

P19.11**The effects of heavy metals exposition on the activity of recombinant plant plastidic glucose 6P dehydrogenase**

A. De Lillo, M. Cardi, M. Lentini, S. Esposito
Dept. Biology, Univ. Federico II of Napoli, Italy

Heavy metals (HMs) play an important role in plant metabolism: some are essential micronutrients, but their excess may become highly toxic to plants., HMs toxicity causes changes in cell structure and functionality, alterations of key metabolic processes, exchanges in enzymatic cofactors, and generation of free radicals. Furthermore, enzyme inhibition is always considered an important biomarker of response., Our study is focused on heavy metals effects on the activity of plastidic Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49), the key enzyme of the oxidative pentose phosphate pathway., This reaction provides the reductants for many metabolic processes in plant cells., These effects were analyzed by measuring the activity variations on recombinant P2-G6PDH isoform from *Populus trichocarpa* exposed to different levels of different heavy metals (Ni, Cd, Pb, Cu, Zn)., Ni and Cd caused a marked decrease in PtP2G6PDH activity, whereas Pb was almost ineffective. Interestingly, both copper and zinc exposition resulted in a strongest decrease in enzyme activity., These results are discussed in order to define a biochemical mechanism of inhibition of plant G6PDH by inorganic cations.

P19.12**Identification and molecular characterization of the sucrose synthase 2 gene in durum wheat**

I. Fanizza¹, Lr. Ceci², G. Galante², B. Gattulli², C. Leoni¹, A. Gadaleta³, D. Nigro³, G. Mangini³, A. Blanco³, M. Volpicella¹

¹*Department of Biosciences, Biotechnology and Biofarmaceutic, University of Bari "A. Moro", Via Amendola 162/A, 70126 Bari (Italy)*

²*Institute of Biomembranes and Bioenergetic – CNR, Research Division Bari, ³Department of Soil, Plant and Food Sciences Section Genetics and Plant Breeding, "A. Moro", Via G. Amendola 165/A - 70126 - Bari, Italy*

Starch in the wheat endosperm is the major storage reserve of proteins, lipids and carbohydrates. The sucrose synthase (SUS) enzyme controls the flow of carbon into starch biosynthesis, catalyzing the first reaction in the conversion of sucrose to starch. The availability of genetic maps and phenotypic data of segregating population allows to map important genes, and to identify closely associated molecular markers to be used in MAS and positional cloning. The strategy adopted to identify the *Sus2* gene in the genomes of the durum wheat cvs. Ciccio and Svevo is showed. In order to obtain the complete sequence of the *Sus2* gene, a bioinformatic analysis starting from the cDNA sequence of *Sus2* gene in *Triticum aestivum* was performed. Specific primer combinations were designed and used in specific PCR reactions on the genomic DNA from leaf tissue of Ciccio and Svevo. The entire genomic structure of the *Sus2* genes were determined, and several SNPs, INDELs and variants were found in the comparison of the two homeologous alleles. A recombinant inbred lines developed from a cross the Svevo and Ciccio durum wheat cultivars will be used to genetic map the *Sus2* gene.

P19.13**Evolutionary role of a vacuolar metal transporter for hypertolerance/hyperaccumulation in *Arabidopsis halleri***

E. Fasani, G. DalCorso, A. Furini
Dept. Biotechnology, Univ. of Verona, Verona, Italy

VMT is a tonoplast metal transporter participating in vacuolar sequestration that is involved in metal hyperaccumulation/hypertolerance. The gene is constitutively expressed in metal hyperaccumulators, as *Arabidopsis halleri*, with higher transcript levels than in the corresponding non-accumulator species. In *A. halleri*, three different promoter sequences were identified for *VMT*, suggesting the presence of multiple gene copies, while a single copy is present in *Arabidopsis thaliana*. *VMT*

promoter activity was compared in *A. thaliana* and *A. halleri* by GUS assay. All promoters are active in roots and guard cells, but *A. halleri* members drive GUS expression also in leaf mesophyll and trichomes. *In silico* analysis highlights, in the 5'UTR of the *A. halleri* promoters, a dimer of MYB-binding motifs, which is mutated in a single nucleotide in the *A. thaliana* sequence. Promoter mutation analysis indicates that this motif is likely involved in trichome-specific expression. The high *VMT* transcription levels observed in trichomes of *A. halleri*, counteracted by its absence in *A. thaliana* trichomes, suggest a putative evolutionary role of VMT in the hypertolerance/hyperaccumulation trait.

P19.14**Proline metabolism in salt-shocked versus salt-adapted rice seedlings**

M. Bertazzini, R. Nansi Tchapnda, M. Zarattini, G. Forlani

Dept of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy

Proline accumulation was investigated in rice (*Oryza sativa* L. cv Loto) seedlings grown in the presence of increasing concentrations of salt. A remarkable difference was found between salt-adapted and salt-shocked plants. In seedlings grown under normo-osmotic conditions proline levels rapidly raised following the treatment with NaCl. However, also the level of other free amino acids increased likewise, and the percentual content of proline varied only slightly. In contrast, under the same conditions tobacco seedlings showed a specific increase of free proline. On the contrary, in rice seedlings directly sown in the presence of inhibitory concentrations of salt the homeostatic level of most amino acids was maintained, with the only exception of proline and asparagine. In this case, proline percent values increased significantly. Results suggest that, contrary to other plant species, proline accumulation in rice is not a rapid mechanism for osmotic adjustment, but may represent a long-term adaptation to cope with the effects of excess salt in the environment. This work was supported by AGER Foundation in the frame of the Risinnova project, grant # 2010-2369.

P19.15**Peroxydase and polyphenoloxidase activities and isoforms distribution in fruits of sweet cherry from Campania Region (Italy)**

A. Mirto, F. Iannuzzi, P. Woodrow, L. Ciarmiello, P. Carillo, A. Fuggi

Dept. Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Caserta, Italy

Peroxidases and polyphenoloxidases play key roles in plant physiologic process, like lignin biosynthesis, responses to biotic and abiotic stresses, fruit ripening. Such enzymes are of main concern along the food supply chain, causing the deterioration of organoleptic properties of fruit and vegetable foods. Peroxidases and polyphenoloxidases occurred in many isoforms, that have been used also as genetic markers in the identification of ecotypes and cultivars. In the aim of conservation and valorization of the agrobiodiversity, in this study peroxidases and polyphenoloxidases isoforms, have been characterized in fruits of sweet cherry landraces of Campania Region. The enzyme preparation were obtained from ripened cherry fruits harvested at commercial maturity. Soluble and bound peroxidases and polyphenoloxidases activities were measured in partially purified enzyme preparations. Peroxidase activities were about 10 times higher than polyphenoloxidase ones. They changed depending on the ecotype and on the phenolic substrate. Acidic, neutral and basic peroxidase isoforms were found, differently distributed among the ecotypes. Peroxidase enzymes from different landraces showed also different sensitivity to thermal denaturation. Financial support was obtained by "Regione Campania (Italy), PSR 2007/2013, Misura 214, Azione f2, progetto Agrigenet".