

FISV - Federazione Italiana Scienze della Vita

Program and Abstracts of the XIV FISV CONGRESS

Sapienza University of Rome, Italy

September 20 - 23, 2016

Disclaimer

This abstract book has been produced using author-supplied copy through the website *fisv2016.azuleon.org*.
Editing has been restricted to some corrections of spelling and style.

CONTENTS

Welcome Letter	4
Member Societies	5
Committees - Secretariat	6
Session Organisers	7
Map	8
Programme Overview	9
PROGRAMME	10
ABSTRACTS	
Plenary Lecture	29
Plenary Symposium	30
PS1 - Cancer Stem Cells	30
PS2 - CRISPR/CAS: from a prokaryotic immune system to a powerful tool for biomedical and agricultural research	31
PS3 - Oxygen Sensing and Redox Signalling: common themes of aerobic life	32
PS4 - Systems Biology: from Genetic Networks to Organismal Functions	33
PS5 - New roles and molecular pathophysiology of mitochondria	34
The EMBO Keynote Lecture	35
Parallel Symposia	36
S1 - The (recent) evolution of human evolution	36
S2 - From Reverse- to Structural-Vaccinology and beyond. Current challenges against infectious diseases	37
S3 - Nitrogen: Nutritious and Noxious	38
S4 - Shaping the Cancer Genome: from pathways to mutational signatures	39
S5 - Unfolding truth: making sense of intrinsically disordered proteins	40
S6 - Plant adaptation and phenotypic plasticity to climate change	41
Poster and Selected Short Talks	42
1 - Environmental Microbiology and Biotechnology	42
2 - Genomics, Proteomics and Systems Biology	50
3 - Chromosome Biology, Cell Division and Cell Cycle	57
4 - Epigenetics and Epigenetic Therapies	60
5 - Oncogenes and Tumor suppressors	64
6 - Plant Metabolism and Environmental Stress	70
7 - Genetics of Microorganisms	78
8 - Transcription Mechanisms and Networks	81
9 - DNA replication, Repair and Recombination	83
10 - Non-coding RNA	87
11 - Environmental and Molecular Mutagenesis	89
12 - Plant Nutrition	91
13 - Cellular Stress, apoptosis and autophagy	94
14 - Development, Differentiation and Aging	98
15 - Metabolism and its regulation in health and diseases	100
16 - Human Genetics and Genomic Diversity	106
17 - Neurobiology	112
18 - Immunology and Host-Pathogen Interaction	114
19 - Protein Synthesis, Degradation and Homeostasis	117
20 - Stem Cells, iPS, Cancer Stem Cells	119
21 - Nutrition Biochemistry	122
22 - Evolution	125
23 - Cell Communication, Cell Adhesion and Membrane Trafficking	127
24 - Plant Development and Disease	129
Author Index	135

treated mutants. *AtCuAOδ* over-expressing plants showed an increase in stomatal closure level along with a detectable H_2O_2 production in guard cells under normal growth conditions. These data suggest that *AtCuAOδ* may play a role as H_2O_2 source in ABA-induced stomatal closure.

P6.6

Population traits shape the elevation effect on non-structural carbohydrates (NSC) and flavonoids of *Vaccinium myrtillus* stands in Alpine tundra

V. Casolo, F. Boscutti, E. Petrusa, A. Filippi, E. Buffon, M. Zancani, E. Braidot

Environmental stressors induce plants to acclimate by both morphological and physiological modifications. Along an elevation gradient (as a proxy for temperature stress), we demonstrated that changes of two ecophysiological stress indicators (NSC in underground stems and leaf flavonoids) of *Vaccinium myrtillus* were affected not only by altitude, which, in some cases, acts as secondary player. In particular, plant traits (age, width of xylem rings, length of stem shoot) of the populations and interspecific competition (shrub density) can shape the effect of elevation. Glucose content was positively correlated with altitude, but negatively with competition. Instead, sucrose decreased at high altitude and in older populations. Starch content increased along with ring width and decreased with high shrub density. Flavonoid content was mainly related to elevation and plant trait of the population. NSC and flavonoids exhibit different patterns with respect to elevation and plant traits. In conclusion, we suggest that bulk NSC can not be considered as indicators of stress and that plant traits would represent modulators of species response.

P6.7

Expression Analysis of Stress-Related genes involved in the response of Durum wheat to salinity and high light

L. F. Ciarmiello, P. Woodrow, A. Mirto, F. Conte, V. S. De Lucia, E. Dell'Aversana, L. D'Amelia, L. De Simone, A. Fuggi, P. Carillo
Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche (DISTABIF)- Seconda Università degli Studi di Napoli (SUN)- Caserta, Italia

The study of stress-related genes is critical to understand the molecular mechanisms of stress tolerance in plants. Several studies have demonstrated the important role of asparagine synthetase, glutamate decarboxylase and Δ^1 -pyrroline-5-carboxylate synthase genes signalling in response to environmental stresses. In order to investigate the expression changes of these genes in durum wheat under salinity and high light, a semi-quantitative RT-PCR analysis was performed. High light increased the gene expression level of *TdAsn1* alone (2.6 fold) and in combination with salinity (2 fold) in comparison with the control at low light. The isoforms *TdAsn2* was expressed at low levels compared to *TdAsn1* and the transcript was present only in leaves in control conditions or simultaneous stresses. A trend similar to that of *TdAsn1* was observed for *P5CS* expression. On the contrary, *GAD* expression was decreased by salinity and high light (1.1 and 1.5-fold, respectively) and even more under the two combined stresses (3.7 fold) compared to control. The expression levels were compared to the respective enzymatic activities. Our expression data confirmed the pivotal role of the studied genes in the response to abiotic stresses in durum wheat.

P6.8

Metabolic engineering of the phenylpropanoid pathway in *S. lycopersicum* using CRISPR/Cas9 mediated genome editing

F. D'Orso^{1,2}, T. Lawrenson¹, W. Harwood¹, Y. Zhang¹, J. Li¹, L. Tomlinson³, G. Morelli², C. Martin¹

¹John Innes Centre, Norwich Research Park, Colney, Norwich, UK, ²Food and Nutrition Research Centre, Council for Agricultural Research and Economics, Rome, Italy, ³The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

CRISPR/Cas9 system is a powerful tool enabling efficient and precise genome editing in many organisms and with many applications in several fields, including metabolic engineering. In plants one of the most important secondary metabolic pathways is the phenylpropanoid pathway that produces many nutraceutical compounds. To modify this pathway, we edited the *SIHQT* gene involved in the biosynthesis of caffeoylquinic acids (CQAs), the most abundant polyphenols in many plant species. We induced mutagenesis of the *SIHQT* gene at two different positions directing the cas9 cut in the *SIHQT* genomic sequence. Undertaking full genotypic analysis of a large number of T0 transformed tomato plants, we observed a very high mutation frequency but also considerable variability in terms of the number of alleles and types of mutations in any one plant; this variability correlated very well with the CQA accumulation. Here we show that the CRISPR/cas9 system can be used successfully for metabolic engineering of the phenylpropanoid pathway affecting the caffeoylquinic acid biosynthesis and possibly altering the production of other phenolic compounds with potential impacts on nutritional value.

P6.9

Subcellular localization analyses of a *Hordeum vulgare* P2-G6PDH isoform by transient expression of reporter fusion proteins

A. De Lillo¹, M.C. Lutterbey², H. Lansing², A. von Schaewen², V. Paradisone¹, S. Esposito¹

¹Dept. of Biology – University of Napoli “Federico II” – Naples, (Italy), ²Institute for Plant Biology and Biotechnology (IBBP) – Münster, (Germany)

In plants different glucose 6-phosphate dehydrogenases (G6PDH) exist. Among them, plastidic P2 isoforms are considered an essential source of NADPH in heterotrophic plastids. Barley P2-G6PDH displays an unusually long plastidic transit peptide (>95aa), considering these sequences generally comprise less than 60aa. We investigated the subcellular localization of *HvP2-G6PDH* reporter fusions in *Arabidopsis* protoplasts and tobacco leaves; specifically, whether this protein may be directed to heterotrophic plastids only, or to other compartments as well. The results obtained in protoplasts suggest that *HvP2-G6PDH* with GFP fused to the C-terminus may localize to chloroplasts. However, upon *Agrobacterium* infiltration, the fusion protein was detected in heterotrophic plastids of the epidermis and in the cytosol of mesophyll cells. Interestingly, with GFP fused to the N-terminus, the fusion protein partially co-localised with peroxisomes, indicating presence of a peroxisomal targeting signal. Moreover, initial co-expression experiments suggest interaction with inactive *HvP0-G6PDH*. Further studies are needed to confirm the P2-P0 interaction, and to specify localization of the heterodimers.

P6.10

Transgenerational responses to nitrogen deprivation in *Arabidopsis thaliana*

M. Massaro¹, L. Zanin¹, N. Tomasi¹, E. De Paoli¹, M. Morgante^{1,2}, R. Pinton¹

¹Dept Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy, ²Istituto di Genomica Applicata, Udine, Italy

Nitrogen deprivation represents one of the major stresses to which plants are exposed. As plants respond to this stress, it would be profitable to retain a memory of the response to prime following generations. Therefore, an experimental setup considering successive generations of control or stressed *Arabidopsis* plants was used to evaluate the establishment of a transgenerational memory of N-deficiency stress response. Results demonstrated a faster induction of a high nitrate uptake capability as a result of multigenerational stress exposures. This behaviour was paralleled by changes in the expression of nitrate responsive genes. Transcriptional analyses revealed an enduring modulation of genes in downstream generations, despite the lack of stress stimulus in these plants. Using genome-wide detection of DNA methylation we could

show a correlation between stress treatments and DNA methylation changes. This suggests the involvement of epigenetic responses, while the mechanism underlying the maintenance of expression changes in the progeny of stressed plants remains elusive and compels further investigation.

P6.11

Cyclic AMP deficiency negatively affects thermotolerance by altering redox homeostasis

A. Paradiso¹, C. Lapenta¹, W. Sabetta², S. Fortunato, L. Viggiano¹, E. Blanco³, M.C. de Pinto¹

¹Dept Biology, Univ. of Bari "Aldo Moro", Bari, Italy; ²Spin-off SINAGRI srl, Univ. of Bari "Aldo Moro", Bari, Italy; ³Inst. Bioscience and Bioresources, CNR, Bari, Italy

Heat stress (HS), affecting different physiological, biochemical and transcriptional pathways can have deleterious effects on plants. To cope with high temperatures, plants have a number of defense mechanisms, including the synthesis of heat shock proteins (HSPs) and the maintenance of an opportune redox balance. Cyclic AMP is involved in the acquisition of thermotolerance; in *Arabidopsis*, cAMP increases during HS and activates CNGC6, inducing Ca²⁺ influx and HSPs expression. To get more insight into the role of cAMP in HS response, tobacco BY-2 cells have been transformed with the "cAMP-sponge", a genetically encoded tool, able to selectively bind cAMP. The cAMP content in the transgenic (cAS) lines is lower than in WT lines. At optimal temperature, inhibition of cell division and enhancement of antioxidants occur in cAS cells, suggesting that cAMP deficiency is sensed as a stress condition. HS inhibits cell division both in WT and cAS cells. However, cAMP deficiency makes BY-2 cells more susceptible to HS, determining an increase in cell death. The failure in the control of redox homeostasis in response to HS seems to be the cause of the low thermotolerance in condition of cAMP deficiency.

P6.12

Glutamate dehydrogenase isoenzyme 3 (GDH3) of *Arabidopsis thaliana* is less thermostable than GDH1 and GDH2 isoenzymes

L. Marchi¹, E. Polverini², E. Degola¹, E. Baruffini¹, F. M. Restivo¹

¹Dipartimento di Bioscienze, Università di Parma, Parma, Italy;

²Dipartimento di Fisica e Scienze della Terra, Università di Parma, Parma, Italy

NAD(H)-glutamate dehydrogenase (GDH; EC 1.4.1.2) is an abundant and ubiquitous enzyme that may exist in different isoenzymic forms. Variation in the composition of the GDH isoenzyme pattern is observed during plant development and specific cell, tissue and organ localization of the different isoforms have been reported. However, the mechanisms involved in the regulation of the isoenzymatic pattern are still obscure. In *Arabidopsis thaliana*, three genes (*GDH1*, *GDH2*, *GDH3*) encode three different GDH subunits (β , α and γ) that randomly associate to form a complex array of homo- and hetero-hexamers. In order to assess if the different *Arabidopsis* GDH isoforms may display different structural properties we have investigated their thermal stability. Differences among the various GDH isoforms were observed. In particular, the γ subunit containing isoforms were less stable than the α or β containing isoforms. The stability of GDH1 ($\beta\beta$) and GDH3 (6γ) isoenzymes was then studied using site-directed mutagenesis in a heterologous yeast expression system. It was established that the carboxyl terminus of the GDH subunit is involved in the stabilization of the oligomeric structure of the enzyme

P6.13

Ultrastructural and physiological changes in barley seedlings exposed to cadmium

M. Lentini¹, S. Sorbo², A. De Lillo¹, V. Paradisone¹, C. Arena¹, A. Basile¹, S. Esposito¹

¹Dipartimento di Biologia, Università di Napoli "Federico II" – Italy;

²CESMA, Università di Napoli "Federico II" – Italy

Cadmium pollution is one of the main menaces for the natural ecosystems, because its relatively high solubility, and its strong toxicity for the living organisms. Barley (*Hordeum vulgare*) is one of the first 5 crops cultivated worldwide, and it is particularly resistant to abiotic stress. Therefore, barley plants could represent an interesting model in the evaluation of cadmium accumulation in a widely cultivated crop. The effects of exposition for 7 days at different Cd levels were analysed on barley seedlings. Morphological, and TEM - SEM analyses revealed a massive damage to leaf chloroplasts. Cadmium exposed roots show irregular vacuoles and damaged endomembrane system structure. Physiological and biochemical analyses were related to leaf water content, ultrastructural damage, measurements of photochemical indices, content in pigments and starch accumulation. Nitrate reductase activity, proline and Heat Shock Protein 70 levels suggested a dose-dependent effect of Cd. Finally, the cadmium change root structure by increasing the number of lateral roots. In conclusion, our data suggest that barley can be used as a useful tool to assess and monitor pollution by cadmium.

P6.14

Comparison of functional properties of δ^1 -pyrroline-5-carboxylate reductases from plants and bacteria

G. Forlani

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

Besides being needed for protein synthesis, a wide array of plants and microorganisms accumulate high intracellular levels of free proline to cope with osmotic stress conditions. Proline production is tightly regulated at both the transcriptional and the translational levels, yet the mechanisms for post-translational regulation of the enzymatic activities involved have not been fully elucidated. Two main routes lead to proline synthesis using glutamate or ornithine as the precursor, respectively. These two pathways share the terminal step, the conversion of δ^1 -pyrroline-5-carboxylate (P5C) to L-proline, catalyzed by P5C reductase (EC 1.5.1.2). The genes coding for P5C reductase in *Bacillus subtilis*, *Streptococcus pyogenes*, *Arabidopsis thaliana*, *Oryza sativa* and *Medicago truncatula* were isolated and expressed in *E. coli*. The functional properties of the affinity-purified proteins were characterized and compared, with special emphasis on the use of NADH or NADPH as the electron donor and the occurrence of inhibitory or stimulatory effects by increasing concentrations of products (proline, NAD⁺ and NADP⁺), cations and anions. This work was supported in part by AGER, grant # 2010-2369.

P6.15

Salt tolerance in an Italian rice variety is provided by rapid and specific stress responses

E. Formentin¹, C. Sudiro¹, E. Barizza¹, G. Perin¹, E. Lavezzo², P. Stevanato³, P. Fontana⁴, G.A. Sacchi⁵, M. Zottini¹, F. LoSchiavo¹

¹Dept Biology, Padova Univ., Padova, Italy; ²Dept Molecular Medicine, Padova Univ., Padova, Italy; ³Dept Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), Padova Univ., Legnaro, Italy; ⁴Research and Innovation Center, Edmund Mach Foundation, S. Michele all'Adige, Italy; ⁵Dept Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Milan Univ., Milan, Italy

Soil salinity is a major constraint for crop production overall the world and rice is the most sensitive to salt among cereals. To identify mechanisms involved in salt tolerance, we compared the response of two contrasting Italian rice genotypes at physiological and molecular level. Upon salt stress the susceptible cultivar, Vialone Nano, showed growth arrest and leaf yellowing, due to Na⁺ accumulation in leaves leading eventually to a drop in photosynthetic efficiency. The tolerant variety, Baldo, sacrificed the oldest leaves, excluded Na⁺ from the new leaves and resumed growth after two days. This variety seems to react promptly to salt stress by stopping growth and closing stomata rapidly. Therefore, these plants put in place an adaptation programme by changing root architecture and activating a NPQ response in new leaves. A quick Na⁺ compartmentalization in root vacuoles along with changes

in hormonal levels seemed to play an essential role in the regulation of this adaptive behaviour. In fact, genes involved in osmosensing and Na⁺ translocation in vacuoles showed expression profiles peculiar of the tolerant plants and might be considered as markers of salt tolerance.

P6.16

Glutathione-Ascorbate cycle and lipid peroxidation in fruit of sweet cherry landraces of Campania region (Italy)

A. Mito, F. Letizia, F. Iannuzzi, L. Ciarmiello, P. Woodrow, P. Carillo, A. Fuggi
Dept. Environmental, Biological and Pharmaceutical Sciences and Technologies

Fresh fruits are living organs that continue their metabolism even after harvest. The advanced stages of ripening culminate into the senescence process, that eventually leads to the death of fruit. Several works highlight the importance of antioxidant metabolism in relation to the fruit quality and shelf-life: fruit ripening is accompanied by a progressive increase in oxidative stress that is controlled by a related induction of the antioxidant scavenging systems. Dysfunctioning of such systems in the later stages of ripening causes an increase of oxidation, that is among the most important factors of fruit decay, favouring also parasite attack and development. High antioxidant metabolite levels, in fact, could delay senescence and preserve nutritional and nutraceutical characteristics, significantly reducing fruit loss and cost. Sweet cherries fruits are excellent sources of phytochemicals: nutraceuticals and antioxidants. It has been demonstrated that the eating of cherries reduces the risk of cancer and the joint pains, and protects from cardiovascular and neurodegenerative diseases. The aim of this work was to characterize glutathione-ascorbate cycle as well as lipid peroxidation in mature fruits of the sweet cherry germoplasm of Campania region and their involvement in post harvest storage. Fruits from cherry landraces of Campania region were collected at commercial maturity and used for the analyses. Glutathione as ascorbate contents differed among the landraces as well as glutathione reductase. Differences were also found in the lipid peroxidation activities using the MDA test. The data of glutathione level and redox state and glutathione peroxidase, ascorbate level and redox state as ascorbate peroxidase activities, tocopherols and polyphenols, two groups of landraces have been evidenced. The first showed high polyphenol oxidase activities, that could indicate a higher risk of developing oxidative stress and, consequently, a higher susceptibility to the oxidative degradation during shelf-life. The second showed high ascorbic acid and tocopherols contents, and low polyphenol oxidase activities. The high metabolites concentration could reduce the risk of oxidative damages during storage, therefore they could show a longer shelf-life than the other tested fruits. These characteristics were probably due to endogenous characteristics, making these landraces particularly interesting for breeding programs aimed to improve sweet cherry shelf-life, highlighting also the value of genetic heritage of sweet cherry of Campania region.

P6.17

Adaptation to metalliferous soils and nutrient use efficiency in populations of *Silene paradoxa* L.

E. Giorni, M. Rabatti, C. Gonnelli
Department of Biology, University of Florence, Florence, Italy

Plants adapted to live in metal-enriched soils have to face both the stress due to the high metal concentrations and to nutrient deficiency. In this work, we investigated the difference in response to 3 macronutrients (Ca²⁺, Mg²⁺, K⁺) deficiency stress by two metalcolous and a non metalcolous population of *Silene paradoxa* in order to see if the adaptation to heavy metal-enriched soils could have modified the physiology of nutrient utilization. Plants were grown in hydroponic solution, totally and partially (1/10) deprived of each nutrient and the nutrient content in roots and shoots was measured. Growth results show that the two metalcolous populations were less sensitive to the nutrient deficiency compared to the non metalcolous one, thus demonstrating that, despite the scarcity of nutrients in the soil, these populations are able to optimize the nutrient

accumulation and allocation in their organs to maintain an adequate development and growth rate. The NUE (nutrient use efficiency) values demonstrated that metalcolous populations are indeed more efficient than the non metalcolous one in facing the nutrient deficiency stress.

P6.18

Pointing out the distinct features of plant and algae phosphoribulokinase

L. Gurrieri¹, A. Del Giudice², S. Fermani³, G. Falini³, M. Zaffagnini¹, S.D. Lemaire⁴, L. Galantini², P. Trost¹, N.V. Pavel², F. Sparla¹
¹Dept of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, ²Dept of Chemistry, University of Roma "Sapienza", Roma, Italy, ³Dept of Chemistry, University of Bologna, Bologna, Italy, ⁴Institut de Biologie Physico-Chimique, Université Pierre et Marie Curie, Paris, France

Phosphoribulokinase (PRK) is an essential and highly regulated homodimeric enzyme of the Calvin-Benson cycle, conserved in all green photosynthetic organisms and required to re-generate the RuBisCO's substrate (Michelet et al., 2013). PRK is the only enzyme of the Calvin-Benson cycle whose 3D-structure is unknown and that cannot be predicted by homology modelling because of lack of similar proteins in other organisms. In the present study, two recombinant forms of PRK, one from the green alga *C. reinhardtii* (CrPRK) and one from the higher plant *A. thaliana* (AtPRK), were analysed and compared in order to highlight possible biochemical and structural differences. The two mature forms of PRK share 75% of amino acid identity. Low resolution structures obtained by Small Angle X-ray scattering (SAXs) showed appreciable differences in shape between CrPRK and AtPRK, the former being more anisotropic and less compact and the second showing a larger contact area between the two subunits. The activity of the two enzymes appeared differently affected by physicochemical parameters such as redox conditions, pH and temperature. Analyses of circular dichroism (CD) indicated a different binding of the substrates. A systematic experimental approach aimed in solving the first crystal structure of a plant PRK is underway.

P6.19

Effects of cGMP constitutive accumulation in *Arabidopsis thaliana* plants challenged with avirulent pathogens

V. Locato¹, J. Hussain², W. Sabetta³, J. Chen², S. Behera⁴, S. Cimini¹, E. Blanco³, A. Costa⁴, D. Bellin², M. C. de Pinto⁵, E. Vandelle², L. De Gara¹
¹Food Sciences and Human Nutrition Unit, University Campus Bio-Medico of Rome, Rome, Italy, ²Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy, ³Institute of Biosciences and Bioresources – CNR, Research Division Bari, Via Amendola 165/A, 70126 Bari, Italy, ⁴University of Milan, Department of Biosciences, Via G. Celoria 26, 20133 Milano, Italy, ⁵Department of Biology, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy

Cyclic guanosine 3',5'-monophosphate (cGMP) has been indicated as a second messenger induced by nitric oxide (NO) in incompatible plant-pathogen response. However, the role of cGMP in the signaling of plant defense response has not yet characterized. In this study a genetic tool has been used in order to constitutively increase cGMP level in plant. In particular, *Arabidopsis thaliana* plants expressing the rat soluble guanylate cyclase (GC) have been produced. In order to evaluate cGMP contribution in local and systemic acquired resistance, the transgenic plants have been subjected to the infection of an avirulent strain of *Pseudomonas syringae*. Even if phenotypic local response of GC plants did not show any difference compared to wild type line, glutathione metabolism resulted modified suggesting a crosstalk between cGMP and redox signaling. Large-scale transcriptomic and proteomic analysis highlighted significant modulation between wild type and GC overexpressing plant of both gene expression and protein abundance at the infection site. Moreover, high constitutive cGMP level in GC plants appeared to affect systemic acquired response of the plants to the pathogen.

P6.20**Chemical characterization and standardization of bioactive boswellic acids from *Boswellia* (Frankincense) species by HPLC-ESI-MS/MS**G. Mannino¹, A. Occhipinti¹, M.E. Maffei¹¹Dept Life Sciences and Systems Biology, Turin Univ., Turin, Italy

Plant extracts are a rich source of secondary metabolites able to exert biological activities on humans. They also have a high economic impact on the dietary supplements market. Environmental factors and extraction methods can strongly affect the chemical composition of plant extracts; therefore, the use of accurate and sensitive analytical techniques for the characterization and quantification of bioactive molecules is a compulsory quality standard to assure to costumers both safety and bioactivity. Traditional medicine uses *Boswellia* spp. resin-gum extracts for anti-inflammatory, anti-proliferative, antiseptic and neuro-protective effects because of the presence of pentacyclic triterpenoids known as boswellic acids (BAs). *B. sacra* and *B. serrata* are characterized by significant amounts of 3-*O*-Acetyl-11-keto- β -boswellic acid (AKBA), α - and β -BAs and their acetylated derivatives. In market products, BAs percentages are often misinterpreted and it is not unusual to find claims of 70% BAs content. This study aims to quantify BAs content by using HPLC coupled to Tandem Mass Spectrometry in two commercial *Boswellia* species to achieve the accurate standardization of bioactive BAs.

P6.21**Sirtuin-mediated DNA damage response by modulation of glutamate dehydrogenase activity in *Arabidopsis thaliana***M.L. Mauro¹, G. Bruscalupi¹, P. Costantino¹, C. Failla²¹Dept Biology Biotechnology, Sapienza Univ., Rome, Italy; ²IDI-IRCCS, Pomezia, Italy

Sirtuins, ClassIII NAD-dependent deacetylases, play a central role in many metabolic pathways related to cell survival and are evolutionary conserved from bacteria to mammals. Among the seven human sirtuins, SIRT4 and SIRT6 share homology domains with the two sirtuins present in *Arabidopsis thaliana* plants, AtSRT2 and AtSRT1 respectively. With the aim to evaluate sirtuin functions in phylogenetically distant organisms, we report data on a corresponding role between *Arabidopsis* SRT2 and human SIRT4 genes. We find that AtSRT2 is involved in a defence process already known to be regulated by SIRT4. In fact the DNA Damage Response (DDR) in human cells induces SIRT4 that in turn limits proliferation via repression of glutamine metabolism (Jeong et al, Cancer Cell 2013, 23:450). In *Arabidopsis* seedlings, the induction of DNA damage promotes transcriptional activation of SRT2 gene and decreased activity of glutamate dehydrogenase (GDH), one of the enzymes that catalyze α -ketoglutarate (α KG) production from glutamine. As α KG is a major anaplerotic component of TCA cycle in proliferating cells, the decreased GDH activity is coherent with the slowed cell proliferation that we observed. Moreover, in plants knock out for SRT2, GDH activity and cell proliferation are less affected by DNA damage, confirming the role of AtSRT2 in this metabolic pathway.

P6.22**Molecular insights on the role of *Arabidopsis thaliana* NAOD in fruit set**B. Molesini¹, S. Zanzoni¹, G. Mennella², G. Francese², A. Losa³, G. L. Rotino³, and T. Pandolfini¹¹Department of Biotechnology, University of Verona, Verona, Italy,²Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per l'Orticoltura, Pontecagnano-Faiano (Salerno), Italy; ³Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di ricerca per l'Orticoltura (ORL), Montanaso Lombardo (Lodi), Italy

Ornithine (Orn), an intermediate of arginine and polyamines (PAs) biosynthetic pathways, is produced in plants by N²-acetylornithine:N-

acetylglutamate acetyltransferase (NAOGAcT). In enteric bacteria, Orn is synthesised also by N-acetylornithine deacetylase (NAOD) via a linear pathway. The plants seem to be unable to use this pathway despite the presence of many NAOD-like genes identified in various plant species. We have studied the role of the putative NAOD of *Arabidopsis* (*At4g17830*) by analysing the effects of its downregulation *in vivo*. *AtNAOD*-suppressed plants displayed an impaired fruit setting. *AtNAOD* downregulation determined a reduced Orn content and altered PAs levels. To elucidate the role of *AtNAOD* in fruit setting, we compared the mRNA profile of fertilised flowers of *AtNAOD*-downregulated plants with that of wild-type. We found 63 genes significantly changed (fold change $\geq |2|$). Our analysis revealed that the altered Orn and PAs metabolism in the reproductive organs of the *AtNAOD*-downregulated plants is associated with an impaired transcription of cysteine-rich signalling peptides involved in male-female cross-talk and perturbation of genes involved in regulating N:C status.

P6.23**Salt-tolerant related protein: a new protein involved in plant adaptation to low temperature**

C. Muzi, A. Fiorillo, L. Camoni, P. Aducci, S. Visconti

Plants are constantly exposed to different environmental stresses such as extreme temperatures, high salinity, excess or lack of water, light and nutrients that strongly limit plant growth and significantly reduce crop yield. Plants respond with changes in their pattern of gene expression and protein products when exposed to these stresses. Thus, the ability to adapt has an impact on the distribution of the plant and crop yield. In a proteomic analysis of temperature stress-responsive proteins of *Arabidopsis thaliana* rosette leaves, an uncharacterized protein, known as salt-tolerant related protein (STRP), resulted to be strongly increased by cold treatment. The aim of this work was to clarify the role of this protein in *Arabidopsis* response to cold stress. By using polyclonal antibodies obtained against recombinant STRP protein, it was demonstrated that STRP is expressed in the cytosol but is also associated to the plasma membrane. Low temperature treatment greatly increased STRP levels in the cytosol and this effect was due to an enhanced protein stability rather than to activation of gene transcription. Moreover, STRP knock out mutant showed increased susceptibility to oxidative damage induced by cold stress, as demonstrated by the higher levels of lipid peroxidation and ion leakage. These results suggest that STRP may play a protective role in the plant response to cold stress.

P6.24**Identification and quantification of flavonoids in fresh leaves and fruits of *Cyclanthera pedata* Scrabs (Caigua) and in their commercial food supplement preparations**E. Orsini¹, D. Corradini², I. Nicoletti², L. De Gara¹¹University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy; ²CNR, Institute of Chemical Methodologies, Area della Ricerca di Roma 1, Via Salaria Km 29,300, Montelibretti (Rome), Italy

Cyclanthera pedata Scrabs (Caigua) is a plant belonging to the Cucurbitaceae family largely cultivated in South America. The plant is used as food and in popular medicine to treat diabetes and to control high blood pressure and cholesterol. Hypoglycemic properties of plant extracts have been frequently related to their content in flavonoid glycosides which are known to possess antihyperglycemic activity. This poster reports the results of a study carried to identify and quantitate flavonoid glycosides extracted from fresh fruit and leaves of *Cyclanthera pedata* Scrab and from commercially available food supplements based on Caigua extracts. Plants of Caigua were grown either in a greenhouse or in an open field located in the vicinity of our University, whereas the food supplements were of commercial origin. Flavonoids were extracted using methanol or methanol-water mixtures and their separation and quantification were carried out by reversed phase high performance

liquid chromatography (RP-HPLC), using both UV spectrophotometry and mass spectrometry as the detection systems. The different occurrence and quantity of flavonoids in the investigated samples are reported and discussed.

P6.25

Can chemical xylem sap changes trigger grapevine isohydric and anisohydric behaviors under environmental water deficit?

C. Pagliarani¹, S. Cavalletto¹, M. Ashofteh Beiragi¹, I. Siciliano^{1,2}, M. Vitali¹, T. Strano¹, A. Schubert¹, M. L. Gullino^{1,2}, F. Secchi¹

¹Dept of Agricultural, Forest and Food Sciences, University of Torino, Italy, ²Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Italy.

Grapevine cultivars have been classified as isohydric or anisohydric on the base of their different stomatal kinetics experienced in response to water deficit conditions. In this work, dynamic changes of stomatal responses to leaf water potential were monitored in six *Vitis vinifera* varieties in order to investigate a possible correlation between physiological parameters and biochemical features of xylem sap [apoplastic pH, abscisic acid (ABA) and soluble sugar concentrations]. Sap samples were collected in an experimental vineyard during the summer season at different levels of environmental water deficit: low (early summer), moderate (middle of the season) and recovery (one day after rainfall in late summer). Preliminary results showed that all varieties were characterized over the season by: a progressive xylem sap alkalization, a decrease of soluble sugar content and an increase in ABA level. However, when near-isohydric (Grenache) and anisohydric (Barbera) cultivars were compared, distinct profiles of all the analyzed parameters of xylem sap were observed, thus providing peculiar physiological responses of grape varieties upon progressive soil water scarcity conditions.

P6.26

Effect of zinc deficiency in *Hordeum vulgare*: are changes in morphological and physiological aspects related to alterations in nitrate uptake?

V. Paradisone, M. Lentini, A. De Lillo, S. Esposito

Zinc (Zn) is an essential micronutrient for living organisms, involved in a wide variety of metabolic processes. Despite of the evidence that Zn is the second most abundant transition metal in nature, more than 3 million people worldwide suffer from Zn deficiencies. The main aim of this work was to analyse the effects of Zn deficiency in a plant of high agronomic relevance as barley (*Hordeum vulgare*). Thus plants were grown for 7 days in hydroponic culture in control, toxicity and deficiency conditions. We measured the levels of heat shock protein 70, growth inhibition factor, water content, photosynthetic pigments content, starch accumulation, nutrient uptake and expression and activity of several basal metabolism enzymes, such as glucose6 phosphate dehydrogenase. The results show under Zn deficiency several changes in both morphological aspects and in biochemical processes. Moreover, NO₃⁻ uptake changed suggesting that under Zn deficiency, nitrogen metabolism was altered inducing structural and physiological changes in barley plants.

P6.27

The existence of sirtuin activity in purified durum wheat (*Triticum durum* Desf.) mitochondria

M. Alfaro¹, M. Soccio¹, M. Laus¹, D. Trono², D. Pastore¹

¹Dept. of SAFE, Univ. of Foggia, Italy, ²CREA-Cereal Research Centre, Foggia, Italy

Sirtuins are a family of NAD⁺-dependent deacetylases able to regulate proteins through the N^ε-deacetylation of lysine residues. Recently, it has been demonstrated that the *Arabidopsis* SRT2 (At5g09230) resides in the inner mitochondrial membrane and is involved in regulation of energy metabolism and metabolite transport. In *silico* analysis suggested that a functional SRT2 may exist in durum

wheat having an N-terminal signal peptide that putatively directs it to mitochondria. So, we tried to assay for the first time a sirtuin activity in a highly purified mitochondrial fraction. To do this, we used the luminescent SIRT-Glo™ assay (Promega), based on the luciferin/luciferase reaction. We were able to measure a sirtuin activity that resulted *i)* linearly dependent on mitochondrial protein, *ii)* inactivated by protein denaturation by boiling mitochondria and *iii)* completely abolished by 100 mM nicotinamide, a known sirtuin inhibitor. On the basis of calibration by using human SIRT1 (Sigma-Aldrich) an unexpectedly high activity of durum wheat mitochondria (DWM) sirtuin equal to 0.165 ± 0.013 µg SRT1 eq./mg DWM was calculated, thus suggesting relevant role/s of this enzyme in DWM.

P6.28

From an expression-based reverse genetic study to the functional characterization of two determinants of osmotic stress tolerance

P. Punzo¹, A. Ruggiero¹, R. Nurchio¹, M. Possenti², G. Morelli², S. Grillo¹, G. Batelli¹

¹Institute of Biosciences and Bioresources (CNR-IBBR) Research Division Portici, Via Università, 133 80055 Portici (Italy), ²Council for Agricultural Research and Economics, Food and Nutrition Research Centre (CREA-NUT), Via Ardeatina 546 00178, Rome, (Italy)

Adaptation to osmotic stress requires an extensive alteration of gene expression. Previously, we identified several genes regulated in cells adapted to polyethylene glycol (PEG). Here, the functional role of fifty of these genes was verified. Using a large-scale phenotype screening, we have identified two genes: the splicing factor *IAG1* (*INSENSITIVE TO ABA IN GERMINATION1*) and the putative TOR-pathway component *XSA1* (*EXTRA SENSITIVE TO ABA1*). *IAG1* is induced upon long-term exposure to abscisic acid (ABA) and PEG and is mainly expressed in trichomes and stomata, organs controlling transpiration. Germination analysis of plants with altered expression of *IAG1* and protein interaction with the splicing factor *SUA*, suggest that *IAG1* may be involved in pre-mRNA splicing of effectors of ABA response leading to germination inhibition. *XSA1* possibly affects pathways in ABA-mediated response to stress. *XSA1* is expressed in vascular tissues and is up-regulated by long-term exposure to NaCl and ABA. *xsa1-1* is ABA hypersensitive, indicating alteration in ABA biosynthesis and/or perception. Taken together, our results reveal promising mechanisms of plant adaptation to osmotic stress.

P6.29

Key role of pectin methylesterases in controlling ascorbic acid content in tomato fruit

M. M. Rigano¹, A. Raiola¹, V. Lionetti², E. Fabri², D. Bellincampi², A. Barone¹

¹Dept. of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy, ²Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy

The potential increase of L-ascorbic acid (AsA) content in tomato is a common goal in breeding programs due to its beneficial effect on human health. In a previous study, one *Solanum pennellii* introgression line (IL12-4-SL) harbouring one quantitative trait locus that increases the content of AsA in the fruit was identified. In the present study, we confirmed the higher content of total AsA and of the reduced form of AsA in IL12-4-SL compared to the cultivated line M82 at three developmental stages (mature green, breaker and mature red). Genomic and transcriptomic analyses indicated in IL12-4-SL the involvement of genes controlling pectin de-methyl esterification/degradation in AsA accumulation. The expression of the identified candidate genes during fruit ripening was studied in relation with pectin methylesterase (PME) activity, degree of pectin methyl esterification and AsA content. A PME and a polygalacturonase were identified that may affect pectin-derived D-galacturonic acid (GalUA) content leading to AsA biosynthesis as metabolic precursor. This knowledge could provide novel tools for marker-assisted selection of tomato genotypes with a higher content of antioxidant in the fruit.

P6.30**Molecular insights into the interaction between KAT1 channels and their regulatory protein 14-3-3**

A. Saponaro¹, A. Porro¹, A. Chaves Sanjuan¹, M. Tomasi¹, B. Introini¹, M. Nardini¹, G. Thiel², A. Moroni¹

¹University of Milan, Italy, ²TU-Darmstadt, Germany

The voltage-gated inward rectifier potassium channel KAT1 of *Arabidopsis thaliana* plays a key role in stomatal movement, thus controlling carbon dioxide uptake for photosynthesis and water balance of the entire organism. In previous work we have shown that the cytosolic regulatory proteins 14-3-3 interact with the C-terminal portion of the KAT1 channel and modulate both its voltage dependent gating and the trafficking to the plasma membrane (1,2). By a combination of high resolution structural and functional studies we are now able to detail the molecular and structural basis for the channel/14-3-3 interaction. Biochemical binding assays with isothermal titration calorimetry (ITC), structural studies (crystallography) and electrophysiological (patch-clamp) monitoring of KAT1 function support a molecular model in which the 14-3-3 protein is binding to the C-terminus of KAT1 via a mode III binding motif. Strikingly, this interaction is strongly stabilized by the fungal toxin fusaric acid (FA) thereby potentiating the regulatory effect of 14-3-3 on the channel. Based on this detailed insight on protein/protein interaction we are now investigating the molecular basis of gating modulation that presumably involves the recruitment of additional partner proteins. Since 14-3-3 binds exclusively to KAT1 but not to the very similar channel KAT2, we are using KAT2 as a functional tool to investigate the molecular mechanism of 14-3-3 modulation of KAT1 channel opening.

[1] Sottocornola B. et al. *J Biol Chem.* 281(47):35735-41

[2] Sottocornola B. et al. *Plant Biol (Stuttg).* 10(2):231-6

P6.31**First evidence of a Ca²⁺- induced loss of membrane potential in durum wheat mitochondria**

M. Soccio¹, D. Trono², M. Alfàrano¹, M.N. Laus¹, D. Pastore¹

Durum Wheat (*Triticum durum* Desf.) Mitochondria (DWM) were recently shown to possess a Ca²⁺-activated PLA₂ that releases FFAs and, under stress conditions, may activate both uncoupling protein (UCP) and mitochondrial potassium channel (PmitoK_{ATP}), thus damping protonmotive force and excess ROS production. Nevertheless, so far, no information is available about how the DWM-PLA₂ activity increases under stress. One possibility is that changes of Ca²⁺ concentration might regulate the enzyme. To study this possibility, DWM oxidizing succinate were added with CaCl₂ (0.1 and 0.5 mM) and a biphasic depolarization was observed under conditions contrasting possible occurrence of permeability transition (5 mM Mg²⁺, no phosphate). A rapid membrane potential decrease ranging from 10 to 25 mV (depending on Ca²⁺ concentration) is observed, followed by a Slow Continuous Depolarization (SCD). Interestingly, 1 mM ATP (in the presence of atractyloside, oligomycin and Ap5A), able to inhibit UCP and PmitoK_{ATP} together with 0.1% BSA, able to bind FFAs, were able to rapidly reverse the SCD phase. This is compatible with the hypothesis that PLA₂/UCP/PmitoK_{ATP} pathway might be involved in the SCD phase.

P6.32**Calcium signaling in response to abiotic stress during plant evolution**

M. Storti¹, S. Golin¹, M. Zottini¹, A. Costa², T. Morosinotto¹, A. Alboresi¹

¹Department of Biology, University of Padova, 35121 Padova, Italy;

²Department of Bioscience, University of Milan, 20133 Milan, Italy

The colonization of land environment has been a key step in the evolution of photosynthetic organisms. Plants acquired novel abilities that were not essential to their aquatic progenitors, such as resistance to dehydration. Such a massive change clearly involved an adaptation of the interaction of organisms with the environment. In order to investigate the evolution of mechanisms of external stimuli perception we exploited

Physcomitrella patens, a moss belonging to the phylum bryophytes, though to maintain the characteristics of the first plants colonizing land. In particular we focused on the study of the response to osmotic stresses in term of Ca²⁺ dynamics, as univocal signals activating metabolic response. To this aim we generated mosses stably expressing the YC3.6 Cameleon variant, a ratiometric FRET-based Ca²⁺-probe, targeted to different subcellular compartments. By confocal microscopy, we verify the specific localization of Cameleon in the cytosol, mitochondria or nuclei. By analyzing the plants at the protonema stage, we could observe, in all cell compartments analyzed, a transient increase in Ca²⁺ concentration triggered by both dehydration and rehydration treatments.

P6.33**Does carbon starvation during prolonged drought prevent embolism repair pushing trees toward irreversible hydraulic failure?**

P. Trifilò¹, V. Casolo², F. Raimondo¹, E. Petrusa², M.A. Lo Gullo¹, A. Nardini³

¹Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences, Univ. Messina, Italy, ²Dept. of Agricultural, Food, Environmental and Animals Sciences, Univ. Udine, Italy, ³Dept. of Life Sciences, Univ. Trieste, Italy

Recent episodes of anomalous drought and heat waves have caused, on a global scale, widespread mortality of plants. Drought-induced tree death is a complex event and recent hypotheses suggest that hydraulic failure and carbon starvation are co-responsible of tree decline. Progressive decline in soil water reserves increases xylem tension inducing xylem embolism and stomatal closure. Reduced CO₂ uptake can lead to impoverishment of non-structural carbohydrates (NSC) reserves. Plants can cope with hydraulic failure by reversing xylem embolism via refilling upon stress relief. Embolism reversal is likely based on an osmotic mechanism, and thus requires availability of soluble sugars. We tested the hypothesis that embolism reversal represents the mechanistic link between carbon starvation and stem hydraulics. Measurements were performed on laurel plants exposed to carbon starvation caused by a prolonged water stress. Plant water status, gas exchange, leaf water potential isotherms, loss of hydraulic conductivity and NSC content of stems were measured in control versus drought-treated plants, before re-irrigation and eventual recovery. Our findings suggest a strong correlation between NSC availability and embolism reversal ability, opening interesting new scenarios on mechanisms responsible for tree resistance and resilience to severe water shortage.

P6.34**Strigolactones are needed for miR156 inducibility by drought in tomato**

I. Visentin, C. Pagliarini, A. Caracci, A. Schubert, F. Cardinale
Dept Agriculture, Forestry and Food Science (DISAFA), Univ. Torino, Grugliasco (TO), Italy

Strigolactones (SL) are terpenoid hormones influencing many aspects of plant development, biotic interactions and responses to environmental stress, namely drought. MicroRNAs (miRNAs) are a set of small, non-protein-coding RNAs that regulate gene expression at the post-transcriptional level. Given the inducibility of certain miRNAs by environmental stress, and the pervasive effects of SL on plant acclimatization to drought, we asked whether the latter may have a role in miRNA metabolism in such conditions in tomato. The results show that SL synthesis in the shoot, but not in the root, is needed for the accumulation of miR156 (but not miR166) in both organs under drought. A preliminary analysis of pre-miR156 levels indicates that stress triggers precursor accumulation only in shoots, which implies that mature miR156 moves rootwardly under drought. Additionally, pre-miRNA inducibility by drought seems to be only marginally affected by SL availability, suggesting that the latter may rather play their role later in miRNA maturation - a process whose efficiency towards specific miRNAs is sensitive to cellular context.