal is to elucidate the origin and biological role of plant cryptic viruses. Cryptoviruses do not induce any symptoms in the host, and up to now no experimental transmission to other host plants was successful, therefore exact analysis of their effects on host's physiological responses is very difficult. According to our current knowledge cryptoviruses are only dispersed by seed and pollen, and remain associated with their host plants during its whole life cycle. Plant cryptoviruses characterized up to now belong to the *Alphacryptovirus* genus of Partitiviridae family. Their genomes consist of 2-3 dsRNA segments ranging from 1-3 kbp in size, the genomic dsRNA are packaged in isometric virions. The RNA segments are monocistronic, one of them always encodes the replicase (RdRp), the other (two) dsRNA the capsid protein(s) (CP) of virions. Some experimental results indicate that cryptoviruses are mutualistic. In 2005 Nakatsukasa-Akune *et al.* observed that overexpressing the WCCV1 capsid protein in *Lotus japonicus* inhibited the nodulation, altered the abscisic acid content and confered protection against other infections and stresses at the same time[2]. Such adventageous effects might explain the widespread distribution and life-long persistance of cryptoviruses in the host.

In the *Beta* genus three alphacryptoviruses have been described: *Beet cryptic virus* 1, -2 and -3 (BCV1, -2, -3). The sequence of BCV1 and BCV2 was determined at our department[3]. BCVs were found in all *Beta* species and convarietases of the genus, such as chard (*Beta vulgaris* L. convar. *vulgaris)*, sugarbeet (*Beta vulgaris* convar. *crassa* provar. *altissima*), fodder beet (*Beta vulgaris* convar. *crassa* provar. *crassa*), beetroot (*Beta vulgaris convar. crassa provar. conditiva),* sea beet (*Beta maritima* subsp. *maritima)* and *Beta macrocarpa*. However, the distribution of the various BCVs is different in different taxons. In sugarbeet mainly BCV2 and BCV1 are present, while for chard the presence of BCV1 and BCV3 and for beetroot the occurrence of BCV2 are characteristic. In addition, we observed that in earlier cultivars of sugarbeet BCV1 and BCV2 could be found nearly in the same frequency, while in recently cultivated varieties BCV1 became very rare and in most varieties only BCV2 can be detected.

According to our present knowledge cryptoviruses are spread only by seed and pollen. Since the RNA-dependent RNA-polymerase has no proof-reading activity, one's expectation is that without a strong selection preassure the virus should evolve quickly and adapt to the host. Two papers in the literature suggest that cryptoviruses have a strong tendency for sequence conservation. By studying sequence variations of VCV1 in different *Vicia faba* cultivars[4] or that of

fig cryptic virus in different geographical locations[5] very low sequence variability was observed. Since the evolutionary distance between *Beta* convars is probably much larger than that among *Vicia* cultivars, we investigated sequence variability in *Beta* taxons. We determined the nucleotide sequences of BCV1 occuring in the chard cultivar 'Lucullus' and those of BCV2 occuring in the beetroot cultivar 'Bíborgömb' and then compared them to the corresponding sugarbeet virus sequences.

To determine the sequences viral genomic dsRNA was purified by CF11 column chromatography, reverse transcribed to cDNA and than amplified by PCR using High Fidelity DNA polymerase. The BCV1 sequences were determined after cloning, while the sequences of BCV2 were determined directly from the PCR products. In the case of BCV1 RdRp we detected 4,9% difference at the nucleotide level among sugarbeet and chard sequences, but only 6 mutations out of the observed 89 resulted in amino acid changes. The BCV1 capsid protein (CP) seems to be more conserved. Here 24 nucleotide changes and only one amino acid change was observed. BCV2 sequences are also highly conserved. Here we detected less mutations than in BCV1. In the case of the RdRp 5 nucleotides were mutated in comparison to sugarbeet BCV2, from these three led to amino acid change. The mutations didn't affect the RdRp motifs of the Partitiviridae family. In the BCV2 CP1-encoding dsRNA region we found 7 nucleotide changes from which only two caused an amino acid change. In the case of BCV2 CP2 we determined until now only a partial, 500 basepair long sequence which contains 4 silent mutations. Taken together, we conclude that the BCV1 and BCV2 cryptovirus sequence are strongly conserved at the amino acid level suggesting a selection preassure acting in the host plant.

The newest *in silico* analyses, partly confirmed by PCR results, showed that partitiviral sequences or whole genes can be found and expressed not only in plants, but in other eukaryotes as well. In 2012, Liu Huiquan's research group searched plant EST databases for cryptovirus occurence. Their work revealed that, apart from the cryptoviruses described to date, many new dsRNA virus have coevolved with host supergroups. Interestingly, endogenization of the „host's own" cryptoviral sequences, i.e. sequences derived from present day cryptoviruses of the given host, has not been reported. Therefore we made an attempt to detect putative endogenized BCV2 and BCV1 sequences in beetroot genomic DNA. This was done by applying seventeen BCV2-specific and seven BCV1-specific primer pairs. Using BCV2-specific primers we detected PCR products in five cases, these are being sequenced.

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DIFFERENT RESPONSES OF RICE VARIETIES TO AEROBIC CONDITIONS IN HUNGARY

M. Jancsó, P. Zrena, J. Pauk
Doctoral School of Plant Sciences, Szent István University, Gödöllő
Research Institute for Fisheries, Aquaculture and Irrigation, Szarvas
Faculty of Agriculture, University of Szeged, Hódmezővásárhely
Cereal Research Non-profit Ltd., Szeged

Paddy rice (*Oryza sativa* L.) is the largest consumer of irrigation water worldwide. The rice plant needs massive amount of water during the growing season therefore water scarcity is a serious problem in rainfed rice growing areas. Especially, in Asia where rice is a staple food and 23 million hectares of rice are affected by different levels of drought stress reported by the International Rice Research Institute (IRRI 2012). Since rice is also a model plant for agronomy, genetics and plant physiology, research on drought tolerance has a great role to maintain food security.

Hungary is the northernmost border of rice growing in Europe. Because of the marginal climatic conditions (e.g. short season, low temperature and long day length) breeding for abiotic stress (cold, salinity and drought) tolerance is of high importance. In Hungary, an aerobic rice technology (SANORYZA) was developed in the late eighties and was patented in 1992. Several aerobic rice varieties ('Karmina', 'Ringola' ('HSC55'), 'Sandora') were also released by the breeders of the Irrigation Research Institute (today HAKI, Szarvas) (Simon-Kiss 2001). The SANORYZA system was used to produce rice without permanent flooding where irrigation was carried out using mainly sprinkle irrigation. The water demand of rice production could be decreased by 45-55 percent without any loss of quality and quantity.

In our experiments, drought tolerance of rice varieties was tested in controlled environments and under field conditions from 2005 to 2011 in Szarvas (Lysimeter Research Station and Galambos Rice Research Station, Szarvas, Hungary). Small-scale experiments were setup to determine drought tolerance of ten Hungarian state released temperate rice varieties ('Ábel', 'Janka', 'Bioryza H', 'Sandora', 'Marilla', 'M 60', 'M 488', 'Fruzsina', 'Unggi-9' and 'Dáma') and internationally well-known standard varieties ('IRAT 109', 'APO (NISC RC9)' and 'IR60080-46A'). To the investigation of genetic and physiological background of drought tolerance of these genotypes, agronomical, genetic and breeding aspects were combined and several characteristics (shoot and root growth, yield, canopy temperature (CT), chlorophyll content, dry matter, water consumption, milling quality) were studied both under aerobic and flooded conditions. The flooded conditions were maintained as standard rice growing technology with direct dry sowing (paddy rice), while aerobic rice was irrigated via drip irrigation. In 2010 and 2011 the small scale experiments were setup at both sites (4 replications, random block design). During the seasons the amount of natural precipitation in the growing season was 513.7 mm and 248.9 mm, respectively. The irrigation water for the aerobic rice was 220 mm and 238 mm respectively. Comparing to the conventional rice production (average 1000 mm per season) the irrigation water could be lower as nearly 75 %.

Differences in drought tolerance were resulted in significant differences of paddy yield performance, what is the main agronomical character for the measurement of production. 'Sandora', 'Bioryza H', 'Ábel' and 'Unggi-9' were found the most drought-tolerant Hungarian

cultivars. The highest average yield under aerobic conditions were 2.93 t ha^{-1} ('Sandora'), 2.72 t ha^{-1} ('Bioryza H') and 2.59 t ha^{-1}('Ábel'). However, 'IRAT 109' had significant difference of plant type and mainly in case of canopy temperature it was found more tolerant to drought than the other varieties tested. The harvest index (HI) is an important parameter for yield performance. Highest HI's were registered for the varieties 'Bioryza' (37.8), 'Sandora' (36.8) and 'Ábel' (35.6).

During the seasons, the development of the rice plants under different conditions was registered weekly. Based on the average plant height and the dry matter content, 'Sandora', 'Unggi-9' and 'IRAT 109' were the most tolerant to drought while 'Marilla', 'APO (NISC RC9)' and 'Fruzsina' were susceptible to limited water supply. However, our results also showed that at the beginning of the growing season, until the active tillering rice needs no more water than other cereal crops, but depending on the varieties after that developmental phase significant differences are occurred.

The chlorophyll content of varieties was monitored using the SPAD value (Minolta SPAD-502 leaf chlorophyll meter). The CT of the varieties under aerobic conditions was measured by an infrared thermometer (Cole Parmer 39800). 'IRAT 109' and 'Sandora' had significantly lower CT than the other varieties especially comparing to 'Marilla' and 'Fruzsina'.

Based on field experiments, model cultivars ('Sandora' and 'Marilla') were selected for further genetic analysis focusing on flow-cytometric analysis and on transcript profiling of roots on oligonucleotide DNA chip. Comparing the tolerant and sensitive varieties several gene categories were found which exhibits drought induced changes (Zombori *et al.* 2008)

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UP-REGULATION OF DEFENSE GENES IN PEPPER LEAVES INOCULATED WITH TWO DIFFERENT TOBAMOVIRUSES

Csilla Juhász[1], István Tóbiás, Gábor Gullner
[1]Ph.D. School in Biology, University of Szeged
Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest

Virus infections lead to substantial alterations of gene expression patterns in infected plants including the up-regulation of a wide variety of defense-related genes. These defense reactions are controlled by a complex, multilayered regulatory network in which various transcription factors and defense-related plant hormones play critical roles. In addition, plant membrane lipids also substantially influence virus replication. Upon infection, positive-strand (messenger-sense) RNA viruses induce significant modifications in intracellular plant membranes in order to create protected viral replication compartments. During this process the structure of membrane lipid bilayers are markedly altered including both protein and lipid modifications. Replication of *Brome mosaic virus* was severely inhibited in yeast by a mutation in *OLE1*, an essential gene encoding delta 9 fatty acid desaturase. These results showed that viral RNA synthesis is highly sensitive to lipid composition and particularly to the level of unsaturated fatty acids.

In recent years our research has been focused on the defense reactions of pepper *(Capsicum annuum* L.*)* plants following virus inoculations. We have used two different viruses in order to compare compatible and incompatible pepper-virus interactions. Inoculation of pepper leaves with *Obuda pepper virus* (ObPV) led to the appearance of hypersensitive necrotic lesions (incompatible interaction), while *Pepper mild mottle virus* (PMMoV) caused no symptoms or very mild chlorotic spots on the inoculated leaves and mild mosaic on the non-inoculated upper leaves (compatible interaction). Both viruses are positive-strand RNA viruses and they belong to the genus *Tobamovirus*. During earlier investigations the 51-fold induction of total lipoxygenase (LOX, E.C. 1.13.11.12) enzyme activity and the up-regulation of *LOX* genes were observed in ObPV-inoculated pepper leaves. LOX isoenzymes catalyze the peroxidation of both free and membrane lipid-bound polyunsaturated fatty acids, and therefore they can potentially interfere with virus replication. ObPV inoculation led also to a massive induction of a divinyl ether synthase (*DES*) gene. The expression of *LOX* and *DES* genes increased only slightly in PMMoV-inoculated, susceptible leaves. To learn more about the defense mechanisms of virus-infected pepper plants, we examined the expression of pepper genes belonging to diverse defense pathways with a special attention to LOX-dependent reactions.

Seeds of the pepper *(Capsicum annuum* L.*)* cultivar TL 1791 harbouring the L^3 resistance gene were planted into soil and grown under normal greenhouse conditions. For each experiment 55-60 d old plants were used. The third and fourth true leaves of plants were inoculated with a suspension of an ObPV strain isolated in Hungary, or with an L^3-resistance-breaking strain of PMMoV isolated in Louisiana, USA. Mock-inoculated plants were used as controls. For gene expression analysis on the mRNA level, a reverse transcription – polymerase chain reaction (RT-PCR) procedure was applied. All PCR products of the expected length were ligated into pGEM-T vector (Promega, Madison, WI,

USA), cloned in competent *E. coli* cells and sequenced. Quantitative real-time RT-qPCR analysis were carried out by using iQ SYBR Green 2x Supermix (Bio-Rad, Hercules, CA, USA) in a DNA Engine Opticon 2 instrument (MJ Research, MA, USA). Expression of a pepper actin gene (GenBank accession AY572427) served as a constitutive control.

ObPV inoculation of pepper leaves resulted in the appearance of necrotic lesions 3 days after inoculation, while PMMoV inoculation caused no visible symptoms or very mild chlorotic spots on the inoculated leaves in accordance with earlier studies. ObPV inoculation markedly induced the expression of several genes encoding pathogenesis-related (PR) proteins including a basic CaPR-1, CaPR-4 and the ribonuclease CaPR-10. In PMMoV inoculated or mock-inoculated leaves the up-regulation of these *PR* genes was negligible.

At the beginning of our studies the GenBank database contained only one entirely sequenced pepper *LOX* gene (*CaLOX1*, GenBank accession FJ377708), which encoded a protein with 9-LOX positional specificity. Therefore, we set out to clone and sequence novel pepper *LOX* genes. We have determined the complete coding sequences of a *13-LOX* (*CaLOXa*, GenBank accession JF304313), a *9-LOX* gene (*CaLOXb*, deposition in progress), and the partial sequence of an other, distinct *13-LOX* gene (*CaLOXc*, deposition in progress). The protein coding nucleotide sequences showed high similarity to each other within the 9- and 13-LOX groups. Therefore during the design of *LOX* gene-specific PCR primers we selected such primer pairs, in which the reverse primer annealed to the less conserved 3' untranslated regions of LOX cDNAs. By using these specific primer pairs we examined the expression of four individual *LOX* genes in ObPV and PMMoV-inoculated pepper leaves by real-time RT-qPCR. In ObPV inoculated leaves the expression of *CaLOX1* and *CaLOXb* genes were substantially induced, which encode proteins with 9-LOX positional specificity. The same gene was only slightly induced by PMMoV inoculation. Weaker inductions were found in the case of *13-LOX* genes following ObPV or PMMoV inoculations. Plant hormones and an ethylene precursor (salicylic acid, methyl-jasmonate and 1-aminocyclopropane-1-carboxylic acid) induced very differently the expression of these four *LOX* genes.

The plant-specific WRKY transcription factor proteins have been extensively studied in recent years because of their involvement in plant defense reactions against various pathogenic microorganisms including viruses. We have observed the early and significant up-regulation of *WRKY2* and *WRKY RKNIF2* genes (GenBank accessions DQ402421 and GQ253367, respectively) following ObPV and, to a lesser extent, PMMoV inoculation.

In summary, our results showed that the rapid and massive up-regulation of defense genes encoding PR-proteins, LOXs and WRKY transcription factors in the incompatible pepper-ObPV interaction contributes to antiviral resistance. We suppose that the rapid up-regulation of *9-LOX* genes contributes to the alteration of intracellular plant membranes in order to inhibit the replication of invading *Tobamoviruses*.

UPF1 PHOSPHORYLATION IS ESSENTIAL FOR NONSENSE-MEDIATED mRNA DECAY (NMD) IN PLANTS

Farkas Kerényi, Izabela Wawer, Joanna Kufel, Dániel Silhavy
Plant RNA Biology Group, Agricultural Biotechnology Center, Gödöllő

Nonsense-mediated mRNA Decay (NMD) is a conserved eukaryotic quality control mechanism that degrades mRNAs containing in-frame premature termination codons (PTC), thereby preventing the accumulation of potentially harmful truncated proteins. It is also involved in regulation of expression level of wild-type mRNAs that contain cis-acting NMD elements. These cis-acting elements are different: it could be an unusually long 3'UTR or an intron located in 3'UTR placed at least 50-55 nt downstream from the stop codon. The core trans-acting NMD factors UPF1, UPF2, UPF3 and SMG7 are conserved from yeast to mammals. In eukaryotes, two types of NMD system were described, the long 3'UTR-based NMD that operates in yeast and invertebrates and the intron-based NMD, which functions in mammals. If the 3'UTR is too long, the termination factors – on stalling ribosome at the stop codon – interact with UPF1 instead of PABP on polyA-tail. UPF1 binds UPF2 and UPF3 forming the NMD surveillance complex leading to rapid mRNA decay (long 3'UTR-based NMD). In mammals, during intron splicing an exon-junction complex (EJC) is deposited onto the mRNA, which serves as a binding platform for central NMD factors Upf3, Upf2. Normally the translating ribosome removes EJC from the mRNA unless it locates in the 3'UTR. If the ribosome can't remove the EJC Upf1 binds to UPF2 to form an NMD surveillance complex, leading to rapid mRNA decay.

NMD is well examined in mammals, yeast and *Drosophila*. There are two main part of NMD: early steps and late steps. The early steps involve identification of PTC-containing mRNAs and binding of UPF proteins to the mRNA. During the late steps the SMG6 and SMG7 NMD factors bind to phosphorylated UPF1 and induce the rapid degradation of the mRNA. The *Hs*SMG6 binds to the phosphorylated T28, the *Hs*SMG7 binds to the phosphorylated S1096 of *Hs*UPF1. These phosphorylations are made by *Hs*SMG1 in very strict circumstances. SMG6 cuts the mRNA leading free 5' and 3' ends. SMG7 remobilize the mRNA to the P-body (processing body) where the mRNA goes through deadenylation and decapping causing free 5' and 3' ends. Free mRNA 5' and 3' ends are attacked by XRN1 and exonuclease, respectively, leading the rapid degradation of mRNA.

At the beginning of the work of our group little was known about plant NMD. Previously our group has elaborated an efficient agroinfiltration-based transient NMD test system combined with virus induced gene silencing (VIGS) to define and examine the cis-acting elements and trans factors of plant NMD. They have found that in plants both long 3'UTR-based NMD and intron-based NMD act efficiently, which appear to be unique among eukaryotes. Furthermore they have confirmed, that the core NMD factors (Upf1, Upf2, Upf3, Smg7) are required for both types of plant NMD, whereas the plant orthologs of the components of EJC are involved only in intron-based plant NMD. They confirmed that the N-terminal and C-terminal S/TQ-rich regions of UPF1 are functionally redundant and that both regions are phosphorylated.

In our work we wanted to further examine the role of UPF1 phosphorylation in plant NMD. We mapped which potential S/TQ sites are really phosphorylated and which are involved in NMD. So we made several mutant constructs in the N-terminal and in the C-terminal region mutating (by alanin-substitution) or deleting some of the potential S/TQ phosphosites. Our mutants was examined in VIGS-complementation test. In UPF1 VIGS plants the NMD doesn't work. Agroinfiltration with a functional UPF1 mutant that can complement the UPF1-deficency of VIGS plant leads to restoration of the NMD in the infiltrated patch. If we agroinfiltrate with a non-functional UPF1 mutant, it can't complement the UPF1 VIGS, thus the NMD is not restored in the infiltrated spot. These processes are observable by co-infiltrating an NMD-sensitive reporter gene (e.g. GFP containing an intron in its 3'UTR – Gc-I). If there is no NMD, the GFP will illuminate strong. If the NMD is restored, the GFP will illuminate weak. The Gc-I mRNA level was examined by Northern-blot.

The N-terminal region of UPF1 is strongly conserved. It contains four potential S/TQ phospho-sites: three serines (S3, S13, S105) and a threonin (T29). The T29 is the homolog of human T28 which binds the SMG7. Therefore we examined whether this phospho-site is only needed for plant NMD. The results of our experiments show that the T29 is the most important phspho-site in the N-terminal region because it is able to complement the UPF1 VIGS alone. However the others together are also able to do. Next, we examined whether these potential S/TQ phosphor-sites are really phosphorylated. We phospho-stained our mutants with the following results: First, there is a highly phosphorylated region between T29 and S105 but this region is not needed for NMD. Second, the examined potential S/TQ phospho-sites are really phosphorylated. These results were verified by mass spectrometry.

The C-terminal region is less conserved. The only conserved in different species' UPF1 that there are lots of S/TQ sites in this region. In plants, there are 18. Our group previously showed that the C-terminal region truncated from the 3'end can complement the UPF1 VIGS well enough. We examined these segments separately and we found that they are redundant. So we examined further only the first four potential S/TQ phospho-site containing segment. Our result show that the first, the second and the fourth sites are not or less phosphorylated and are not involved in NMD, while the third one is phosphorylated and is needed for NMD.

Taken together, our results show that, in contrast to human UPF1, there is no strict site-specific phosphorylation to bind SMG7. Instead, there is a mass-phosphorylation, and there are important S/TQ sites and more or less important ones and there are S/TQ sites that are not needed for NMD.

UPTAKE AND TRANSLOCATION OF A HUNGARIAN *ERWINIA AMYLOVORA* BACTERIOPHAGE ISOLATE THROUGH ROOTS – SIGNIFICANCE IN BIOLOGICAL CONTROL OF FIRE BLIGHT

Judit Kolozsváriné Nagy[1,2], Ildikó Schwarczinger[2]
[1]Doctoral School of Horticultural Science, Corvinus University of Budapest
[2]Centre for Agricultural Research, Hungarian Academy of Sciences, Plant Protection Institute, Budapest

Fire blight, caused by *Erwinia amylovora* (Burill) Winslow *et al.*, is the most destructive disease of several species of the family *Rosaceae* inducing considerable economic losses in orchards. At present, there is no truly effective way to control this disease. The most effective protection method, *i.e.* the timed application of the antibiotic streptomycin to open blossoms, is not allowed in Hungary, similarly to most European countries. Furthermore, removal of infected plant parts and sprayings with currently available chemicals do not provide adequate protection against the pathogen. All these challenges, however, led to the research and development of alternative control strategies. Although, the idea of using bacteriophages against plant pathogenic bacteria emerged in 1924, the first phage-containing pesticide (AgriPhage™, U.S.) was registered only in 2005.

Bacteriophages, or shortly phages, are the viruses of bacteria. Recently, bacteriophages have been found to be effective for control of different plant diseases in several cases. They have many advantageous characteristics, but some of their features make their application as a biopesticide a real challenge. Phages can only replicate in the presence of bacterial cells and they are especially sensitive to environmental conditions, they can be easily inactivated by changing temperatures, extreme pH, precipitation, or UV irradiation. Phage persistence in the plant leaf surface environment can be significantly improved by applying them to the phyllosphere in proper time – before sunrise or after sunset – in sufficient high concentrations, together with light protective carriers, or non-pathogenic host bacteria supporting phage survival and multiplication. The rhizosphere environment is less harsh but it should be considered that, depending on soil conditions, adsorption to soil particles decreases the number of phages that are able to lyse host bacteria.

The ability of viruses to penetrate through leaves and roots is known for a long time. Several reports claim that bacteriophages are also capable to penetrate via the roots and then to translocate in the vascular tissues to distal parts of the plants, maintaining their viability even for days. It has been also revealed that following soil-application of phages specific to the given plant pathogens, the phages reduced the severity of certain plant bacterial diseases such as crown gall or bacterial wilt of tomato. The possible reasons of this effect could be the translocation ability of phages and the proper timing of phage treatment. In fact, in case of bacterial wilt of tomato the best control efficacy was achieved when a phage mixture was applied to the soil at the time of bacterial inoculation.

In the present work our aim was to clarify whether *E. amylovora*-specific bacteriophages introduced near the root zone are able to penetrate and systemically spread into apple plants.

For this purpose, the phage ΦEa5k was used, which we have isolated in 2006 from infected shoots and flowers of a quince (*Cydonia oblonga*) tree from Hungary. This phage isolate belongs to the *Myoviridae* and forms clear plaques of an average size of 3-4mm with a halo of 0.8-2mm on agar layers containing *E. amylovora* strain Ea1/79Sm. We tested the root uptake of this phage isolate on five-month-old apple (*Malus* x *domestica* 'Pinova') seedlings grown in sterile circumstances in hydroponic culture or perlite medium. Five plants per treatment were placed separately in small glass containers. A phage suspension at a concentration of 10^{13} PFU/ml was added to the plants: 30 ml into the hydroponic culture and 10 ml in case of seedlings set in perlite previously moistened with 2 ml of sterile water. Control plants were treated with sterile distilled water only. Subsequently we took samples at two time points (from three seedlings per treatment on the second day, and from two plants per treatment on the sixth day) by extracting the upper stem parts and leaves together. Considering that the titer of phage suspensions applied will possibly drop below levels detectable with direct re-isolation, 200µl of samples taken after a two-day incubation were shaken for a further four days in liquid medium with bacterial culture (*E. amylovora* strain Ea1/79Sm). For the detection of phages the modified Adams' droptest was used: 100 µl of phage suspension was dropped onto the top agar layer containing 100 µl of test bacteria.

In general, perlite medium was more favourable for phage detection. Following incubation for two days, we managed to detect phages by direct sampling from one plant sample grown in perlite. However, we could detect phages in all plants grown in perlite and in one plant grown in hydroponic culture by applying the so-called multiplication sampling method. In case of phages isolated from plants grown in perlite plaques were translucent and coalesced. During incubation for six days, we could detect phages in all plants grown in hydroponic culture and in one plant grown in perlite. All water-treated controls were phage-free. Plaque morphology of the isolated phages was the same as that of the phage cultures used for inoculation. Taken together, *E. amylovora*-specific bacteriophages were capable of penetrating the roots and to systemically spread in apple plants and could maintain viability for six days without presence of the host bacterium. However, phage titer was significantly reduced under such circumstances.

The present study is the first to demonstrate *in planta* translocation of an *E. amylovora*-specific bacteriophage isolate. Furthermore, this investigation is the first step in clarifying whether phages of *E. amylovora* are more effective biocontrol agents when penetrating roots or when applied to the plant surface above soil level. However, it needs to be emphasized that the mechanism of *in planta* bacteriophage translocation is so far unknown. Future research should focus on further investigations on penetration and translocation of phages into plants.

ASCORBATE, AS ALTERNATIVE ELECTRON DONOR TO PHOTOSYSTEM II, PROTECTS PLANTS AGAINST PHOTOINHIBITION AND STIMULATES THE PHOTOPRODUCTION OF HYDROGEN IN GREEN ALGAE

Valéria Nagy, Szilvia Z. Tóth, Roland Tengölics, Gert Schansker, Gábor Rákhely, Kornél L. Kovács, Győző Garab
Ph.D. School in Biology, University of Szeged
Institute of Plant Biology, Biological Research Center of the HAS, Szeged

Oxygenic photosynthetic organisms produce organic materials by using light energy and water as terminal electron donor. The oxygen evolving complex (OEC) is one of the most vulnerable components of the photosynthetic electron transport chain. It has been shown that when the OEC is damaged, alternative electron donors, present in large amounts, donate electrons to photosystem II (PSII) *in vivo*[1]. In the present study we have demonstrated that the alternative electron donor is ascorbate and investigated the importance of this novel role of ascorbate in plant physiology and its possible biotechnological applications.

The alternative electron donor function of ascorbate were studied on heat treated wild type and ascorbate deficient and ascorbate overproducing mutant *Arabidopsis thaliana* plants. The effect of ascorbate on hydrogen production were examined on wild type *Chlamydomonas reinhardtii* cells. The main techniques applied were the measurement of chlorophyll fluorescence transient to assess PSII activity, the absorbance change at 820 nm to monitor the activity of PSI rection centre and gas chromatography to follow the H_2 and O_2 accumulation in algal cultures.

Our studies carried out on higher plant leaves and algal cells have shown that ascorbate serves as an alternative electron donor to PSII; the rate of electron donation depends on the ascorbate content of leaves: $t_{1/2}$ is approximately 25 ms in wild type *Arabidopsis* plants and about 55 ms in the ascorbate-deficient mutants[2]. The rate of electron donation can be accelerated by infiltration of leaves with ascorbate solution confirming that the source of alternative electron donor of PSII is the ascorbate pool of the thylakoid lumen. When the OEC is damaged by heat stress, highly oxidising components (Tyr_Z^+ and $P680^+$) accumulate in PSII in the light, leading to the fast inactivation of PSII. We have demonstrated[3] that under these conditions ascorbate has a protective function by providing electrons to PSII and slowing down the harmful accumulation of Tyr_Z^+ and $P680^+$.

Based on these results, it was reasonable to assume that ascorbate, by replacing OEC and supporting the electron transport without oxygen evolution, can enhance the photoproduction of hydrogen in *Chlamydomonas reinhardtii* cells. Photoproduction of hydrogen is known to depend on the activity of PSII; however, the oxygen evolution associated with PSII activity strongly inhibits the hydrogenase. It has earlier been shown that *Chlamydomonas* cells are able to evolve considerable amounts of hydrogen under anaerobic conditions following their sulphur deprivation, which suppresses their PSII activity[4]. Our experiments have shown that the addition of 10 mM ascorbate to sulphur-deprived cell

culture accelerates significantly the linear electron transport via PSII to PSI and to the hydrogenase, leading to a three-fold increase in hydrogen production[5]. Similar results were obtained by using diphenylcarbazide (DPC), an artificial electron donor to PSII. The stimulation of hydrogen production was sensitive to diuron and dibromothymoquinone (inhibitors of PSII and the cytochrome b_6f complex, respectively), which proves that the enhancement of the hydrogen evolution by ascorbate and DPC can indeed be accounted for by their functioning as alternative PSII electron donors.

Ascorbate, as the alternative electron donor of PSII, can provide protection against donor side photoinhibition and may be used to improve H_2 production of algal cultures.

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FUNCTIONAL ANALYSIS OF CUCUMBER MOSAIC VIRUS 2b PROTEIN

Katalin Nemes[1,4], Ákos Gellért[3], Ervin Balázs[3], Katalin Salánki[2]
[1] Szent Istvan University, PhD School of Biological Sciences, Gödöllő
[2] Agricultural Biotechnology Center, Gödöllő
[3] Centre of Agricultural Research, Hungarian Academy of Sciences, Martonvásár
[4] Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Plant Pathology, Budapest

Cucumber mosaic virus (CMV) is an economically important plant virus with a broad host range. Unlike other members of the family Bromoviridae, the strains of CMV have a very broad, collective host range, infecting more than 800 plant species in over 100 families, including fruit crops, vegetables and ornamentals, both monocots and dicotyledonous. The genome of CMV consists of three single-stranded, positive-sense RNA molecules, which encodes five proteins. The smallest one (110 aa) is the multifunctional 2b protein encoded by the RNA2. It has roles in symptom induction, virus movement, evasion of the defense mechanism mediated by salicylic acid and jasmonic acid. The 2b protein could also suppress the antiviral RNA silencing, it was among the first viral protein described as an RNA silencing suppressor.

RNA silencing mediated by short-interfering RNAs (siRNAs) is a potent antiviral defence mechanism, and many plant viruses encode VSRs, although there is great diversity in the mode of action of these factors (Csorba et al., 2009). 2b protein is unique among the known plant and animal VSRs because it directly interacts with both the RNA and protein components of the RNA silencing machinery (Zhang et al., 2006; Goto et al., 2007; González et al., 2010; Hamera et al., 2012, Duan et al., 2012). 2b binds ARGONAUTE1 (AGO1) (González et al., 2010; Zhang et al., 2006), which is an RNA 'slicer' enzyme known to be involved in plant antiviral RNA silencing (Baumberger and Baulcombe, 2005; Harvey et al., 2011) The interaction of CMV 2b with AGO1 and AGO4 from Arabidopsis has been demonstrated in vitro and in vivo by coimmunoprecipitation and bimolecular fluorescence complementation assays, which is consistent with the observed activity of CMV 2b to suppress the *in vitro* slicer activity of AGO1 and AGO4 (Zhang et al., 2006; Gonza´lez et al., 2010; Hamera et al., 2012). Intriguingly, although the positive-strand RNA genome of CMV replicates exclusively in the cytoplasm, 2b is predominantly localized to the nucleus by single or double nuclear localization signals (NLSs) in subgroup II and I strains of CMV, respectively (Lucy et al., 2000; Wang et al., 2004).

The 2b proteins of different CMV strains and other cucumoviruses share a number of conserved amino acid sequence domains, suggesting their important protein functions. Known or suspected functional domain include nuclear localization signals (NLS), an RNA binding domain (overlapping the NLSs), putative phosphorylation sites, as well as the N and C termini (involved in DNA binding). We have analyzed systematically the effect of mutations in these domains and also in other parts of the protein if other substantial domain in the virus life cycle could be identified. Alanine scanning is simple and widely used technique for determination of the functional role of protein residues. During our work three by three consecutive amino acids have been replaced to alanine. Wild-type (WT)

and mutant RNA 2 were generated by *in vitro* transcription and were combined with *in vitro* synthesized RNAs 1 and 3 using the plasmids pRs1 and pRs3 as templates (Diveki et al., 2004). N. clevelandii plants were inoculated mechanically at three leaves stages, and symptom development was observed. In all host-virus combinations, the infection was monitored by Northern analysis of the inoculated, and the systematically infected leaves. Systemic accumulation of the virus was confirmed by Northern hybridization. Stability of the mutations within the viral progeny was confirmed by DNA sequencing of RT-PCR products.

The majority of the mutant viruses caused similar symptoms as the original Rs-CMV. In these cases the sequence analysis confirmed the stability of the mutations. In the case of two mutants symptoms were not observed and the presence of viral RNA was not detected in the non-infected leaves. In six cases the symptoms developed later and were milder, and in two further cases the test plants recovered.

The crystal structure of the homologue *Tomato aspermy virus* (TAV) 2b protein has been determined in 2008. The known part of the 2b protein contains two long a-helices. The helical axes rotate 120° angle to each other. The 2b protein forms a pair of hook-like dimers to bind the siRNA duplex. The a-helices fit into the major groove of the siRNA in a sequence-independent and length-preference manner. The biologically active form is tetramer: four 2b protein molecules and two siRNA duplexes. The C-terminal domain (aa. 69-110) of 2b protein is missing from the X-ray structure therefore a reliable, full-length R-CMV 2b protein model was generated with molecular modelling methods. The active siRNA bound tetramer form was also constructed. The 1.(MEL_{1-3}) construction does not cause systemic infection. This mutation is localized at the forepart of the first α-helix, which is involved in the leucine-zipper-like tetramerization mechanism. The active tetrameric structure was most likely not being able to form because of this mutation. The 8.(KKQ_{22-24}) mutation is in the middle of the first α-helix, while the 12.(RER_{34-36}) mutation is located at the end of the first α-helix. Both constructions showed systemic symptom, in spite of the infected plants showed only slightered symptoms, and the systemic maintance of theese mutants was detectable later compared to the wild type virus. The ribonucleo-protein complex may have been able to evolve, but it has not got long-term lifetime. Therefore, after a while the PTGS can predominate. Mutations 11.(QNR_{31-33}) and 19.(LPF_{55-57}) are localized at the end of the first and second α-helix, respectively. These residues immersed into the major groove of the siRNA duplex. The experimental data suggest that in the case of these constructs the systemic symptoms evolved very slowly. Based on the tetramer structure can be rendered probable, that these mutations produce reduced stability siRNA-protein complexes without losing its function. The 14.(SPS_{40-42}) mutation is in the forepart of the second α-helix. Most likely this mutation disrupts the integrity of the second a-helix. The 24.(RHV_{70-72}) mutation which does not cause systemic infection localize at C-terminal domain of 2b protein. Putative function of this domain is stabilizing the binding of the double-stranded RNA. Recent structural information is assumed that these mutations make the C-terminal domain partially unstructured.

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