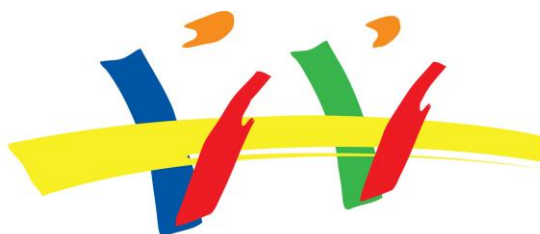
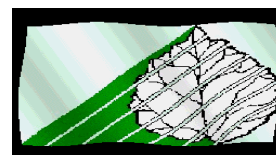




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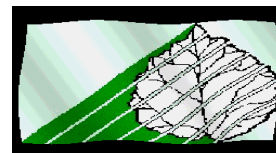
IPA Cross-border Co-operation Programme

HUSRB/1002/214/036 “OXIT”

ABSTRACTS OF OPENING CONFERENCE

*Good neighbours
creating
common future*





Use of cDNA library transformation for the identification of stress tolerance genes.

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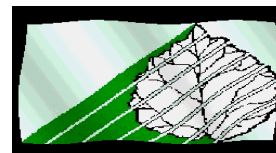
Plants are exposed to constantly changing environments and often have to survive in adverse conditions. Although numerous genes have been identified which control stress responses, the regulatory pathways by which plants tolerate salinity are still not well understood. To identify novel factors which control stress tolerance, cDNA libraries of *Arabidopsis thaliana* and its halophyte relative *Thellungiella salsuginea* were generated in a plant expression vector under the control of the estradiol-dependent *lexA* promoter (Conditional Overexpression System, COS). Transgenic plant population was generated with the COS library and screened for enhanced or reduced salt tolerance, ABA insensitivity or activation of the stress-induced reporter gene construct ADH1-LUC. Several *Arabidopsis* lines were identified with significant differences in their tolerance to salt, sensitivity to ABA or capability to trans activate the reporter gene construct. We are characterizing several genes identified in selected COS lines.

Overexpression of a previously unknown *Arabidopsis* gene could enhance germination in high salt or high osmotic conditions. The encoded protein was targeted to the nucleus and had close similarity to a subunit of the RNA polymerase II. Transcript profiling with RNAseq technology identified numerous stress-regulated genes with enhanced transcript levels in the overexpressing plants. Selection for ABA insensitivity lead to the identification of a previously unknown zinc finger transcription factor, which altered seed development and germination, ABA sensitivity, hypocotyl elongation and light responses in early seedling development. Nuclear targeting of the cloned transcription factor was verified. Transcript profiling showed that the identified factor controlled the expression of a number of genes, including those which were induced by light or ABA.

In order to explore genetic diversity for stress tolerance, cDNA library of *T. salsuginea* was introduced randomly into *Arabidopsis* plants. Screening the transgenic plant population on high salt media lead to the identification of 15 lines with enhanced salt tolerance. Inserted cDNA were rescued, cloned and sequenced to determine the identity of the encoded proteins. Salt tolerance was confirmed by germination and growth assays in five lines, which were selected for further characterization. One of the identified cDNA encoded an unknown protein with high similarity to a translation initiation factor. Enhanced expression of this *Thellungiella* cDNA improved growth and tolerance to high salinity and osmotic stress conditions. Our results showed, that novel genetic approaches can be valuable for the identification of regulatory genes in different plant species, which can improve tolerance to extreme environmental conditions.



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The Arabidopsis heat shock factor HSFA4A controls tolerance to different environmental stresses.

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Previously we have constructed the COS cDNA library to identify cDNA which, upon overexpression can modulate stress responses in transformed cells or plants. In order to identify stress regulatory factors at cell level, Arabidopsis cell culture was transformed with the COS library and the transformed cells were selected for salt tolerance on culture media supplemented with NaCl. Four cell colonies showed superior growth on selective medium, and used to rescue cDNA inserts. One of the cloned cDNA encoded a novel heat shock factor, the overexpression of which could improve salt tolerance of transgenic Arabidopsis plants as well. When the selected transgenic line was tested on different conditions, we found that overexpression of the inserted transcription factor could improve tolerance to heavy metals, osmotic and oxidative stresses. The heat shock factor was targeted to nuclei and could mediate the transcriptional activation of a set of genes, which respond to oxidative stress. We showed that dimerization of the transcription factor took place in plant cells, which was suggested to be essential for the activation of target genes. Our experimental results confirmed, that selection for certain traits can be performed at cell level, which offers an efficient way to identify novel factors able to increase stress tolerance in plant level.



Characterization of two poplar homologs of *GRAS/SCL TF*, a gene encoding a transcription factor with relevance draught and salt tolerance

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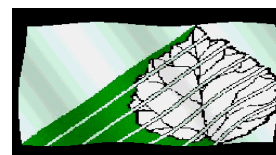
According to scientific publications GRAS proteins are unique to plants and are members of a major protein family and homologs have been found besides in Arabidopsis also in many other higher plants. Although the Arabidopsis genome encodes at least 33 GRAS protein family members only few of them have been characterized so far. Those investigations has shown that GRAS proteins plays an important roles in signal transductions, meristem maintenance and development.

Recent publication published in 2010 of a salt and draught-inducible poplar GRAS protein SCL7 showed that this gene is potentially useful for engineering draught and salt tolerance in trees, thus focus our attention to a GRAS/SCL transcription factor (TF) as a candidate gene for our research work. Thus, understanding the molecular basis will be helpful in developing selection strategies for improving salinity tolerance.

Identification of molecular markers linked to salinity/draught-tolerance traits has provided plant breeders a new tool for selecting cultivars with improved salinity/draught-tolerance. Information on salt-responsive proteins/genes is crucial for improving salt-tolerance through genetic engineering techniques. Research which can contribute much to reveal the molecular basis of adaptation (e.g. tolerance to abiotic stress) are those involving the candidate gene sequence diversity at nucleotide level. A prerequisite for the determination of nucleotide polymorphisms in candidate genes is the knowledge of their sequence.

The aim of this research was to identify and characterize candidate genes (CG) of salinity/draught stress tolerance as the first step. Nucleotide diversity in these CG were screened in several poplar clones to search for polymorphisms putatively correlated to salt stress tolerance. Using different strategies the knowledge about nucleotide diversity in CG in Serbian poplar clones was broaden.

Key words: poplar, GRAS gene, salt/draught stress, sequence diversity



Reactive oxygen species and antioxidants in abiotic stress tolerance in poplar plants

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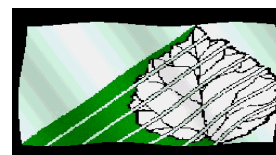
Various abiotic stresses lead to the overproduction of ROS ($O_2\cdot^-$, H_2O_2 , $OH\cdot$ and 1O_2). $O_2\cdot^-$ can also react with another very influential signaling free radical species, $NO\cdot$ to give up peroxynitrite ($OONO\cdot$) in plants which are highly reactive and toxic and ultimately results in oxidative stress. The production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attacks. Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications. Oxidative stress is a condition in which ROS or free radicals, are generated extra- or intra-cellularly, which can exert their toxic effects to the cells. These species may affect cell membrane properties and cause oxidative damage to nucleic acids, lipids and proteins that may make them nonfunctional. However, the cells are equipped with excellent antioxidant defense mechanisms to detoxify the harmful effects of ROS. The antioxidant defenses could be either non-enzymatic (e.g. glutathione, proline, α -tocopherols, carotenoids and flavonoids) or enzymatic (catalase (CAT), superoxide dismutase (SOD), guaiacol and ascorbate peroxidases (GPOX, APOX) and glutathione reductase (GR).

Copper, zinc and cadmium are the pollutants, which could produce oxidative stress. Their different concentrations induce of positive or negative impact on stimulation or inhibition of the most important antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), guaiacol and ascorbate peroxidases (GPOX, APOX) and glutathione reductase (GR) as well as increasing MDA (malondialdehyde) quantity. Finally, three thresholds of Cu, Ni and Cd concentrations resulting in increasing toxicity against plants could be proposed, splitting the ranges of the non toxic presence of these metals into soil.

There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant and scavenging activities of plants and increased resistance to drought stress. Accumulation of proline under stress protects the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment. It also can interact with some antioxidant enzymes and stabilize their structure and function. Higher proline accumulation was observed in drought-tolerant than in drought sensitive species. The accumulation of antioxidants is necessary for scavenging reactive oxygen species and to protect lipid membrane from oxidative stress in plants subjected to drought stress. Protection against drought and oxidative stress could be induced by various physiological and biochemical changes like activation of antioxidant system and accumulation of some secondary biomolecules with antioxidant activity what depends on plant species.



The project is co-financed by the
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Therefore, present study was designed to examine ROS quantities, enzymatic and non - enzymatic defense mechanisms as well antioxidant (using FRAP method) and free-radical scavenging capacity (using DPPH method), lipid peroxidation intensity and proline accumulation in roots and leaves of poplar clones in order to examine their potential in enhancing protection from oxidative stress caused by heavy metals and drought. Obtained results should be used in selection of drought tolerant poplar species with interesting perspectives of application in phytoremediation.

Mitochondria and oxidative stress in plants

Laura Zsigmond

Department of Plant Biology, University of Szeged, Szeged, Hungary

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Mitochondrial respiration is sensitive to environmental conditions and can be influenced by oxidative stress. Previously we described the mitochondrial protein PPR40, and our results suggest a close link between regulation of oxidative respiration and environmental adaptation in Arabidopsis. Overexpression of the PPR40 gene in Arabidopsis resulted in enhanced germination and superior plant growth in saline conditions. Respiration increased in PPR40 overexpressing plants during salt stress. Reduced antioxidant homeostatic status accompanied salt tolerance. The PPR40 can diminish the generation of reactive oxygen species by stabilizing the mitochondrial electron transport and protecting plants via reducing oxidative damage during stress. Protection of mitochondrial functions by genetic engineering might offer valuable tools for plant protection and can be a promising strategy for biotechnological applications to increase stress tolerance of plants.



General introduction, experimental design and preliminary results

Erdei László, Tari Irma, Gallé Ágnes

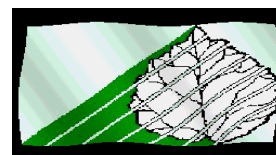
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In the period of 01.01.2012 – 30.04. 2012 maintain management and communication among project partners were maintained in order to conduct experimental work and prepare special experimental designs concerning the growth of poplar clones of different tolerance of osmotic stress and heavy metals, especially copper (Cu^{2+}) for the next period.

Experimental work for comparative analysis of oxidative stress responses in contrasting genotypes of model (*Arabidopsis*) plant has been carried out (presentation in this meeting by Laura Zsigmond). Cuttings of poplar clones, as provided by Project Partner 1 (Novi Sad) will be rooted hydroponically using polyethyleneglycol 6000 (PEG) as osmoticum. Alternatively, cuttings will be rooted in perlite system which allows the administration of different water stress treatments. Leaves of the sprouts will be used for biochemical analysis. Since during phytoremediation process poplar trees are exposed to heavy metal stress, the ability of the detoxification of the toxic pollutant and of the excess reactive oxygen species (ROS) is a key component of the remediation efficiency. As glutathione transferases (GST) take part in both the plant cell detoxification and the elimination of excess ROS (through peroxidase activity), the timing and rate of the induction of the GSTs can be an important factor in the defence reactions. In poplar species heavy metal stress induces relevant changes in the GST activity and phytochelatin synthesis, which is in correlation with the different HM stress tolerance of genotypes. Until now copper stress caused the induction of one tau group GST gene (GSTU19, GQ377230.1) was published. Detecting the changes of this gene and identification of other stress related genes would improve the understanding of the plant cell defence system. This kind of investigation is well prepared in the reporting period.

Implementation of tenders for purchase of equipments is also in progress.



Characterization and Oxidative stress tolerance in plants: from models to trees (OXIT)

Opening conference

Szeged, 9. March 2012.

Location:	Biological Research Centre, HAS Szeged
9.30 – 10.00	Registration
10.00 – 10.10	Welcome Dr. László Szabados, MTA SZBK Sasa Orlovic, INŠŽS
10.10 – 10.40	Use of cDNA library transformation for the identification of stress tolerance genes Dr. László Szabados, MTA SZBK
10.40 – 11.10	General introduction, experimental designs and preliminary results at the University of Szeged Dr. László Erdei, Dr. Irma Tari, Dr. Ágnes Gallé, SZTE
11.10 – 11.40	Reactive oxygen species and antioxidants in abiotic stress tolerance in poplar plants Prof. Dr Dubravka Štajner, INŠŽS
11.40 – 12.10	Coffee break
12.10 – 12.40	The Arabidopsis heat shock factor HSFA4A controls tolerance to different environmental stresses Imma Pérez Salamó, MTA SZBK
12.40 – 13.10	Characterization of two poplar homologs of GRAS/SCL TF, a gene encoding a transcription factor with relevance to draught and salt tolerance Dr Vladislava Galovic, INŠŽS
13.10 – 13.40	Mitochondria and oxidative stress in plants Dr. Laura Zsigmond, SZTE
13.40 – 14.00	Discussion
14.00	Lunch, Conference closing