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Oral Communication Abstract – 2C.01

HYDRAULIC SYSTEMS AND SUGAR TRANSPORT IN PLANTS: AN ECOLOGICAL SCALING PERSPECTIVE

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Research on phloem transport has seen a resurgence in recent years, partly for the interest of this topic in the context of ecological research on the carbon transfer from plants to soil and partly because of recent methodological and conceptual advances.

I will highlight some of the most important underlying themes of these current research efforts with en amphasis on showing the integrative nature of xylem and phloem transport, which act as natural message conveyor belts between sources and sinks of water and carbon in the plant.

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Oral Communication Abstract – 2C.02

DETECTION OF MITOCHONDRIAL CA^{2+} DYNAMICS IN ARABIDOPSIS PLANTS EXPRESSING THE FRET-BASED CAMELEON PROBE

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Calcium homeostasis, plant mitochondria, genetically encoded Ca^{2+} specific probes, fluorescence resonance energy transfer (FRET)-based indicator, abiotic stress

Mitochondria represent a key organelle in plant cells being involved in many aspects of the plant life: normal cell metabolism, stress response and programmed cell death regulation. Despite the pronounced metabolic connection among mitochondria and the other cellular compartments, little is known about the signals that mediate this communication or about the coordination of the activities by signalling molecules. Many factors have been suggested to be involved in the retrograde signalling control, ranging from ROS, cellular carbohydrate status, the mETC reduction state and calcium. In this work we describe the unprecedented use of FRET-based mitochondria-targeted calcium specific probe Cameleon to produce stably transformed Arabidopsis plants that enable the analysis of mitochondrial Ca2+ dynamics in planta and reveal independent regulation of [Ca2+]m resulting from physiological or environmental stimuli. Moreover, by crossing plant expressing the Cameleon in the nucleus with the mitochondria-targeted one, we were able to monitor in vivo, at the same time, the Ca2+ dynamics in two different subcellular compartments.

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Oral Communication Abstract – 2C.03

EXPRESSION OF FUNGAL AND PLANT PHOSPHATE TRANSPORTERS IN ARBUSCULATED CELLS: A COMPETITION FOR PI UPTAKE?

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Mineral nutrition, phosphate uptake, arbuscular mycorrhizal symbiosis

Phosphorus (P) is an essential plant nutrient and is limiting for plant growth in most natural and agricultural ecosystems throughout the world. In an evolutionary context, the development of mutualistic interactions with arbuscular mycorrhizal fungi (AMF) is considered the most important adaptation of terrestrial plants to face mineral nutrition requirements (Bonfante and Genre, 2008). Although being a major benefit of the symbiosis, the molecular mechanisms underlying fungal-mediated uptake, translocation and assimilation of inorganic phosphate (Pi) from the soil to the colonized root cells of the plant remain poorly known. Current data suggest that Pi, taken up by the extraradical mycelium (ERM) from soil solutions through high-affinity Pi transporters (PT), is translocated along AM fungal hyphae as polyphosphate (poly-Pi), and after hydrolysis, in the arbuscule, Pi is exported from the AM fungus to the periarbuscular space where is taken up by plant cortical cells thanks to mycorrhiza-inducible PTs (Javot et al., 2007).

The aims of this study were to analyze the expression profile of the high-affinity PT gene (*GintPT*) of the AMF *Glomus sp. DAOM 197198* in different fungal structures (spores, ERM and arbuscules) and to investigate the influence of different environmental conditions on its expression level. Mycorrhizal roots of *Medicago truncatula* were exposed to low (32 mM) or high Pi concentrations (300 mM). After a morphological analysis of the mycorrhization level, we collected arbusculated cortical cells using the laser microdissection technology and we performed semi-quantitative RT-PCR experiments to monitor the expression profiles of both *GintPT* and the *M. truncatula* PT, *MtPT4*, which is known to be exclusively expressed in arbusculated cells. We also evaluated *GintPT* modulation in ERM grown in monoxenic solid culture over the interaction with non-host (*Arabidopsis thaliana*) plants by qRT-PCR assays. Our findings show that *GintPT* is constitutively expressed along all the steps of the fungal life cycle suggesting that P uptake is necessary for fungal viability and metabolism. Interestingly, changes in Pi concentrations in the nutrient solution do not lead to modulation of *GintPT* expression level in the arbuscules. By contrast, the plant *MtPT4* shows enhanced transcript levels at 300 μM Pi concentration. The findings open new scenarios to the current view of the Pi export from fungus towards plant at the arbuscule interface, since they suggest a competition for the Pi uptake between the two symbionts.

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Oral Communication Abstract – 2C.04

PCP1 AND ATOSA1: PLASTIDIAL PROTEINS INVOLVED IN OXIDATIVE STRESS RESPONSE AND METAL HOMEOSTASIS IN *ARABIDOPSIS* CHLOROPLASTS

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Activity-of-bc1-complex family, chloroplast, oxidative stress, metals, photosynthesis

The Activity-of-bc1-complex protein family was firstly characterized in *Saccharomyces cerevisiae,* in which the protein acts as a chaperone-like protein essential for the proper conformation and the efficient functioning of the mitochondrial *bc1* complex. In *Arabidopsis thaliana* 17 genes cluster as Activity-of-bc1-complex proteins, even though knowledge about their putative functions is still limited.

This work is focusing on the characterization of two proteins in *A. thaliana* belonging to this family, in order to understand their involvement in plant development or stress responses. The first, PCP1 (putative chloroplast protein 1) is encoded by an homolog of a gene of *Brassica juncea* modulated upon cadmium treatment. The second, AtOSA1 is an oxidative stress-related protein involved in plant response to stress generated by Cd^{2+}, hydrogen peroxide and excessive light. These proteins share 45% aminoacidic identity, and both hold the domain characteristic of the family and two transmembrane spans at the C-terminus. Under standard growth conditions, single mutants and the crossed-double mutant did not show morphological or developmental abnormalities, with the exception of the pale-green phenotype showed by *atosa1* and double mutants. This was supported by pigment analysis that revealed a reduced total chlorophyll content and an increased *chl a/b* ratio in these mutants. Since AtOSA1 is involved in plant response to oxidative stress, we tested the hydrogen peroxide effect on mutants. *pcp1* and *pcp1/atosa1* plants showed a reduced root length, suggesting that root growth in mutants is more sensitive to hydrogen peroxide than in WT.

The analysis of cellular localisation of FLAG-tagged AtOSA1 and PCP1 proteins revealed that both are localised in chloroplasts. To address whether photosynthesis might be affected in mutant plants, analysis of the photosynthetic machinery was performed by Western-Blot and electron transport in thylakoid membrane was investigated. No particular differences in protein composition in PSI and PSII were observed, but protein content of the *b6f* complex is severely reduced in mutants. Analysis of chlorophyll fluorescence showed that mutant and WT plants have similar maximum quantum yield, effective PSII quantum yield and excitation pressure (1-qP value). An increase in light intensity caused a NPQ induction in all genotypes, particularly marked in the *atosa1*mutant.

Due to the difference in chlorophyll content, mutant plants could be impaired in transport of metal ions that are required for photosynthetic apparatus building. We are currently measuring the content of metals in chloroplasts, comparing WT to mutants, with particular concern to the major metals involved in the chloroplast physiology and oxidative stress-counteracting mechanisms.

Plastidial co-localization and high sequence similarity suggest that AtOSA1 and PCP1 may have evolved functional redundancy. Their overlapping function is observed in root length,

analysing plant response to oxidative stress. Nevertheless, the two single mutants do not display similar pigment composition and are characterised by a different NPQ induction. The latter results suggest that either the two proteins do not share a complete functional redundancy or that their different expression level partially complement the lack of gene function of the single mutants.

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Oral Communication Abstract – 2C.05

EFFECT OF K^+ CHANNEL ACTIVITY ON THE OXIDATIVE PHOSPHORYLATION IN DURUM WHEAT MITOCHONDRIA FROM CONTROL AND HYPEROSMOTIC-STRESSED SEEDLINGS

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ATP synthesis, durum wheat mitochondria, membrane potential, K^+ channel, environmental stress

Durum wheat mitochondria (DWM) possess an ATP-inhibited K^+ channel, the Plant mitoK_{ATP} (PmitoK_{ATP}), that catalyses the electrophoretic uniport of K^+ through the inner mitochondrial membrane; the co-operation between PmitoK_{ATP} and the K^+/H^+ antiporter, very active in plant mitochondria, allows the operation of a K^+ cycle, whose capacity in DWM is so high to collapses membrane potential ($\Delta\Psi$), the major component of protonmotive force (Δp) in plant mitochondria. Under environmental stress conditions the decrease in $\Delta\Psi$ (and Δp) due to PmitoK_{ATP} activation may dampen the generation of reactive oxygen species (ROS) that is known to increase under these conditions. This suggests that the channel may play a role in the defence of cell from oxidative stress occurring when plants suffer adverse environmental conditions.

In order to establish whether activation under stress of PmitoK_{ATP} entails a loss of ATP synthesis, here, we investigated how this channel may affect oxidative phosphorylation (OXPHOS) in DWM purified from control seedlings and from seedlings subjected both to severe mannitol and NaCl stress.

ATP synthesis via OXPHOS (oligomycin sensitive) by succinate-oxidising DWM was followed in continuous, by using a spectrophotometric ATP-detecting system, in the absence or presence of KCl to activate the channel. As expected, severe osmotic and salt stress caused a decrease in the rate of ATP synthesis compared to the control condition. This result is in line with the decrease in the ATP content observed in seedling tissues as a consequence of the hyperosmotic stress imposition. When PmitoK_{ATP} was activated by KCl the ATP synthesis via OXPHOS was about 90% inhibited in severely stressed DWM. Contrarily, in control DWM, although PmitoK_{ATP} collapsed $\Delta\Psi$, ATP synthesis as well as coupling (RC and ADP/O ratios) checked by oxygen uptake experiments were found to be unaffected. Similar results were obtained using DWM from moderately mannitol stressed seedlings. Measurements of ΔpH and $\Delta\Psi$ demonstrate that no ΔpH increase occurs able to compensate the $\Delta\Psi$ decrease and maintain Δp and suggest that the driving force for ATP synthesis is a no-bulk phase Dp, retained by DWM as a consequence of braking of PmitoK_{ATP} by its inhibitor ATP.

We suggest that PmitoK_{ATP} may play an important defensive role at the onset of the environmental/oxidative stress by preserving energy in a crucial moment for cell and mitochondrial bioenergetics. Consistently, under moderate mannitol stress, miming an early stress condition, the channel may efficiently control ROS generation (about 35 fold from fully open to closed state)

without impairing ATP synthesis. Anyway, if the stress significantly proceeds, the $PmitoK_{ATP}$ becomes fully activated by decrease of ATP concentration (25-40%) and increase of activators (FFAs and superoxide anion), thus impairing ATP synthesis.

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Poster Communication Abstract – 2C.06

LEAF HYDRAULIC VULNERABILITY CORRELATES WITH DROUGHT RESISTANCE IN *ACER* AND *QUERCUS* SPECIES

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Water availability, vein density, leaf hydraulic conductance, gas exchange

Leaves represent the major hydraulic bottleneck in long distance water transport systems of terrestrial plants [1]. Previous studies have shown that leaf hydraulic evolution was among the major factors leading to high photosynthetic productivity of Angiosperms [2]. Indeed, gas exchange rates are correlated to leaf hydraulic conductance (k_{leaf}) across a wide range of vascular plants [3]. In turn, k_{leaf} has been shown to be correlated to structural traits like vein density and xylem conduit dimensions [4]. Leaves are the plant organ most vulnerable to drought-induced hydraulic dysfunction. Leaf water potential (Y_{leaf}) values between -1.0 and -2.5 MPa commonly induce 50% loss of leaf hydraulic conductance (P50) in broad-leaved plants. P50 has been shown to be not correlated to maximum k_{leaf} [5] suggesting that no trade-off exists between hydraulic efficiency and vulnerability at the leaf level. Recent studies have identified a number of leaf structural traits correlated to P50 across several vascular plants. The major limit of published studies resides in the very heterogeneous species' assemblages used to test leaf structural-functional relationships. In fact, trait correlations can be confounded by interactions between environmental adaptation and phylogenetic relationships between species. In order to test the adaptive value of leaf hydraulic and structural traits in closely related taxa, three *Acer* (*A. pseudoplatanus, A. campestre, A. monspessulanum*) and three *Quercus* (*Q. petraea, Q. pubescens, Q. ilex*) species were studied. Within each genus, the species selected represent increasing levels of adaptation to dry habitats. Leaf hydraulic capacity was measured using the rehydration kinetic technique over a wide range of initial leaf water potentials to assess water stress-induced changes in k_{leaf}. Minimum seasonal Y_{leaf} values were recorded in the field. The following leaf structural traits were also measured: specific leaf area, vein density, midrib xylem conduit diameter, mesophyll thickness, stomatal density. Overall, our data suggest that species from drought-prone habitats have lower maximum k_{leaf} and are more resistant to drought-induced xylem dysfunction. The possible structural bases of such differences and ecological consequences are highlighted and discussed in the poster.

REFERENCES

[1] Nardini A, Tyree MT. 1999. Root and shoot hydraulic conductance of seven *Quercus* species. *Annals of Forest Science* 56: 371-377.

[2] Brodribb TJ, Feild TS. 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters* 13: 175-183.

[3] Brodribb TJ, Holbrook NM, Zwieniecki MA, Palma B. 2005. Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *The New Phytologist* 165: 839-846.

[4] Nardini A, Gortan E, Salleo S. 2005. Hydraulic efficiency of the leaf venation system in sun- and shade-adapted species. *Functional Plant Biology* 32: 953-961.

[5] Blackman CJ, Brodribb TJ, Jordan GJ. 2010. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *The New Phytologist* 188: 1113-1123.

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Poster Communication Abstract – 2C.07

ION-MEDIATED REGULATION OF XYLEM HYDRAULICS IN THE GENERA *FRAXINUS* AND *ACER*: RELATIONSHIPS WITH XYLEM ANATOMY AND DROUGHT ADAPTATION

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Water availability, vessel grouping, ionic effect

Leaf gas exchange and photosynthetic rates are limited by plant hydraulic properties and in particular by xylem hydraulic conductance (K_{XYL}) [1]. Under drought stress conditions, cavitation-induced embolism can lead to significant reductions of K_{XYL}, consequent stomatal closure and impairment of carbon balance [2]. Plants can transiently up-regulate K_{XYL} by modulating xylem sap ionic concentration, a phenomenon known as 'ionic effect' [3]. In particular, it has been suggested that enhanced potassium (K^+) concentration might interfere with the negative charges of the pectic matrix at the interconduit pit membrane, thus inducing pectin shrinking and consequent increase of pit membrane pore dimensions and, finally, of K_{XYL}. In drought-stressed plants, up-regulation of residual K_{XYL} would partially compensate for embolism-induced loss of hydraulic efficiency [4]. The magnitude of the ionic effect is correlated to intervessel connectivity [5]. In particular, the ionic effect was found to be related to vessel grouping index. Interestingly, high vessel grouping is an anatomical trait more commonly observed in drought-adapted taxa [6]. Hence, it can be hypothesized that high vessel grouping in drought-adapted plants translates into high ionic effect and consequent possibility for plants to compensate for drought-induced embolism and maintain positive net carbon exchange under stressful conditions. We tested this hypothesis in *Acer* and *Fraxinus* species adapted to different levels of aridity. In the genus *Acer*, the ionic effect was higher in the xerophylous than in the mesophylous species, and the magnitude of ion-mediated enhancement of K_{XYL} was positively correlated to vessel grouping index. In the genus *Fraxinus*, vessel grouping was higher in the xerophylous species than in the mesophylous one, with no apparent relationship to the magnitude of ionic effect. Overall, our data offer only partial support to the hypothesis that high vessel grouping is a trait conferring higher drought resistance to woody plants through enhanced potential for ion-mediated regulation of K_{XYL}.

REFERENCES

[1] Tyree MT. 2003. Hydraulic limits on tree performance: transpiration, carbon gain and growth of trees. *Trees* 17: 95-100

[2] Nardini A, Lo Gullo MA, Salleo S. 2011. Refilling embolized xylem conduits: is it a matter of phloem unloading? *Plant Science* 180: 604-611

[3] Zwieniecki MA, Melcher PJ, Holbrook NM. 2001. Hydrogel control of xylem hydraulic resistance in plants. *Science* 291: 1059-1062

[4] Trifilò P, Lo Gullo MA, Salleo S, Callea K, Nardini A. 2008. Xylem embolism alleviated by ion-mediated increase in hydraulic conductivity of functional xylem: insights from field measurements. *Tree Physiology* 28, 1505-1512

[5] Jansen S, Gortan E, Lens F, Lo Gullo MA, Salleo S, Scholz A, Stein A, Trifilò P, Nardini A. 2011. Do quantitative vessel and pit characters account for ion-mediated changes in the hydraulic conductance of angiosperm xylem? *New Phytologist* 189, 218-228

[6] Carlquist S. 2009. Non-random vessel distribution in woods: patterns, modes, diversity, correlations. Aliso 27: 39-58

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Poster Communication Abstract – 2C.08

PHLOEM COOLING AS A TOOL FOR INHIBITING XYLEM REFILLING AFTER CAVITATION IN LAUREL

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A robust although circumstantial evidence shows that xylem recovery from cavitation-induced embolism relies on phloem activity. In particular, xylem-associated cells undergo starch depolymerization resulting in sugar transport into air-filled vessels thus drawing water into them. Overall, these cells act as strong sinks to phloem inducing a continuous radial mass flow. The exact role of phloem is, however, matter of debate. The present work represents an attempt at blocking phloem radial unloading without causing wounding as girdling which can *in se* introduce further variables. Phloem was at least partly blocked through cooling stems up to about 6°C. Polypropilene glycol sealing bags were used and stems were maintained at low temperature for 20 min. Both well-watered and water stressed plants were tested for loss of xylem hydraulic conductivity (PLC) using perfusion solutions mimicking native potassium concentrations in xylem sap as measured preliminarly. Other plants were induced to cavitate using a pressure collar applied to 1-year-old stems and then tested for short-term refilling 2 and 20 min after pressure release. Stems of some of these plants were cooled as above for 20 min after pressurization and then tested for PLC as above. To test starch depolymerization of xylem associated-cells and ray cells, the percentage of cells with high starch content was counted under microscope. Our data show that xylem recovery from cavitation really is phloem-dependent in hat stem cooling reduced the drop in PLC without influencing starch depolymerization.

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Poster Communication Abstract – 2C.09

MISTLETOES AND ALBINO LEAVES AS TERMINAL SINKS FOR ELEMENTS NORMALLY RECYCLED BETWEEN XYLEM AND PHLOEM

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Mistletoe, albino leaves, mineral nutrients, phloem structure

Conspicuous physiological similarities between hemiparasitic mistletoes and albino shoots were first demonstrated for the water relations of white orange shoots by Lo Gullo *et al*. (Tree Physiology, 27, 219-217, 2007). Our presentation extends the evidence to specifics of mineral nutrition by a comparison between leaves of an Asiatic mistletoe (*Scurrula elata* Edgew. Danser) on four hosts (*Rhododrendron arboreum* Sm, *Lyonia ovalifolia* (Wall.) Drude, *Lindera pulcherrima* (Nees) Benth. ex Hook.f. and *Viburnum erubescens* Wall.*)* from different families, and white shoots of orange (*Citrus sinensis* L.) and oleander (*Nerium oleander* L.) on otherwise normal green trees.

Leaves of *Scurrula* and its hosts, as well as white and green leaves of *Citrus* and *Nerium* were collected in the field and analyzed for cations, sulfur and nitrogen with standard laboratory techniques.

Mistletoes and albino shoots had increased contents of potassium, phosphorus and copper when compared to host leaves or green leaves. The phloem of albino leaves shows anatomical characteristics of release phloem as defined by van Bel (Plant, Cell & Environment**,** 26, 125- 149, 2003).

The common denominator for these two otherwise very different systems is the inability to export via the phloem carbohydrates from the leaves of the hemiparasite into the host stems or from white into green shoots. Hemiparasites have no phloem connection with their hosts, while white leaves cannot load ions into the phloem because of a lack of sugars indispensable for co-transport across membranes.

The findings are examples of a "terminal sink phenomenon", an obviously general tendency toward the accumulation of ions normally recycled between xylem and phloem, in organs not capable to re-export them via the phloem. Other examples can be found among holoparasitic plants as well as in a number of edible bulbs, tubers, and fruits.

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Poster Communication Abstract – 2C.10

PRELIMINARY CHARACTERIZATION OF THE *LOTUS JAPONICUS* NITRATE TRANSPORTER GENE FAMILIES

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Transport, signalling, N metabolism, symbiosis

Nitrate availability may strongly affect the plant developmental programs and in particular the root architecture. Nitrate can act either as a nutrient by affecting systemically the plant nutritional status through its assimilation process, and as a signal by affecting locally root development through specific signaling pathways. Leguminous plants are capable to perform a unique symbiotic interaction with soil bacteria of the genus rhizobia having as final goal the fixation of the atmospheric nitrogen (N). N fixation occurs into a new root organ, the nodule, where bacteria find a perfect environment to catalyze the N reduction to ammonia. The nodule organogenesis program is triggered by bacteria compounds, the Nodulation factors (Nod factors) secreted in the rhizosphera in response to excreted plant flavonoids. High nitrate concentration is known to strongly inhibit the nodule organogenesis program and the signaling pathway involved in the long distance process governing this type of control has been only recently started to be elucidated.

In higher plants, two types of nitrate transporters NRT1 and NRT2, have been identified. In Arabidopsis there are 53 *NRT1* genes and 7 *NRT2* genes. A complete characterization has been performed only for a few members of the nitrate transporter families and in the case of AtNRT1.1 and AtNRT2.1, an involvement in either uptake and signalling functions have been reported. We started a molecular characterization of the NRT1 and NRT2 gene families in the model legume *Lotus japonicus*. The observed response in terms of gene expression to different biotic and abiotic factors allowed a discrimination between different gene categories that may be involved in different functions.

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Poster Communication Abstract – 2C.11

THE *LOTUS JAPONICUS* PII PROTEIN IS INVOLVED IN A DROUGHT RESPONSE SIGNALLING PATHWAY CONTROLLING THE STOMATA MOVEMENT

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Signalling, N metabolism, stress response

PII in prokaryotic organisms is a crucial integrator of cellular carbon, nitrogen and energy levels. In higher plants, however, its role remains significantly less clear. Previous findings indicate that PII–N-acetylglutamate kinase (NAGK) complex formation controls l-arginine biosynthesis, whereas other work implicates PII in regulating chloroplastic nitrite uptake. Furthermore, a recent report reveals a seed tissue-specific expression of PII in higher plants, associated to a role in the tuning of fatty acid biosynthesis and partitioning in seeds. Together, these findings indicate that PII has evolved from a central metabolic role in prokaryotes towards a more specialized role in eukaryotes. Here we report the molecular characterization of the *L. japonicus* PII protein with a exaustive analysis of the gene expression and protein localization in different tissues and organs, in response to different plant treatments. Furthermore we provide evidences that PII may play a role in the plant response to drought conditions by regulating the stomata movement in *L. japonicus* leaves. Lotus transgenic plants over-expressing the PII protein show a reduction of the stomata aperture in epidermal peels in hydric stress conditions with a consequent lower rate of water loss in detached leaves. Possible mechanisms and signalling pathways underlying this type of response will be discussed.

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Poster Communication Abstract – 2C.12

INTERACTION STUDIES OF DIFFERENT *ARABIDOPSIS* 14-3-3 ISOFORMS WITH THE PLASMA MEMBRANE H^+-ATPASE

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14-3-3 protein, H^+-ATPase, Arabidopsis thaliana, protein-protein interaction

The eukaryotic regulatory proteins 14-3-3 are involved in many important plant cellular processes including regulation of electrochemical gradient across the plasma membrane through the stimulation of H^+-ATPase activity. In both animals and plants, 14-3-3 proteins are present as multiple isoforms whose overall amino acid sequence is highly conserved. In *Arabidopsis thaliana* 12 isoforms are known to be expressed and, based on phylogenetic analysis, they are divided in two groups: epsilon and non-epsilon group. So far it is not fully clarified whether different isoforms may accomplish different functions. To verify a possible specificity of 14-3-3 isoforms towards the H^+-ATPase, two Arabidopsis isoforms, GF14omega and GF14epsilon, characterized by a highly sequence divergence at their C-terminal domain, have been assayed for the ability to interact with the H^+-ATPase. Results demonstrate that GF14omega has a higher affinity towards the enzyme than GF14epsilon and it is more active also in stimulating the H^+-ATPase activity. Interaction studies performed with other 14-3-3 target proteins confirmed the higher binding ability of GF14omega. In order to understand whether the observed different binding ability of GF14omega and epsilon was limited to these isoforms or was instead shared by all isoforms belonging to epsilon and non-epsilon group, other Arabidopsis 14-3-3 isoforms were expressed and tested in binding assays with the H^+-ATPase.

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Poster Communication Abstract – 2C.13

INORGANIC ARSENIC SPECIATION ANALYSIS IN DIFFERENT TOMATO CULTIVARS (*SOLANUM LYCOPERSICUM* L.) AND INFLUENCE OF SILICON (SI) EXPOSURE

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Arsenic, tomato, crop safety, silicon

Elevated arsenic in soils raises concern regarding plant uptake and entry into wildlife and human food chains. Arsenic in water and agricultural land has arisen from application of pesticides. Silicon is added to tomato plants to improve water stress resistance. In this work we have evaluated the effect of As (III) and As(V), with or without Si, on the germination of eight cultivars of *Solanum lycopersicum* L. We have determined the number of seeds germinated and root lengths to find the most resistant cultivar and the more toxic species of arsenic and how Si affected germination and growth. All the tomato cultivars were also grown in garden soil for three months, then supplemented with As species, with or without Si. After 2 weeks they were harvested and fresh biomass was measured and As content was determined. The cultivars showed a remarkably different behavior towards the treatments; in general As uptake is low, so is the translocation with few exceptions. In a further experiment, As was supplemented (with or without Si) to the plants at the fruit production stage. The ripened fruits were collected after three months and the content of As in tomato fruits was measured.

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Poster Communication Abstract – 2C.14

HAS THE "IONIC EFFECT" A ROLE IN PLANT SALT TOLERANCE?

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Salt tolerance, tomato, ionic effect

Recent studies have shown that xylem hydraulic conductance (Kx) is enhanced by increase in xylem sap cation concentration. This phenomenon (hereafter named 'ionic effect') would be caused by shrinking of the pectic matrix in the intervessel pit membranes and consequent increase in the dimensions of pores in them. The ionic effect has been described in different species and seems to play important functional roles in *planta* like compensation for Kx loss induced by xylem embolism and modulation of water delivery to branches exposed to different light conditions. This study reports experimental evidence of the potential role of the ionic effect on plant resistance to salinity

Measurements were performed in plants of *Solanum lycopersicum* L. cv Naomi, a moderate salt-tolerant species. Plants were either grown in half-strength Hoagland hydroponic solution (Control plants, C) or in the same solution enriched with 35mM NaCl (NaCl- plants).

Salt treatment induced increase of leaf succulence as well as of fruit dry mass whilst leaf conductance to water vapour (g_L) and evapotranspiration rate (E_L) were similar for both growth conditions.

Potassium and sodium concentrations as recorded in the xylem sap were higher in plants subjected to salt treatment thus suggesting that increase in external [Na+] favoured K+ uptake. An increase of hydraulic conductance (DKh) of about 10% was recorded in shoots of C-plants when perfused either with 25 mM NaCl or with 25 mM KCl solutions. Shoots of NaCl-plants when perfused with the above solutions gave substantially higher DKh values with respect to control plants. Accordingly, Kx was higher in NaCl- than in C-plants using the evaporative flux method.

In conclusion, tomato plants grown under high salinity seemed to improve water transport to the leaves through Na^+-mediated enhancement of stem hydraulic conductance.

Preliminary observations under microscope suggest that the ionic effect recorded in NaCl-treated plants resulted in increased volume of leaf cells. This may facilitate Na^+ segregation in vacuoles with the final consequence of better tolerance of salt stress conditions.

Present findings would confirm that the ionic effect has potentially important functional implications for plant interaction with environmental factors.

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Poster Communication Abstract – 2C.15

EFFECT OF UREA ON GENE EXPRESSION AND ACTIVITY OF ENZYMES INVOLVED IN NITROGEN METABOLISM IN MAIZE SEEDLINGS

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Nitrogen metabolism, urea, nitrate reductase, glutamine sinthetase, Zea mays

Urea is one of the three forms widely used for nitrogen soil fertilization in agriculture. Even if urea in the soil is degraded to ammonium and CO_2, an appreciable amount could still be present and would represent an alternative source of nitrogen. Indeed, the transport of urea through the plasma membrane in *Arabidopsis* has been recently described (Kojima et al., *Plant Journal*, **52**, 30-40, 2007). Once urea enters the plant, it could follow two main pathways, being directly metabolized in the root or translocated to the epigeal part. Therefore, in this work we examined the effects of different form of nitrogen supply, including urea, on two enzymes of the soluble cellular fractions involved in nitrogen metabolism, namely nitrate reductase (NR) and glutamine synthetase (GS), in roots and leaves of maize. Five-day old seedlings were transferred for 24h in growing media under different nitrogen supply: control (no N); nitrate; nitrate+ammonium; urea; urea+nitrate. *Real time* RT-PCR experiments showed that expression levels of gene coding for NR2 and GS2 enzyme isoforms in roots were transiently induced by treatments with nitrate and urea+nitrate. Furthermore, root exposition to urea+nitrate showed higher transcripts levels and a faster response with respect to the sole nitrate treatment. The enzymatic activity of GS was not significantly modified, both in roots and leaves in all nitrogen supply treatments. Nevertheless, the activity of NR was increased in all nitrogen treatments, but not in the presence of urea, that was even lower than the control. Remarkably, after both 8 and 24h incubation, the plantlets showed a significant increase of NR activity in the samples grown in the presence of urea+nitrate. Results of the present work indicate that the contemporary supply of nitrate and urea to maize seedlings might favour nitrogen assimilation through an enhancement of gene transcription and activity of key enzymes.

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