Progress Report WG1 and WG2 December 2008 –December 2009

WG1 is mainly concentrated on understanding the regulatory roles of two major classes of protein kinases (SnRKs and MAPK), the activities of which are differentially controlled by specific abiotic stress stimuli. WG2 deals with the identification and functional analysis of signaling compounds directing gene expression under stress conditions. The topics developed in WG1 are closely related to the topics in WG2, and therefore a close collaboration between scientists of these two WGs, promoted during the first COST meeting (Matera, Italy), has been continued and several publications have been obtained. An example for this is the training supported through an STMS by a member of the group of **Laszlo Szabados** in the laboratory of **Csaba Koncz**. Here a novel method for isolating low abundant proteins was used and resulted in the partial purification of the transcription factor CAI1. Like the contribution of Prof. Rodriguez, the Szabados/Koncz collaboration demonstrates that the fine tuning of stress response pathways lies in assembly of complexes and a homeostasis of complex components. These are the first steps towards understanding the complex stress network and in the end to manipulate stress reactions towards a more profitable agriculture. Further objective of these WGs are to use the efforts of individual groups to determine the temporal sequence of the molecular events in responses to stress. This should eventually lead to building up molecular networks which can be utilized in a more general system biology approach. Some other examples of relevant work by particular and collaborative work are described below:

Andrzej Jerzmanowski (Laboratory of Plant Molecular Biology. Faculty of Biology. Warsaw University. Poland) and **Csaba Koncz** (Max Planck Institute for Plant Breeding Research. Cologne.Germany)

Genetic analysis of functional redundancy of BRM ATPase and ATSWI3C subunits of Arabidopsis SWI/SNF chromatin remodelling complexes

In yeast and mammals, ATP-dependent chromatin remodelling complexes of the SWI/SNF family play critical roles in the regulation of transcription, cell proliferation, differentiation and development. Homologues of conserved subunits of SWI/SNF-type complexes, including Snf2-type ATPases and SWI3-type proteins, participate in analogous processes in Arabidopsis. Recent studies indicate a remarkable similarity between phenotypic effects of mutations in the SWI3 homologue ATSWI3C and bromodomain-ATPase BRM genes. To verify the extent offunctional similarity between BRM and ATSWI3C, we have constructed atswi3c brm double mutants and compared their phenotypic traits to those of simultaneously grown single atswi3c and brm mutants. In addition to inheritance of characteristic developmental abnormalities shared by atswi3c and brm mutants, some additive brm-specific traits were also observed in the atswi3c brm double mutants. Unlike atswi3c, the brm mutation results in the enhancement of abnormal carpel development and pollen abortion leading to complete male sterility. Despite the overall similarity of brm and atswi3c phenotypes, a critical requirement for BRM in the differentiation of reproductive organs suggests that its regulatory functions do not entirely overlap those of ATSWI3C. The detection of two different transcript isoforms indicates that BRM is regulated by alternative splicing that

creates an in-frame premature translation stop codon in its SNF2-like ATPase coding domain. The analysis of Arabidopsis mutants in nonsense-mediated decay suggests an involvement of this pathway in the control of alternative BRM transcript level.

Publications:

Archacki R, Sarnowski TJ, Halibart-Puzio J, Brzeska K, Buszewicz D, Prymakowska-Bosak M, Koncz C, Jerzmanowski A (2009) Genetic analysis of functional redundancy of BRM ATPase and ATSWI3C subunits of Arabidopsis SWI/SNF chromatin remodelling complexes. Planta 229(6):1281-1292.

Montserrat Pagès (IBMB-CSIC. Barcelona. Spain)

AKIN-betagamma dimerizes through the KIS/CBM domain and assembles into SnRK1 complexes

The SNF1/AMPK/SnRK1 complex is an intracellular energy sensor composed of three types of subunits: the SnRK1 kinase and two regulatory, non-catalytic subunits (designated beta and gamma). We have previously described an atypical plant gamma-subunit, AKINbetagamma, which contains an N-terminal tail similar to the so-called KIS domain normally present in beta-subunits. However, it is not known whether AKINbetagamma normally associates with endogenous SnRK1 complexes in vivo, nor how its unique domain structure might contribute to SnRK1 function. Here, we present evidence that maize AKIN betagamma is an integral component of active SnRK1 complexes in plant cells. Using complementary methodological approaches, we also show that AKINbetagamma associates through homomeric interactions mediated by both, the gamma- and, unexpectedly, the KIS/CBM domain.

Publications:

López-Paz C, Vilela B, Riera M, Pagès M, Lumbreras V. Maize (2009) AKINbetagamma dimerizes through the KIS/CBM domain and assembles into SnRK1 complexes (2009) FEBS Lett. 583(12):1887-1894.

Laszlo Bogre (Institute of Biotechnology, University of Cambridge, UK)

MAPK signalling pathways

Mitogen activated protein kinase (MAPK) pathways are signal transduction modules with layers of protein kinases having c. 120 genes in Arabidopsis, but only a few have been linked experimentally to functions. * We analysed microarray expression data for 114 MAPK signalling genes represented on the ATH1 Affymetrix arrays; determined their expression patterns during development, and in a wide range of time-course microarray experiments for their signal-dependent transcriptional regulation and their coregulation with other signalling components and transcription factors. * Global expression correlation of the MAPK genes with each of the represented 21 692 Arabidopsis genes was determined by calculating Pearson correlation coefficients. To group MAPK signalling genes based on similarities in global regulation, we performed hierarchical clustering on the pairwise correlation values. This should allow inferring functional information from well-studied MAPK components to functionally uncharacterized ones. Statistical overrepresentation of specific gene ontology (GO) categories in the gene lists showing high expression correlation values with each of the MAPK components predicted biological themes for the gene functions. The combination of these methods provides functional information for many

uncharacterized MAPK genes, and a framework for complementary future experimental dissection of the function of this complex family.

Publications:

Menges M, Dóczi R, Okrész L, Morandini P, Mizzi L, Soloviev M, Murray JA, Bögre L. (2008) Comprehensive gene expression atlas for the Arabidopsis MAP kinase signalling pathways. New Phytol. 179(3):643-662

Narendra Tuteja (International Centre for Genetic Engineering & Biotechnology (ICGEB). New Delhi. India.

Lectin receptor-like kinase and salt stress

The plant lectin receptor-like kinases (LecRLKs) are involved in various signaling pathways but their role in salinity stress tolerance has not heretofore been well described. Salinity stress negatively affects plant growth/productivity and threatens food security worldwide. Based on functional gene-mining assay, we have isolated 34 salinity tolerant genes out of one million Escherichia coli (SOLR) transformants containing pea cDNAs grown in 0.8 M NaCl. Sequence analysis of one of these revealed homology to LecRLK, which possesses N-myristilation and N-glycosylation sites thus corroborating the protein to be aglycoconjugate. The homology based computational modeling of the kinase domain suggested high degree of conservation with the protein already known to be stress responsive in plants. The NaCl tolerance provided by PsLecRLK to the above bacteria was further confirmed in E. coli (DH5α). In planta studies showed that the expression of PsLecRLK cDNA was significantly upregulated in response to NaCl as compared to K^+ and Li^+ ions, suggesting the Na^+ ion specific response. Transcript of the PsLecRLK gene accumulates mainly in roots and shoots. The purified 47 kDa recombinant PsLecRLK-KD (kinase domain) protein has been shown to phosphorylate general substrates like MBP and casein. This study not only suggests the conservation of the cellular response to high salinity stress across prokaryotes and plant kingdom but also provides impetus to develop novel concepts for better understanding of mechanism of stress tolerance in bacteria and plants. It also opens up new avenues for studying practical aspects of plant salinity tolerance for enhanced agricultural productivity.

In the context of **WG 4**, a review on polyamines and abiotic stress tolerance has been recently published (see below).

Publications

Joshi, A., Vaid, N., Dang, H. Q. and Tuteja, N. Pea lectin receptor-like kinase promotes high salinity stress tolerance in bacteria and expresses in response to stress in planta. Glycoconjugate J. 2010 (in press)

Gill, S. S. and Tuteja, N. Polyamines and abiotic stress tolerance in plants. Plant Signaling & Behaviour, 5(1): 25-33, 2010.

Teun Munnik and Christa Testerink (Swammerdam Institute for Life Sciences. Amsterdam, Netherlands.

Plant phospholipid signalling

It has been suggested for a long time that phosphatidic acid is involed in stress signaling. Progress had been made through genetic analysis. However, this genetic approach was limited. Now the group of T. Munnik has developed a a phosphatidylinositol biosensor. This work was published by Vermeer et al.

(2009). The implications of this development are discussed by Munnik and Testerink, 2009. The most interesting result is that a connection seems to be present between the biochemical signaling molecules like phosphatidic acid and cellular structures so that vesicle trafficking is affected. This work was also presented in two presentations at the latest INPAS meeting in Tartu. The results of the genetic analysis were published in joined publications of three members (**Bartels, Munnik, Testerink**): (Bargmann et al. 2009a,b).

Publications:

Vermeer, J.E.M.; Thole, J.M.; Goedhart, J.; Nielsen, E.; Munnik, T.; Gadella, T.W.J. (2009) Visualisation of PtdIns4P dynamics in living plant cells. Plant Journal 57: 356-372.

Munnik, T.; Testerink, C. (2009) Plant Phospholipid Signalling - 'in a nutshell'. Journal of Lipid Research, 50: S260-S265.

Bargmann, B.O.R.; Arisz, S.A.; Laxalt, A.M.; ter Riet, B.; van Schooten, B.; Merquiol, E.; Testerink, C.; Haring, M.A.; Bartels, D.; Munnik, T. (2009a) Multiple PLDs required for high salinity- and water deficit tolerance in plants. Plant Cell Physiology, 50: 78-89.

Bargmann, B.O.R.; Laxalt, A.M.; ter Riet, B.; Testerink, C.; Merquiol, E.; Mosblech, A.; Leon-Reyes, A.H.; Pieterse, C.M.; Haring, M.A.; Heilmann, I.; Bartels, D.; Munnik, T. (2009b) Reassessing the role of phospholipase D in the Arabidopsis wounding response. Plant Cell Environment, 32: 837-850.

Dorothea Bartels (University of Bonn. Bonn. Germany)

Molecular mechanisms in desiccation tolerant plants

The desiccation tolerant resurrection plant Craterostigma plantagineum encodes three classes of transketolase transcripts, which are distinguished by their gene structures and their expression patterns. One class, represented by tkt3, is constitutively expressed and two classes, represented by tkt7 and tkt10, are upregulated upon rehydration of desiccated C. plantagineum plants. The objective of this work was to characterize the differentially expressed transketolase isoforms with respect to subcellular localization and enzymatic activity. Using GFP fusion constructs and enzymatic activity assays, we demonstrate that C. plantagineum has novel forms of transketolase which localize not to the chloroplast, but mainly to the cytoplasm and which are distinct in the enzymatic properties from the transketolase enzymes active in the Calvin cycle or oxidative pentose phosphate pathway. A transketolase preparation from rehydrated leaves was able to synthesize the unusual C8 carbon sugar octulose when glucose-6-phosphate and hydroxy-pyruvate were used as acceptor and donor molecules in in vitro assays. This suggests that a transketolase catalyzed reaction is likely to be involved in the octulose biosynthesis in C. plantagineum (Willige et al. 2009).

In another study, the desiccation-tolerant plant Boea hygrometrica was used as a model system to investigate the changes in gene expression and cell wall adaptation that take place during extreme dehydration. A cDNA macroarry analysis of dehydration-inducible genes led to the identification of a gene encoding a glycine-rich protein (BhGRP1). The corresponding transcript was up-regulated during drying in B. hygrometrica leaves. In silico analysis revealed that BhGRP1 is targeted to the cell wall and this was confirmed in planta. Morphological changes in the cell wall architecture were also observed during the process of drying and re-watering. Concomitant with this observation, cell wall profiling by Fourier transform infrared spectroscopy indicated that protein levels increased upon desiccation and remained broadly similar upon re-watering. These findings suggest that the deposition of

BhGRP1 may play a role in cell wall maintenance and repair during dehydration and rehydration in B. hygrometrica (Wang et al. 2009).

Publications:

Willige BC, Kutzer M, Tebartz F, Bartels D. Subcellular localization and enzymatic properties of differentially expressed transketolase genes isolated from the desiccation tolerant resurrection plant Craterostigma plantagineum. (2009) Planta 229(3):659-666.

Wang L, Shang H, Liu Y, Zheng M, Wu R, Phillips J, Bartels D, Deng X. (2009) A role for a cell wall localized glycine-rich protein in dehydration and rehydration of the resurrection plant Boea hygrometrica. Plant Biol (Stuttg) 11(6):837-848.

Pedro L. Rodríguez (IBMCP-CSIC. Valencia. Spain).

Characterization of ABA receptor

Concerning the understanding of the identified ABA receptor major contributions have been made by P.L. Rodriguez who has identified components interacting with the receptor and demonstrated the complex with the abscisic acid substrate. Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs. The abscisic acid receptor PYR1 in complex with abscisic acid. This work indicates that the ABA receptor is cytosolic and nuclear molecule that activates ABA signalling. They propose that enhanced resistance to drought can be obtained through receptor-mediated inhibition of the downstream phosphatase.

Publications:

Santiago, J.; Rodrigues, A.; Saez, A.; Rubio, S.; Antoni, R.; Park, S.Y.; Dupeux, F.; Marquez, J.A.; Cutler, S.R. and Rodriguez, P.L. (2009) Modulation of drought resistance by the abscisic acid-receptor PYL5 through inhibition of clade A PP2Cs. Plant Journal 60: 575-588.

Santiago, J.; Dupeux, F.; Round, A.; Antoni, R.; Park, S-Y.; Jamin, M.; Cutler, S.R.; Rodriguez, P.L.; Marquez, J.A. (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. Nature, 462: 665-668.

Park, S.Y.; Fung, P.; Nishimura, N.; Jensen, D.R.; Fujii, H.; Zhao, Y.; Lumba, S.; Santiago, J.; Rodrigues, A.; Chow, T.S.; Alfred, S.E.; Bonetta, D.; Finkelstein, R.; Provart, N.J.; Desveaux, D.; Rodriguez, P.L.; McCourt, P.; Zhu, J.K., Schroeder, J.I.; Volkman, B.F.; Cutler, S.R. (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of ABA-binding START proteins. Science, 324: 1068-1071.

Claudia Jonak (Gregor Mendel Institute. Vienna. Austria)

A central role of abscisic acid in stress-regulated carbohydrate metabolism.

Abiotic stresses adversely affect plant growth and development. The hormone abscisic acid (ABA) plays a central role in the response and adaptation to environmental constraints. However, apart from the well established role of ABA in regulating gene expression programmes, little is known about its function in plant stress metabolism. PRINCIPAL FINDINGS: Using an integrative multiparallel approach of metabolome and transcriptome analyses, we studied the dynamic response of the model glyophyte Arabidopsis thaliana to ABA and high salt conditions. Our work shows that salt stress induces complex re-adjustment of carbohydrate metabolism and that ABA triggers the initial steps of carbon mobilisation. SIGNIFICANCE: These findings open new perspectives on how high salinity and ABA impact on central carbohydrate

metabolism and highlight the power of iterative combinatorial approaches of non-targeted and hypothesis-driven experiments in stress biology.

Publications

Kempa S, Krasensky J, Dal Santo S, Kopka J, Jonak C (2008) A central role of abscisic acid in stress-regulated carbohydrate metabolism. PLoS One 3(12):e3935.

Julio Salinas (Departamento de Biología de Plantas, Centro de Investigaciones Biológicas, Madrid, Spain)

The role of CBF regulon in cold responses

The elucidation of freezing tolerance has been established by J. Salinas who also collaborated with **A. F. Tiburcio** and showed a link to polyamines which are the subject of WG 4, which led to a joined publication (Cuevas et al. 2009). Besides a highlight is the differentiation of the different regulatory CBF genes. This is important work, because CBF genes have been tested as candidates in transgenic plants for general stress tolerance improvement. Salinas and co-workers reported a specific function for CBF2 in cold stress (Novillo et al. 2007). Recent work has shown that prefoldins play and essential role in salt stress tolerance.

Publications:

Cuevas JC, López-Cobollo R, Alcázar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AF, Ferrando A. Putrescine as a signal to modulate the indispensable ABA increase under cold stress. Plant Signal Behav. 2009 4(3):219-220

Novillo F, Medina J, Salinas J. Arabidopsis CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon (2007) Proc Natl Acad Sci U S A. 2007 Dec 26;104(52):21002-21007

Rodriguez-Milla, M.A.; Salinas, J. (2009) Prefoldins 3 and 5 play an essential role in Arabidopsis tolerance to salt stress. Molecular Plant, 2: 526-534.

Bernd Wollenweber (University of Aarhus, Slagelse, Denmark)

The involvement of the transcription factor ABP9 in abiotic stress responses.

The effects of water deficits (WD), heat shock (HS), and both (HSWD) on photosynthetic carbon- and light-use efficiencies together with leaf ABA content, pigment composition and expressions of stress- and light harvesting-responsive genes were investigated in *ABP9* [ABA-responsive-element (ABRE) binding protein 9] transgenic *Arabidopsis* (5P2). WD, HS, and HSWD significantly decreased photosynthetic rate (*A*) and stomatal conductance (g_s) in wild-type plants (WT). *A* and g_s of 5P2 transgenic plants were slightly reduced by a single stress and were hardly modified by HSWD. Although *A* and electron transport rate (*ETR*) in 5P2 plants were depressed under optimal growth conditions (control) in relation to WT, they were enhanced under HS and HSWD. These results indicate that *ABP9* transgenic plants are less susceptible to stress than the WT. In addition, the increased ABA contents in both WT and 5P2 plants in response to WD and/or HS stresses suggest that declines in *A* and g_s might have been due to ABA-induced stomatal closure. Moreover, compared with WT, 5P2 plants exhibited higher ABA content, instantaneous water use efficiency (*IWUE*), Chl *a*/*b*, *NPQ*, and lower Chl/carotenoid ratios. Finally, altered expression of stress-regulated or light

harvesting-responsive genes was observed. Collectively, our results indicate that constitutive expression of *ABP9* improves the photosynthetic capacity of plants under stress by adjusting photosynthetic pigment composition, dissipating excess light energy, and elevating carbon-use efficiency as well as increasing ABA content, IWUE, and expression of stress-defensive genes, suggesting an important role of *ABP9* in the regulation of plant photosynthesis under stress.

Publications:

Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J. (2008) Water deficits and heat shock effects on photosynthesis of a transgenic Arabidopsis thaliana constitutively expressing ABP9, a bZIP transcription factor. J Exp Bot. 59:839-848.

Rina Iannacone (Metapontum Agrobios, Metaponto Italy)

The role of the transcription factor ATHB7 in abiotic stress tolerance

Drought stress trials were performed on transgenic tomato plants, overexpressing the ATHB7 gene. Plants were exposed to drought stress (no irrigation) for eight days. During the stress trial the content of chlorophylls, carotenoids and xanthophylls (violaxanthin, neoxanthin) and ABA were analysed. Furthermore the number and size of stomata on transgenic and WT tomato leaves was scored.

Results: the amount of all pigments decreased under stress both in transgenic lines and isogenic tomato but the amount of chlorophylls and carotenoids is significantly lower in ATHB7 lines while no significative difference is observed in well watered plants. The ABA content in ATHB7 plants was increased under stress. The observation of stomata on the abaxial side of tomato leaves revealed a lower number of stomata in ATHB7 plants. Furthermore the stomatal aperture was larger in WT plants compared to transgenics in non-stress conditions. The performance of ATHB7 transgenic tomato under drought stress was also studied using the 3D scanalyser platform. Plant phenotype was monitored under visible light and under NIR (Near Infrared). ATHB7 plants showed a faster and complete recover after 1 day of irrigation while WT plants are still wilt and most plants did not recover at all. Moreover the data obtained from the scanalyser platform revealed that the available water in transgenic plants is significantly higher after recovery compared to wild type UC82b. In conclusion ATHB7 plants showed a remarkable tolerance to drought stress compared to WT tomato. ABA levels and number and shape of stomata accounts for a reduction of gas exchange in ATHB7 tomato.

In collaboration with **Kumud Bandhu Mishra** (The Academy of Sciences of the Czech Republic, Institute of Systems Biology and Ecology) a recently developed chlorophyll fluorescence imaging tool have been tested to evaluate drought stress tolerance in tomato through for non-invasive sensing of chlorophyll fluorescence. Preliminary results showed, that plants that over expressed the transcription factor ATHB7 were tolerant to drought-stress and differed in their chlorophyll fluorescence.

In collaboration with **AF Tiburcio**, genes coding for Arginine decarboxylase 1 and 2 (ADC1 and ADC2) were inserted into tomato genome. Several tomato lines containing ADC1 or ADC2 coding sequences were obtained. The presence of the gene, number of copies and the level of expression of the inserted genes were assessed by molecular analyses. Plants will be tested for

their ability to cope with abiotic stress (drought, cold, salt) in the next few months.

Publication:

Deng X, Phillips J, Bräutigam A, Engström P, Johannesson H, Ouwerkerk PB, Ruberti I, Salinas J, Vera P, Iannacone R, Meijer AH, Bartels D. (2006) A homeodomain leucine zipper gene from Craterostigma plantagineum regulates abscisic acid responsive gene expression and physiological responses. Plant Mol Biol. 61(3):469-489

Progress Report WG3 December 2008 –December 2009

WG3 is mainly concentrated to dissect stress-associated signalling cascades that control the accumulation of osmoprotectans and other protective compounds. During 2008-2009, members of WG3 have focused their studies on signalling pathways that induce changes in metabolite profiles including osmolyte accumulation during abiotic stress imposition. All the involved groups have used molecular genetics, physiological and metabolomic approaches along with the development of systems biology strategies relying on mathematical models and bioinformatic analyses. Since each group's study includes various aspects that together converge into a full description of the explored system, this part of the report is organized as a collection of abstracts that summarize the achievements of the groups.
All the groups presented their studies in the annual meeting held in Tartu, Estonia in May 2009. Many scientific collaborations have been established among the groups during the reporting period using STSM fellowships and other sources. Examples of relevant work are described below:

Aviah Zilberstein (Tel Aviv University, Tel Aviv, Israel)
The mitochondrial-cytosolic proline/P5C cycle in plants
We have recently identified the existence of proline/P5C cycle in plants. Free proline is accumulated during the imposition of abiotic and biotic stresses in plants. During stress proline synthesis is enhanced and its breakdown is slowed down. Proline (Pro) two-step-oxidation in all eukaryotes is performed in the inner mitochondrial membrane by the consecutive action of proline dehydrogenase (ProDH) that produces Delta^1-pyrroline-5-carboxylate (P5C) and P5C dehydrogenase (P5CDH) that oxidizes P5C to glutamate (Glu). This catabolic route is silenced in plants during osmotic stresses, allowing free Pro accumulation. Our results show that overexpression of an ectopic ProDH in tobacco and *Arabidopsis* or impairment of P5C oxidation in the *Arabidopsis p5cdh* mutant did not change the cellular Pro to P5C ratio under ambient and osmotic stress conditions, indicating that P5C excess was reduced back to Pro in a mitochondrial-cytosolic cycle. This cycle, involving ProDH and P5C reductase, exists in animal cells and now unraveled in plants. As a part of the cycle operation, Pro oxidation by the ProDH-FAD-linked complex delivers electrons to the mitochondrial electron transport chain. Hyper-activity of the cycle, e.g. when an excess of exogenous L-Pro is provided, generates mitochondrial ROS by delivering electrons to O_2, as was evident by specific MitoSox staining of mitochondrial superoxide ions. The stain has been used to specifically identify mitochondrial ROS in animal cells and is here applied for the first time in plants. In the absence of P5CDH activity, Pro excess led to

higher ROS production under dark and light conditions that also affected the nuclear membrane integrity.
ProDH1 is silenced during stress and up-regulated during the consecutive recovery period, but the mechanism of this silencing is unknown. The results obtained by A. Zilberstein show that G-box consensus sequences in the *ProDH* promoter region are involved in its downregulation. A STMS has been performed between A. Zilberstein and S. Cohen in order to identify regulatory proteins that interact with *cis* elements of the *ProDH1* promoter region to unravel their involvement in the stress signalling cascades. To identify genetic determinants of cold tolerance, genomic interactions in various accessions of *Arabidopsis thaliana* were investigated by **A. Heyer** in a screening for combining ability in the heterosis of cold tolerance. Genetic diversity for cold-tolerance in *Arabidopsis* was also used for setting up a QTL mapping project. A collection of 400 recombinant inbred lines (RIL) was established and genotyped, and QTL for frost tolerance are currently analysed. The RIL population is also available for other members of the action. The group has started a collaboration with **K. Mishra** to evaluate fluorescence based methods in the screening of plant cold tolerance.

Publications:

Miller G, Honig A, Stein H, Suzuki N, Mittler R, Zilberstein A. (2009) Unraveling delta1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. J Biol Chem. 284:26482-26492.

Arnd G. Heyer (University of Stuttgart, Stuttgart, Germany)

Aspects of cold stress tolerance and mathematical models for analyzing changes in metabolite profiles.

In 2008, the group of A.G. Heyer has worked primarily on the role of soluble sugars in cold and drought tolerance. The occurrence of trehalose as a protectant of cell integrity and the structural requirements for fructans as cryoprotective compatible solutes were studied. It turned out that there is a size optimum for fructan oligo saccharides to protect membranes against freezing damage, and synergistic effects can be obtained for molecules of different size classes. In a more general approach to identify genetic determinants of cold tolerance, genomic interactions in various accessions of *Arabidopsis thaliana* were investigated in a screening for combining ability in the heterosis of cold tolerance. Genetic diversity for cold-tolerance in *Arabidopsis* was also used for setting up a QTL mapping project in this important model plant. A collection of 400 recombinant inbred lines (RIL) was established and genotyped, and QTL for frost tolerance are currently analysed. The RIL population is also available for other members of the action. Currently, the role of reactive oxygen species in damage during low temperature exposure is a focus of the work. In this area, the group has started a collaboration with **Kumud Bandhu Mishra** from the Academy of Sciences of the Czech Republic, Institute of Systems Biology and Ecology. A formal agreement on a co-operation was reached, and Prof. Heyer visited the institute in Nove Hrady (Czech Republic) for lecturing and experimental work in July. Dr. Mishra stayed for one week in Stuttgart (Germany) in October to evaluate fluorescence based methods in the screening of plant cold tolerance.

The very promising work will be continued, and joined publications are planned for 2009.
Heyer's group has developed a mathematical modeling approach to emulate plant primary metabolism over diurnal cycles. The response to low temperatures of various natural *Arabidopsis* accessions was estimated using the same approach.

Publications:

Livingston DP 3rd, Hincha DK, Heyer AG. (2009) Fructan and its relationship to abiotic stress tolerance in plants. Cell Mol Life Sci. 66:2007-2023.

Korn M, Peterek S, Mock HP, Heyer AG, Hincha DK. (2008) Heterosis in the freezing tolerance, and sugar and flavonoid contents of crosses between Arabidopsis thaliana accessions of widely varying freezing tolerance. Plant Cell Environ. 31:813-827.

Gad Galili (The Weizmann Institute, Rehovot, Israel)
Principal transcriptional programs regulating plant metabolism in response to abiotic stresses

Amino acid metabolism is among the most important and best recognized networks within biological systems. In plants, amino acids serve multiple functions associated with growth and response to abiotic and biotic stresses. Besides their function in protein synthesis, the amino acids also serve as precursors for multiple metabolic networks, including the production of numerous secondary metabolites, which are important for the response to various stresses. In addition, amino acid metabolism may also cross interact with various genome-wide gene expression programs to coordinate metabolism with physiological response associated with growth and response to the various stresses.
Group of Gad Galili has recently developed a new bioinformatics tool adapted for: (i) analyzing the response of *Arabidopsis thaliana* genes controlling plant metabolism to abiotic stresses; and (ii) identifying novel regulatory genes controlling the operation of the stress-associated metabolism. Using this new approach to analyze publicly available microarray datasets, we have recently identified novel expression coordination patterns between gene modules controlling the operation to central amino acid metabolic networks to various abiotic stresses. We have also further developed this bioinformatics tool to elucidate the transcriptional response of genes encoding the entire set of Arabidopsis metabolic enzymes to the various stress conditions. The „Gene Coordination" tool is used to decipher the cross interaction of genes associated with amino acid metabolism with genome-wide gene expression programs (Less & Galili, 2008).

Publication:

Less, H and Galili, G. (2008) Coordinations between gene modules control the operation of plant amino acid metabolic networks. BMC Syst Biol. 2009 Jan 26; 3:14.

Arnould Savouré (Pierre et Marie Curie University, Paris, France)
Characterization of the signalling pathways involved in the regulation of proline metabolism in both Arabidopsis and Thellungiella
In order to survive and to continue to grow upon environmental changes, plants have developed adaptive strategies. Among them, proline accumulation is one of the most frequently observed responses. Important

knowledge on stress tolerance and stress signalling has been obtained from the plant model *A. thaliana* because of its excellent genetic resources. However this plant is a true glycophyte, this feature limiting the outcome of such a model in plant water stress studies. Over recent years *T. halophila*, a close relative of *A. thaliana,* has attracted growing interest as a model for research in plant salt stress tolerance. *T. halophila* is considered as an extremophile because of its high capacities to tolerate salt and cold stresses. This species shows constitutive elevated transcripts of *A. thaliana* paralogous stress genes and metabolites, which suggests that *T. halophila* is anticipating the stress.

In the last couple of years, we have identified some key lipid signaling elements involved in the tight regulation of the biosynthesis and the catabolism of the compatible solute proline in Arabidopsis thaliana (Thiery et al, J Biol Chem, 2004; Parre et al., Plant physiol., 2007). We addressed the role of these lipid signalling pathways in the halophyte *Thellungiella halophila*. Indeed this plant was described as anticipating the stress with, for example, constitutive higher levels of metabolites such as proline than in *A. thaliana*. The 1-butanol, which diverts part of the PLD-derived PA by transphosphatidylation, has no effect on proline accumulation in non-stress conditions. On the other hand, inhibition of PI-PLCs by the commonly employed specific inhibitor U73122 demonstrated a negative control by PI-PLCs of proline accumulation in non-stress conditions and upon moderate salt stress (200 mM NaCl). At high salt concentration (400 mM NaCl) or upon 400 mM mannitol stress, PLDs exert a positive control on proline accumulation. In conclusion, we provide experimental evidence that positive and negative regulators are involved in the fine regulation of proline metabolism upon water stress. Our study has defined a critical role of lipid signalling pathways in proline accumulation in *T. halophila.* Regulation of these signalling pathways are opposite to those described in *A. thaliana,* which may partially explained the high stress tolerance of this species. In 2009, we further investigated this question to biochemically validate the pharmacological approach that we have previously undertaken. Biochemical and molecular data suggest that the regulation of the proline metabolism in *T. halophila* is different to what was previously described in *A. thaliana*. We believe that the comparison of these two species bring new knowledge in the field of plant stress adaptation and, more generally, of stress signalling cascade.

Publications:

Ghars MA, Parre E, Debez A, Bordenave M, Richard L, Leport L, Bouchereau A, Savouré A, Abdelly C. (2008) Comparative salt tolerance analysis between Arabidopsis thaliana and Thellungiella halophila, with special emphasis on $K^{(+)}/Na^{(+)}$ selectivity and proline accumulation. J Plant Physiol. 165:588-599.

Szabados L, Savoré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci (doi:10.1016/j.tplants.2009.11.009, in press).

László Szabados (Biological Research Center, Szeged, Hungary

Identification of stress-associated regulatory factors that control ABA signalling and proline accumulation

In order to identify genes controlling stress responses and ABA signaling, we have created the conditional overexpression system (COS), which is based on a cDNA library in an estradiol-inducible plant expression vector. Using

large-scale Agrobacterium-mediated transformation, we have generated a transgenic Arabidopsis plant population. Screening for ABA insensitive germination a number of Arabidopsis genes have been identified whose overexpression conferred salt tolerant germination or ABA insensitivity and altered proline accumulation to transgenic plants. The Conditional ABA Insensitive (CAI1) gene encodes a previously unknown zinc finger protein and regulates ABA signalling and proline accumulation upon stress. In order to identify interacting partners of the newly identified CAI1, the Ph.D. student of L. Szabados, M.P. Joseph has visited the partner laboratory of **C. Koncz** in the MPI, Cologne. This purpose of the STSM was the training of M.P. Joseph in isolation and purification of plant nuclear proteins. Several CAI1 interacting partner proteins have been identified. Verification of the interaction of CAI1 and some of the identified proteins is in progress. Collaboration between the group of C. Koncz (MPI, Cologne) and L. Szabados (BRC, Szeged) will continue to characterize the function of CAI1 and several other newly identified genes which control abiotic stress responses and proline accumulation in Arabidopsis.
Collaboration was established with **A. Savouré** to characterize genetic determinants of salt tolerance in extremophiles such as Thellungiella halophila. Special attention is paid to identify regulatory elements which control osmolyte accumulation in salt tolerant and sensitive plants.

Publications:

Papdi Cs, Ábrahám E, Joseph MP, Popescu C, Koncz Cs, Szabados L (2008) Functional identification of Arabidopsis stress regulatory genes using the Controlled cDNA Overexpression System, COS. Plant Physiol. 147: 528–542.

Papdi Cs, Joseph MP, Pérez-Salamó I, Vidal S, Szabados, L (2009) Genetic technologies for the identification of plant genes controlling environmental stress responses. Funct Plant Biol 36:696-720.

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci (doi:10.1016/j.tplants.2009.11.009, in press).

Tihana Teklic (University of J. J. Strossmayer, Osijek, Croatia)

Evaluation of drought and heat tolerance and proline accumulation in field experiments of maize and strawberries

We have performed field experiments with maize hybrids at several localities in Croatia in 2008, where we took pollen, silk and leaf samples at silking stage and determined proline content in plant tissues as well as the activity of P5CS and PDH in leaves. We have the same hybrids at the same localities in this year and we shall repeat these analyses in order to see if agroecological conditions had an influence on the proline synthesis in maize at this developmental stage, when drought and high temperature may restrict grain yield. Plant samples shall be also analyzed by DAF staining and confocal microscopy, in order to investigate the possible role of NO signaling in proline synthesis. This is the cooperation between my faculty and Rudjer Boskovic Institute in Croatia, and the University of the West of England in Bristol, UK (Prof. J.T.Hancock). Thanks to by British Scholarship Trust, my assistant should spend two months in Bristol at the end of this year performing the experiments with Arabidopsis in abiotic stress conditions, where proline and NO signaling should be an important issues.
Secondly, we have the experiments with strawberries, where we investigate the efficacy of biostimulator's treatment on plant resistance to nutrient supply

(mainly nitrogen and potassium in hydroponics conditions) and subsequent salt stress, as well as the environmental conditions in the field (air temperature etc.). Some of the applied biostimulators contain proline, and we shall analyze the effect of these treatments on fruit yield and quality. Similar experiments are ongoing with tomato plantlets.

Alain Bouchereau (Université de Rennes, Rennes, France)
Metabolomic profiling of Arabidopsis and Thellungiella during osmotic stress
Bouchereau's group has compared metabolomic profiles of *Arabidopsis thaliana* and *Thellungiella halophila*. The latter is a highly salt-resistant plant. Differences in water and primary metabolite content were observed under normal and osmotic stress conditions.

Publications:

Lugan R, Niogret MF, Kervazo L, Larher FR, Kopka J, Bouchereau A. (2009) Metabolome and water status phenotyping of Arabidopsis under abiotic stress cues reveals new insight into ESK1 function. Plant Cell Environ. 32:95-108.

Jubault M, Hamon C, Gravot A, Lariagon C, Delourme R, Bouchereau A, Manzanares-Dauleux MJ. (2008) Differential regulation of root arginine catabolism and polyamine metabolism in clubroot-susceptible and partially resistant Arabidopsis genotypes. Plant Physiol. 146:2008-2019.

Ghars MA, Parre E, Debez A, Bordenave M, Richard L, Leport L, Bouchereau A, Savouré A, Abdelly C. (2008) Comparative salt tolerance analysis between Arabidopsis thaliana and Thellungiella halophila, with special emphasis on $K^{(+)}/Na^{(+)}$ selectivity and proline accumulation. J Plant Physiol. 165:588-599.

Kumud B Mishra (Institute of Systems Biology and Ecology, ASCR, Nove Hrady, Czech Republic)
Development of non-invasive methods for screening abiotic stress tolerance in plants
Our current focus is to develop non-invasive methods that could be used for screening of abiotic stress tolerance capacity of the genetically modified plants based on plants optical reporter signals e.g., chlorophyll fluorescence and reflectance. We developed an advanced statistical approach based on classifiers and feature selection methods for the analysis of multidimensional time-resolved chlorophyll fluorescence images. We demonstrated the capacity of the statistical approach for discriminating three species of the same family that was not possible to discriminate using classical fluorescence parameters [Journal of Fluorescence: 19 (5):905-913]. We initiated collaboration with a group of Dr. Rina Iannacone, Agrobios, Metapontum, Italy with the help of STSM program of our COST action. An experiment as a feasibility test to determine the possibility to use chlorophyll fluorescence technique for reveling drought resistance of wild types and tomato trasgenics over-expressing transcription factor ATHB7 was organised during Oct –Nov, 2008. **Dr. Rina Innacone and Dr. Francesco Cellini** were visited our laboratory in January 2009 to discuss the preliminary results of the experiment done at Agrobios and possible ways to extend our scientific cooperation. **Dr. Kumud B Mishra** presented the results of the experiment done at Agrobios in Estonia meeting. We plan to submit the article relate to the experiment in the proposed special issue of our COST consortia. Another scientific co-operation was established

with Prof. Dr. **Arnd G. Heyer**, University of Stuttgart, Germany who visited our laboratory in July 2008. We worked together for a week in October 2008 in Prof. Heyer laboratory to see possibility to use chlorophyll fluorescence for determining cold tolerance of the plants. We found an interesting result during the visit showing evidence for a role of raffinose in protecting the thylakoid membrane at low temperatures. Recently, Anamika Mishra (PhD Student) of the group was awarded with a STSM to work in Prof. Heyer laboratory to see a possibility to develop chlorophyll fluorescence for screening cold tolorence in Arabidopsis accessions.

Publications:

Malenovský Z, Mishra KB, Zemek F, Rascher U, Nedbal L.(2009) Scientific and technical challenges in remote sensing of plant canopy reflectance and fluorescence. J Exp Bot. 60:2987-3004.

Mishra A, Matous K, Mishra KB, Nedbal L. (2009) Towards discrimination of plant species by machine vision: advanced statistical analysis of chlorophyll fluorescence transients. J Fluoresc. 19:905-913.

Arie Altman – The Hebrew University of Jerusalem, Rehovot, Israel

SP1, a novel stress-associated protein, is sumoylated upon salt stress in Populus euphratica, and is consequently relocated from the cytosol to the plasma membrane and to the nucleus

Populus. tremula is salt sensitive whereas *Populus euphratica* is tolerant, as revealed by biomass, growth rate, osmotic potential and chlorophyll data. A 12.4 kDa novel protein (PeSP1), an ortholog of *Populus tremula* SP1 (Wang et al. 2002, Dgany et al. 2005), was identified and characterized in *Populus euphratica*, showing 70.5% similarity and 61% identity to SP1. PeSP1 is also a dodecamer homo-oligomeric protein (148 kDa), highly expressed in the plant, and shares the same biophysical characteristics with SP1. PeSP1(and SP1) were up-regulated upon NaCl stress in both the salt tolerant *P. euphratica* and the salt sensitive *P. tremula* plants; however the expression of PeSP1 in *P. euphratica* was higher than in *P. tremula* under non-stressed conditions. In situ sub-cellular immuno-gold localization of the corresponding orthologs in both P. tremula and P. euphratica leaves shows almost homogenous distribution throughout the cytosol under normal growth conditions. However, under salt stress, PeSP1 is localized to the plasma lemma and the nucleus of the tolerant *P. euphratica*, but no changes were observed in its cellular distribution in P. tremula. Additionally, we show that PeSP1 undergoes a post-translational modification upon stress, due to sumoylation at specific sites. Based on reported studies that SUMO conjugation is involved in altering protein function through changes in activity or cellular localization, we suggest that the present results may indicate a salt-induced sumoylation and redistribution of the protein in a salt tolerant Populus species but not in its related salt sensitive one.

In addition, we have performed a detailed metabolite profiling in 4 salt sensitive and tolerant plants (*Populus tremula* and *Populus euphratica* (both wild type), tomato (wild type and *betA*-trangenic lines), *Arabidopsis thaliana* (wild type and RNAi lines) and wild type *Thellungiella halophyla-salsuginea*)) in response to salt stress and recovery. GC-MS metabolite profiling revealed significant changes in the concentration and organ distribution of about 40 metabolites in response to salt stress in salt-tolerant vs. salt-sensitive plants,

in all 4 plant species mentioned above. The dynamics of metabolite content during the stress and upon recovery from stress was also investigated. We conclude that specific organ accumulation of several mono and oligosaccharides, organic acids, amino acids, osmoprotectants and signaling molecules contribute to plant adaptation to stress. Moreover, we suggest that stress-associated metabolites may in fact drive plant adaptation to abiotic stress.

Virgilija Gavelienė, Institute of Botany, Vilnius, Lithuania

Investigation of cold stress – hardening to wintering protective compounds – saccharides and proline of different winter oilseed rape varieties

We have performed small field experiments with different winter rape varieties in Lithuania in 2009. We investigated very early and middle resistant to wintering v. *Libea*, middle-early, resistant to wintering v. *Sunday* and middle-early and resistant v. *Valesca*. The aim of investigations was to estimate the project – physiological and biochemical peculiarities of winter rape in plants hardening - preparing to wintering period: we estimated the stress protective metabolites – mono-, di-saccharides and proline in root collum and inflorescense tissues in plants, in dynamic under the changes of thermo-photo induction. The obtained data of monosaccharides – glucose and fructose, disaccharides – sucrose and maltose accumulation intensively in plants acclimation - preparing to wintering period exhibit the priority of rape variety *Sunday*. At low positive temperature - rape hardening period the proline content increased in inflorescense and especially in root collum. The proline accumulation was more intensive in rape varieties *Valesca* and *Sunday*. The results of stress protective compounds – mono-, di-saccharides and proline accumulation – in plant tissues are important parameters for rape hardening – preparing to wintering and might be used for selection of winter rape varieties resistant to cold.

Progress Report WG4 December 2008 –December 2009

WG4 aims at integrated molecular analysis of the polyamine (PA) metabolic pathway in response to abiotic stress. Specific objectives of this WG include: (i) identification of molecular and metabolic markers for polyamine (PA)-mediated abiotic responses (ii) assessment of genetic and metabolic variability in various ecotypes and RILs of Arabidopsis regarding PA-responses to abiotic stress (iii) characterization of Arabidopsis PA metabolic loss- and gain-of-function mutants in response to abiotic stress, (iv) global transcriptome and metabolome analysis of loss- and gain-of-function mutants affected in PA metabolism in response to abiotic stress, and (v) physiological and biochemical characterization of PA metabolism in relevant crop plants with improved adaptation to abiotic stress. All the involved groups are working individually or in collaboration in the above mentioned topics and most of them have presented their work in our two previous INPAS Workshops. Several scientific collaborations have been established through STMS and/or other sources. Some examples of relevant work are described below:

Pedro Carrasco (Departamento de Bioquimica y Biologia Molecular, Universidad de Valencia. Valencia. Spain)

Analysis of molecular markers in different tomato cultivars exposed to ozone stress

Three differentially expressed cDNAs have been isolated from ozone treated tomato seedlings. Their level of expression after ozone exposure has been analysed in three tomato cultivars with different sensitivity to ozone (Nikita, Alisa Craig and Valenciano). These comparative analyses have been extended to a number of genes involved in antioxidative, wounding or pathogenesis responses, showing several differences among cultivars that could be related with their different sensitivity to ozone. Gene response to ozone was affected not only by the period and dose of ozone exposure (short time or chronic), but also by growth conditions (controlled growth chamber or field). Comparison of gene expression patterns puts on evidence the needing of validation in field of experiments performed with plants grown under controlled conditions. Our results suggest that changes in gene expression, observed after ozone treatment in field, are affected by additional factors related to environmental clues.

Publication

Marco F, Calvo E, Carrasco P, Sanz MJ. (2008) Analysis of molecular markers in three different tomato cultivars exposed to ozone stress. Plant Cell Rep. 27(1):197-207.

Antonio F. Tiburcio (Universitat de Barcelona. Barcelona. Spain)

Regulatory role of polyamines in abiotic stress tolerance

Polyamines have been found to correlate frequently with biotic and abiotic insults, and their functional involvement in the plant responses to several stresses has been shown genetically with both gain and loss of function mutations. In spite of a large body of physiological and genetic data, the mode of action for polyamines at the molecular level still remains elusive. We have recently performed a detailed integrated analysis of polyamine metabolism under cold stress by means of metabolic studies, quantitative gene expression analyses, and gene inactivations, to characterize in more detail the role of polyamines in response to low temperature. Our data show a unique accumulation profile for putrescine compared to other polyamines, with a progressive increase upon cold stress treatment coincident with a similar transcriptional up-regulation for the two arginine decarboxylase genes *ADC1* and *ADC2.* Loss of function mutants *adc1* and *adc2* display reduced freezing tolerance and alterations in ABA content and ABA-dependent signalling pathways under low temperature, compared to wild type plants. Phenotypical reverse complementation tests for both *adc* and ABA-defective mutants support our conclusion that putrescine modulates ABA biosynthesis at the transcriptional level in response to low temperature thus uncovering a novel mode of action for polyamines as regulators of hormone biosynthesis (Cuevas et. al. 2009). This work was made in collaboration between **4 INPAS groups**. (Tiburcio/Salinas/Koncz/Carrasco). Our group has also shown that over-expression of ADC genes in Arabidopsis confers improved tolerance to drought (Alcázar et al. submitted) and freezing (Altabella T., Tiburcio AF, Ferrando A. Spanish patent application).

Publications

Cuevas JC, López-Cobollo R, Alcázar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AF, Ferrando A. Putrescine as a signal to modulate the indispensable ABA increase under cold stress. Plant Signal Behav. 2009 4(3):219-220

Alcázar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas JC, Vitrian M, Tiburcio AF, and Altabella T (2010) Putrescine accumulation confers drought tolerance in transgenic Arabidopsis plants overexpressing the homologous Arginine decarboxylase 2 gene, Plant Physiology and Biochemistry (special issues "polyamines" in memory of Prof. Nello Bagni). In press

Rubén Alcázar, Teresa Altabella, Francisco Marco, Cristina Bortolotti, Matthieu Reymond, Csaba Koncz Pedro Carrasco, Antonio F. Tiburcio (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta. In press.

Ana M. Fortes (ICAT, BioFIG, Science Faculty, University of Lisbon, Portugal)

The analyses of transcript and metabolic responses during organogenic nodule development in hop indicate the involvement of polyamines and auxins

An integrated molecular and metabolomic approach was used to investigate global gene expression and metabolic responses during development of hop's organogenic nodules in order to gather knowledge on the mechanisms underlying reprogramming of cells through stress and hormone treatments. Hop (*Humulus lupulus* L.) is an economically important plant forming organogenic nodules which can be used for genetic transformation and micropropagation. Transcript profiling using a 3,324-cDNA clone array revealed differential regulation of 133 unigenes, classified into 11 functional categories. Several pathways seem to be determinant in organogenic nodule formation, namely defense and stress response, sugar and lipid metabolism, synthesis of secondary metabolites and hormone signaling. Metabolic profiling using 1H NMR spectroscopy associated to two-dimensional techniques showed the importance of metabolites related to oxidative stress response, lipid and sugar metabolism and secondary metabolism in organogenic nodule formation.

The expression profile of genes pivotal for energy metabolism, together with metabolites profile, suggested that these morphogenic structures gain energy through a heterotrophic, transport-dependent and sugar-degrading anaerobic metabolism. Polyamines and auxins are likely to be involved in the regulation of expression of many genes related to organogenic nodule formation. These results represent substantial progress toward a better understanding of this complex developmental program and reveal novel information regarding morphogenesis in plants.

Publications:

Ana M Fortes, Filipa Santos, Young H Choi, Marta S Silva, Andreia Figueiredo, Lisete Sousa, Fernando Pessoa, Bartolomeu A Santos, Mónica Sebastiana, Klaus Palme, Rui Malhó, Rob Verpoorte and Maria S Pais (2008). *BMC Genomics* 2008, 9: 445 .

Milena Cvikrová (Institute of Experimental Botany, ASCR v.v.i., Prague, Czech Republic)

(i) Polyamine profiles and biosynthesis in Norway spruce somatic embryos through the desiccation phase

Desiccation of zygotic embryos is, in most seeds, the terminal event in their development. In somatic embryos desiccation for three weeks may represent a sort of an osmotic stress. The biosynthetic enzyme activity in mature cotyledonary embryos of Norway spruce declined through the desiccation phase, accompanied by sharp reductions in polyamine contents (with the exception of spermine). Especially the decrease in cellular putrescine contents in embryos through the desiccation phase resulted in a shift in the spermidine/putrescine (Spd/Put) ratio from ca. 2 in early cotyledonary embryos to around 10 after three weeks of desiccation. During the desiccation period the spermine (Spm) level significantly increased. The accumulation of high levels of PAs in somatic embryos might contribute to their “reserves”, consisting predominately of proteins and triglycerides, which are utilized during their germination. Another important aspect that should be considered concerns the involvement of PAs in the synthesis of nitric oxide (NO), which is known to act as a signalling molecule in plant cells. On the other hand, the significantly higher total content of PAs in somatic embryos (two-fold and three-fold higher levels of Spd and Spm, respectively) than in zygotic embryos might be responsible for their lower germinability.

(ii) Heat stress response in tobacco plants

Tobacco plants (wild-type and transgenics constitutively over-expressing proline biosynthetic gene) were used. Proline over-production was associated with faster stress response and defence initiation. Initial phase of heat stress (2 h at 40°C) coincided with an increase in free spermidine, norspermidine and cadaverine, which correlated with a stimulation of the activity of biosynthetic enzymes. After 6 h of heat stress, decrease in putrescine, spermidine and norspermidine coincided with down-regulation of the activity of PA biosynthetic enzymes and stimulation of diamine oxidase (DAO). No effect on malondialdehyde (MDA), product of lipid peroxidation, was observed within 6 h of heat stress.

Publication

Gemperlová L., Fischerová L., Cvikrová M., Malá J., Vondráková Z., Martincová O., Vágner M. (2009) Polyamine profiles and biosynthesis in somatic embryo development and comparison of germinating somatic and zygotic embryos of Norway spruce. Tree Physiol. 29(10), 1287-1298.

Oscar A. Ruiz (Universidad Nacional de San Martin (UNSAM). Chascomus. Argentina)

Polyamine oxidase in response to salt

The possible involvement of apoplastic reactive oxygen species produced by the oxidation of free polyamines in the leaf growth of salinized maize has been studied here. Salt treatment increased the apoplastic spermine and spermidine levels, mainly in the leaf blade elongation zone. The total activity of polyamine oxidase was up to 20-fold higher than that of the copper-containing amine oxidase. Measurements of H(2)O(2), *O(2)(-), and HO* production in the presence or absence of the polyamine oxidase inhibitors 1,19-bis-(ethylamine)-5,10,15 triazanonadecane and 1,8-diamino-octane suggest that, in salinized plants, the oxidation of free apoplastic polyamines by polyamine oxidase by would be the main source of reactive oxygen

species in the elongation zone of maize leaf blades. This effect is probably due to increased substrate availability. Incubation with 200 microM spermine doubled segment elongation, whereas the addition of 1,19-bis-(ethylamine)-5,10,15 triazanonadecane and 1,8-diamino-octane to 200 microM spermine attenuated and reversed the last effect, respectively. Similarly, the addition of MnCl(2) (an *O(2)(-) dismutating agent) or the HO* scavenger sodium benzoate along with spermine, annulled the elongating effect of the polyamine on the salinized segments. As a whole, the results obtained here demonstrated that, under salinity, polyamine oxidase activity provides a significant production of reactive oxygen species in the apoplast which contributes to 25-30% of the maize leaf blade elongation.

Publication:
Rodríguez AA, Maiale SJ, Menéndez AB, Ruiz OA. (2009) Polyamine oxidase activity contributes to sustain maize leaf elongation under saline stress. J Exp Bot. 60(15):4249-4262.

Riccardo Angelini (University "Roma Tre". Rome. Italy) and **Kalliopi Roubelakis-Angelakis** (University of Crete, Heraklion, Greece)

Characterization of polyamine and diamine oxidases

The until now best characterised plant polyamine oxidases (PAO), such as the apoplastic maize PAO (ZmPAO), are involved in the terminal catabolism of spermine (Spm) and spermidine (Spd), conversely to the animal PAOs which oxidise Spm and Spd through a polyamine back-conversion pathway. In Arabidopsis thaliana, five PAO genes (AtPAO1-5) are present with a varying sequence homology to ZmPAO and subcellular localization (cytosolic or peroxisomal). Sequence analysis indicated that AtPAO2-4 derivate from a common ancestor and biochemical characterization of recombinant AtPAO1, AtPAO2 and AtPAO4 showed that these enzymes oxidise the common polyamines Spd and Spm and the stress-related uncommon polyamines norspermine and thermospermine through a back-conversion pathway. AtPAOprom::GUS transgenic Arabidopsis plants for AtPAO1, AtPAO2 (representative member of the AtPAO2-4 subfamily) and AtPAO5 were analysed and data suggest a distinct tissue-specific expression pattern for each AtPAO. Inducible expression following abscisic acid treatment was also evidenced.

In another study, the analysis of physiological roles of AtCuAOs has been approached through the phenotypic analysis of CuAO insertional mutants of the model organism Arabidopsis thaliana. In Arabidopsis a CuAO gene (At4g14940) was already identified in 1998. The product of this gene, designated as AtCuAO1 (formerly ATAO1), has been demonstrated to be localized in the apoplast and to posses amine oxidase activity. This gene is expressed during early stages of vascular tissue development as well as in lateral root cap cells and its expression is induced by nematodes invasion. A BLAST analysis with the At4g14940 sequence as query sequence has led to identify ten putative CuAO genes. Prediction of sub-cellular localization of these ten proteins performed with P-sort tool revealed four proteins (including AtCuAO1) with predicted extracellular localization. In order to investigate the physiological roles of the four putative extra-cellular CuAOs, the genotypic and phenotypic analysis of the respective insertional mutant has been performed. First, insertional mutant lines for each studied CuAO gene

have been identified in TAIR (The Arabidopsis Information Resource) database. Omozigous mutant lines have obtained through PCR analyses for three genes, because of technical problems with one mutant line. Macroscopic analysis of atcuao1 plant phenotype didn't reveal any evident differences between atcuao1 and wild type plants. Analysis of AtCuAO1 promoter sequence in Plant CARE database has revealed the presence of hormone responsive elements (abscissic acid, auxin, methyl-jasmonate, giberellin). According to microarray data, obtained from ARABIDOPSIS eFP Browser, AtCuAO1 expression is induced by methyl-jasmonate, but not by abscisic acid (ABA), auxin and giberellin.

Publication:

Angelini. R, Cona A., Federico R, Fincato P., Tavladoraki P., Tisi A. (2010) Plant amine oxidases "on the move": an update. Submitted to Plant Physiology and Biochemistry (special issues "polyamines" in memory of Prof. Nello Bagni)

Tomonobu Kusano (Tohoku University. Japan)

Spermine signalling pathway in plant defense responses

We have proposed that the polyamine spermine (Spm) functions as a signaling molecule to evoke defense reactions/cell death in avirulent pathogen-attacked tobacco plants. To understand its molecular basis in depth, Spm-responsive genes in Arabidopsis thaliana were identified by SuperSAGE analysis. Close to 90% of the Spm-responsive genes also responded during cucumber mosaic virus (CMV)-elicited hypersensitive response. Spm modulated the expression of genes of redox components, and genes involved in protein folding and secretion, protein degradation and defense. Two other prominent changes, the coordinately enhanced expression of members of the photorespiration pathway and a diversion in electron flow from the primary electron transfer chain of respiration to an alternative oxidase pathway, occurred in response to Spm. Spm activated the expression of 6 transcription factor genes including ZAT7, ZAT12, AtWRKY40 and AtbZIP60, of which the former three genes' products are currently assigned as components of H_2O_2 signaling pathway, suggesting the involvement of H_2O_2 in Spm-triggered responses. Since AtbZIP60 plays a proven master role in the unfolded protein response in Arabidopsis thaliana, it may function to control the expression of genes participating in protein folding and secretion, which were mentioned above. Spm induction and CMV-triggered up-regulation of the genes described mainly coincided and their induction was suppressed by inhibitors of Spm oxidation. Furthermore, treatment with those inhibitors prior to CMV inoculation allowed higher viral multiplication in Arabidopsis thaliana plants. These results support the existence of a Spm-signaling pathway in Arabidopsis thaliana and its significant role in defense against CMV.

Publication

Mitsuya Y, Takahashi Y, Berberich T, Miyazaki A, Matsumura H, Takahashi H, Terauchi R, Kusano T. (2009) Spermine signaling plays a significant role in the defense response of Arabidopsis thaliana to cucumber mosaic virus. J Plant Physiol. 166(6):626-643

It should be pointed out that this report is just a summary of WG contributions.

Some more relevant work is listed in our **publications (see list of publications 2009 in INPAS website)**. We consider that **the scientific objectives original proposed for this second year of the Action have been successfully fulfilled**. As indicated in the next Section, the number of countries and participants have significantly increased, so the scientific task force of the Action is much bigger than it was initially expected.