Second Congress
Roma 12th – 14th July, 2010

PROGRAMME

Università degli Studi di Roma
“la Sapienza”

P.le A. Moro 5 - Roma
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00 – 13:30</td>
<td><strong>REGISTRATION, POSTER MOUNTING, POSTER VIEWING</strong></td>
<td>AULA GIACOMINI, GIARDINO DBV</td>
</tr>
<tr>
<td>13:30 – 15:30</td>
<td><strong>PLENARY SESSION: PLANT NUTRIENTS FOR HUMAN HEALTH</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>CHAIR: LAURA DE GARA</strong></td>
<td></td>
</tr>
<tr>
<td>13:30 – 14:00</td>
<td><strong>ENGINEERING PHENYLPROPAANOID PRODUCTION FOR HEALTHY FOODS</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>14:00 – 14:30</td>
<td><strong>LOW PHYTIC ACID MUTANTS: USEFUL TOOLS EITHER FOR RESEARCH IN THE</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>FIELD OF SEED PHYSIOLOGY, OR FOR FOOD AND FEED BIOFORTIFICATION</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>14:30 – 14:50</td>
<td><strong>PI-02 ROLE OF ANTHOCYANIN-RICH FOOD IN CARDIOPROTECTION AND</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>OBESITY.</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>14:50 – 15:10</td>
<td><strong>PI-04 AN EMMER (TRITICUM DICOCCON SCHRANK) TETRAMERIC INHIBITOR OF</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>ALPHA AMYLASE.</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>15:10 – 15:30</td>
<td><strong>PI-06 TRANSCRIPTIONAL-METABOLIC NETWORKS IN BETA-CAROTENE-</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>ENRICHED POTATO TUBERS: THE LONG AND WINDING ROAD TO THE</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td></td>
<td><strong>“GOLDEN” PHENOTYPE.</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>15:30 – 16:00</td>
<td><strong>COFFEE BREAK AND POSTER VIEWING</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:00 – 18:00</td>
<td><strong>PARALLEL SESSION: OMICS IN PLANT BIOLOGY</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:00 – 16:20</td>
<td><strong>PIII-01 IDENTIFICATION AND CHARACTERIZATION OF THE GRAPEVINE</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>DEFENSIN-LIKE GENE FAMILY.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:20 – 16:40</td>
<td><strong>PIII-02 PROFILING THE HYPOXIC RESPONSE OF ARABIDOPSIS THALIANA ROOTS</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>FOR CHANGES IN TRANSCRIPTION FACTOR AND PRI-MIRNA EXPRESSION.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:40 – 17:00</td>
<td><strong>PIII-03 TRANSCRIPTOMIC APPROACHES FOR A STRUCTURAL AND FUNCTIONAL</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>CHARACTERIZATION OF GRAPEVINE MIRNAS.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>17:00 – 17:20</td>
<td><strong>PIII-05 GENOMICS AND FUNCTIONAL GENOMICS OF SYMBIOTIC FUNGI.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>17:20 – 17:40</td>
<td><strong>PIII-07 BIOCHEMICAL, PROTEOMIC AND PHYSIOLOGICAL ANALYSIS OF</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>CHLOROPLAST-CHROMOPLAST TRANSITION IN SOLANUM LYCOPERSICUM.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>17:40 – 18:00</td>
<td><strong>PIII-10 ER AND GOLGI PROTEOME DYNAMICS DURING TOMATO FRUIT</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>RIpening.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:00 – 18:00</td>
<td><strong>PARALLEL SESSION: MEMBRANE DYNAMICS AND FUNCTIONS</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>16:00 – 16:40</td>
<td><strong>PRODUCTION OF REACTIVE OXYGEN SPECIES AT THE PLASMA MEMBRANE AND</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td></td>
<td><strong>THEIR ROLE IN GERMINATION AND ELONGATION GROWTH.</strong></td>
<td>AULA GIACOMINI - DBV</td>
</tr>
</tbody>
</table>
**ANJA LISZKAY** (IBITEC-S, CEA SACLAY – FRANCE)

**16:40 – 17:00**

*PII-06* AUXIN-RESPONSIVE GENES AIR12 CODE FOR A NEW FAMILY OF ASCORBATE-REDUCIBLE PLASMA MEMBRANE B-TYPE CYTOCHROMES.

**MARIA RAFFAELLA BARBARO** (UNIVERSITY OF BOLOGNA – ITALY)

**17:00 – 17:20**

*PII-04* DO DIFFERENT P-TYPE ATPASES SHARE A COMMON MECHANISM OF REGULATION?

**LAURA LUONI** (UNIVERSITY OF MILAN – ITALY)

**17:20 – 17:40**

*PII-02* SORTING AND TURNOVER OF TONOPLAST PROTEINS: THE ARABIDOPSIS POTASSIUM CHANNEL TPK1 AS A MODEL.

**MARIE MAITREJEAN** (CNR-IBBA – MILAN, ITALY)

**17:40 – 18:00**

*PII-08* COMMON PLAYERS IN PLANT ORGANELLE FISSION MECHANISMS: MORPHOLOGICAL AND MOLECULAR ANALYSES.

**CRISTINA RUBERTI** (UNIVERSITY OF PADUA – ITALY)

---

**ANJA LISZKAY** (IBITEC-S, CEA SACLAY – FRANCE)

**18:00 – 20:00**

**PLENARY SESSION: EPGENETICS**

**CHAIR: CARLO SOAVE**

**AULA II NEC**

**EPIALLELES FORMATION AND INHERITANCE IN PLANTS.**

**SERENA VAROTTO** (UNIVERSITY OF PADUA – ITALY)

**EPGENETICS REPROGRAMMING IN MAIZE**

**JOSE GUTIERREZ-MARCOS** (UNIVERSITY OF WARWICK WELLESBOURNE – UK)

**IMPACT OF EPGENETICS ON EVOLUTIONARY THEORIES**

**CARLO SOAVE** (UNIVERSITY OF MILAN – ITALY)

---

**TUESDAY, JULY 13th**

**09:00 – 11:00**

**PARALLEL SESSION: PLANT AND ENVIRONMENT**

**CHAIR: LUIGI DE BELLIS**

**AULA II NEC**

**09:00 – 09:15**

DIFFERENTIAL EXPRESSION OF SAPORIN GENES UPON WOUNDING, ABA TREATMENT AND LEAF DEVELOPMENT.

**ANDREA TARTARINI** (UNIVERSITY OF L’AQUILA – ITALY)

**09:15 – 09:30**

EXPLOITATION OF PLANT DIVERSITY FOR ADAPTIVE-RELATED TRAITS A. TONDELLI (GENOMIC RESEARCH CENTRE, FIORENZUOLA D’ARDA, ITALY)

**09:30 – 09:45**

*PV-03* SURFACE NADH PEROXIDASES AND OXYLIPIN PATHWAY ARE INVOLVED IN THE RECOVERY FROM PHYTOPLASMA DISEASE IN APPLE.

**ALBERTO BERTOLINI** (UNIVERSITY OF UDINE – ITALY)

**09:45 – 10:00**

*PV-12* FLOODING TOLERANCE AND PHOTOPERIODIC RESPONSE IN DIFFERENT PRUNUS SPP ROOTSTOCK MUTANTS

**LAURA PISTELLI** (UNIVERSITY OF PISA – ITALY)

**10:00 – 10:15**

*PV-09* EXPRESSED PECTIN METHYLESTERASE INHIBITOR (PMEI) GENES SHOW A DIFFERENT PATTERN OF ACCUMULATION IN WHEAT TISSUE AND FOLLOWING FUNGAL INFECTION

**VALENTINA ROCCHI** (UNIVERSITY OF VITERBO “LA TUSCIA” – ITALY)
<table>
<thead>
<tr>
<th>Time</th>
<th>Session/Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:15 – 10:30</td>
<td><strong>PV-01</strong> ISOLATION AND MOLECULAR CHARACTERIZATION OF TDP1 (TYROSYL-DNA PHOSPHODIESTERASE) GENES FROM BARREL MEDIC INVOLVED IN REPAIR OF DNA TOPOISOMERASE I-INDUCED DAMAGE AND OTHER OXIDATIVE DNA LESIONS. ALMA BALESTRAZZI (UNIVERSITY OF PAVIA – ITALY)</td>
</tr>
<tr>
<td>10:30 – 10:45</td>
<td><strong>PV-22</strong> IMPACT OF IRRADIANCE ON THE C ALLOCATION IN THE COASTAL MARINE DIATOM SKELETONEMA MARINOI SARNO &amp; ZINGONE. ALESSANDRA NORICI (POLITECNICO DELLE MARCHE – ITALY)</td>
</tr>
<tr>
<td>10:45 – 11:00</td>
<td><strong>PV-02</strong> DIFFERENTIAL ACTIVATION OF DEFENCE GENES AND ENZYMES IN MAIZE GENOTYPES WITH CONTRASTING LEVELS OF RESISTANCE TO FUSARIUM VERTICILLIOIDES. ADRIANO MAROCCO (UCSC, PIACENZA - ITALY)</td>
</tr>
<tr>
<td>09:00 – 11:00</td>
<td>PARALLEL SESSION: ENERGY CONVERSION CHAIR: ROBERTO BARBATO AULA GIACOMINI - DBV</td>
</tr>
<tr>
<td>09:00 – 09:40</td>
<td>THE DYNAMICS OF PHOTOSYNTHESIS ELECTRON TRANSPORT: MOLECULAR DETAILS OF REGULATORY MECHANISMS PAOLO PESARESI (UNIVERSITY OF MILAN – ITALY)</td>
</tr>
<tr>
<td>09:40 – 10:00</td>
<td><strong>PVI-03</strong> HEAT DISSIPATION IN THE MOSS PHYSCOMITRELLA PATENS: INSIGHTS ON THE EVOLUTION OF PROTECTION MECHANISMS UPON LAND COLONIZATION. ALESSANDRO ALBORESI (UNIVERSITY OF VERONA – ITALY)</td>
</tr>
<tr>
<td>10:00 – 10:20</td>
<td><strong>PVI-07</strong> MIXOTROPHIC GROWTH OF NEOCHLORIS OLEOABUNDANS AND LIPID SYNTHESIS INDUCTION UNDER NUTRIENT STARVATION. COSTANZA BALDISSEROTTO (UNIVERSITY OF FERRARA – ITALY)</td>
</tr>
<tr>
<td>10:20 – 10:40</td>
<td><strong>PVI-01</strong> SIMPLE ONE-STEP ISOLATION OF A PURE ACTIVE FORM OF PHOTOSYSTEM II DIRECTLY FROM PEA THYLAKOIDS. CRISTINA PAGLIANO (POLITECNICO DI TORINO, ALESSANDRIA – ITALY)</td>
</tr>
<tr>
<td>10:40 – 11:00</td>
<td><strong>PVI-02</strong> REGULATION OF LIGHT HARVESTING IN PLANTS: A STRUCTURAL BASIS FOR THE PH-DEPENDENT XANTHOPHYLL CYCLE. TOMAS MOROSINOTTO (UNIVERSITY OF PADUA – ITALY)</td>
</tr>
<tr>
<td>11:00 – 11:30</td>
<td>COFFEE BREAK</td>
</tr>
<tr>
<td>11:30 – 13:30</td>
<td>PLENARY SESSION: PLANT CELL SIGNALLING CHAIR: FIORELLA LO SCHIAVO AULA II NEC</td>
</tr>
<tr>
<td>11:30 – 12:15</td>
<td>PLASTID-TO-NUCLEUS SIGNALLING – INVOLVEMENT OF DUAL-TARGETED PROTEINS? KARIN KRUPINSKA CHRISTIAN-ALBRECHTS UNIVERSITY OF KIEL, GERMANY</td>
</tr>
<tr>
<td>12:15 – 13:00</td>
<td>A CALCIUM SENSOR / PROTEIN KINASE NETWORK FOR DECODING CALCIUM SIGNALS IN PLANTS. JORG KUHLA UNIVERSITÄT MÜNSTER, GERMANY</td>
</tr>
<tr>
<td>13:00 – 13:30</td>
<td>A HITCHHIKER'S GUIDE OF CA²⁺ HANDLING: BASIC PRINCIPLES OF CA²⁺ MEASUREMENT AND HANDLING IN MAMMALIAN CELLS. ILARIA DRAGO UNIVERSITY OF PADUA - ITALY</td>
</tr>
<tr>
<td>13:30 – 14:30</td>
<td>FREE LUNCH</td>
</tr>
<tr>
<td>14:30 – 16:00</td>
<td>POSTER VIEW</td>
</tr>
<tr>
<td>16:00 – 19:30</td>
<td>GENERAL ASSEMBLY – LECTURE A. BACCARINI MELANDRI</td>
</tr>
<tr>
<td>20:30</td>
<td>SOCIAL DINNER</td>
</tr>
</tbody>
</table>
**WEDNESDAY, JULY 14th**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chair/Presenter</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:50-11:00</td>
<td><strong>PLENARY SESSION: PLANT RECEPTORS AND SIGNALLING</strong></td>
<td><strong>GIULIA DE LORENZO</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>08:50-09:20</td>
<td>SENSING AND SIGNALING IN THE ABSCISIC ACID PATHWAY; MOLECULAR MECHANISMS.</td>
<td><strong>JOSÉ A. MÁRQUEZ</strong> EMBL. GRENOBLE - FRANCE</td>
<td></td>
</tr>
<tr>
<td>09:20-09:50</td>
<td>TO BE ANNOUNCED</td>
<td><strong>SILKE ROBATZEK</strong> (THE SAINSBURY LABORATORY –JIC, NORWICH – UK)</td>
<td></td>
</tr>
<tr>
<td>09:50-10:10</td>
<td>DANGER SENSING IN PLANTS</td>
<td><strong>GIULIA DE LORENZO</strong> (UNIVERSITY OF ROME “SAPIENZA” – ITALY)</td>
<td></td>
</tr>
<tr>
<td>10:10-10:30</td>
<td>TEMPORAL AND SPATIAL REGULATION OF CELL CYCLE GENES BY THE SHR/SCR NETWORK LINKS PATTERNING AND GROWTH</td>
<td><strong>ROSANGELA SOZZANI</strong> DUKE UNIVERSITY, DURHAM, NORTH CAROLINA, USA</td>
<td></td>
</tr>
<tr>
<td>10:30-10:45</td>
<td><em>PVII 03</em> THE RATE OF CELL DIFFERENTIATION CONTROLS THE ARABIDOPSIS ROOT MERistem GROWTH PHASE.</td>
<td><strong>SERENA PERILLI</strong> (UNIVERSITY OF ROME “SAPIENZA” – ITALY)</td>
<td></td>
</tr>
<tr>
<td>10:45-11:00</td>
<td><em>PVII 05</em> FROM PLANT TO HUMAN PHYSIOLOGY: ROLE OF THE PHYTOTOXIN FUSICOCCIN IN THE AGGREgATION PROCESS.</td>
<td><strong>CRISTINA DI LUCENTE</strong> (UNIVERSITY OF ROME “TOR VERGATA” – ITALY)</td>
<td></td>
</tr>
<tr>
<td>11:00-11:30</td>
<td><strong>COFFEE BREAK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30-13:00</td>
<td>Parallel Session: PLANT METABOLISM AND SECONDARY METABOLITES</td>
<td><strong>SERGIO ESPOSITO</strong></td>
<td>AULA II NEC</td>
</tr>
<tr>
<td>11:30-12:00</td>
<td>MARINE PLANT METABOLITES IN THE LIGHT OF ECOLOGICAL AND PHYSIOLOGICAL CONSIDERATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00-12:15</td>
<td><em>PIX-01</em> THE ACTIVITY OF ARABIDOPSIS THALIANA Δ1-PYRROLINE-5-CARBOXYLATE REDUCTASE IS MODULATED BY GLUTAMATE AVAILABILITY, AND BY BOTH THE RATIO AND THE REDOX STATUS OF PYRIDINE NUCLEOTIDE COFACTORS.</td>
<td><strong>DAVIDE PETROLLINO</strong> (UNIVERSITY OF FERRARA – ITALY)</td>
<td></td>
</tr>
<tr>
<td>12:15-12:30</td>
<td><em>PIX-05</em> GREEN COFFEE (COFFEA ARABICA L.) BEANS HARVESTED IN ETHIOPIA, INDIA, KENYA AND TANZANIA SHOW DIFFERENT LIPOLYTIC ACTIVITIES.</td>
<td><strong>SONIA PATUI</strong> (UNIVERSITY OF UDINE – ITALY)</td>
<td></td>
</tr>
<tr>
<td>12:30-12:45</td>
<td><em>PIX-11</em> EFFECT OF STORAGE TEMPERATURE ON VITAMIN E CONTENT (TOCOPHEROLS) IN BROCCOLI BRASSICA RAPA L. SUBSP. SYLVESTRIS.</td>
<td><strong>GIUSEPPINA MASSARO</strong> (2nd UNIVERSITY OF NAPLES, CASERTA – ITALY)</td>
<td></td>
</tr>
<tr>
<td>12:45-13:00</td>
<td><em>PIX-09</em> OVEREXPRESSION, REFOLDING AND CHARACTERISATION OF P2-G6PDH FROM BARLEY ROOTS.</td>
<td><strong>MANUELA CARDI</strong> (UNIVERSITY’ OF NAPLES “FEDERICO” II – ITALY)</td>
<td></td>
</tr>
<tr>
<td>13:00-13:15</td>
<td><em>PIX-13</em> THE CP12-MEDIATED COMPLEX OF PHOTOSYNTHETIC ENZYMES ISOLATED FROM NICOTIANA TABACUM AND ARABIDOPSIS THALIANA.</td>
<td><strong>LUCIA MARRI</strong> (UNIVERSITY OF BOLOGNA – ITALY)</td>
<td></td>
</tr>
<tr>
<td>13:15-13:30</td>
<td><em>PIX-16</em> TRANSCRIPT PROFILING OF TOMATO MUTANTS PRODUCING ANTHOCYANINS IN THE FRUIT.</td>
<td><strong>GIOVANNI POVERO</strong> (SCUOLA SUPERIORE SANT'ANNA, PISA – ITALY)</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30 – 13:30</td>
<td>Parallel session: SIGNALLING AND PLANT GROWTH REGULATORS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHAIR: ALESSANDRO VITALE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AULA GIACOMINI - DBV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30-12:10</td>
<td>PROTON PUMP INTERACTOR 1: A REGULATORY PROTEIN IN SEARCH OF A PHYSIOLOGICAL FUNCTION.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M.I. DE MICHELI S (UNIVERSITY OF MILAN – ITALY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:10-12:30</td>
<td>PX-01 THE DEVELOPMENTAL MUTANTS OF BARLEY: GENETIC AND PHYSIOLOGICAL ANALYSIS TO DESIGN THE PLANT FOR THE FUTURE.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANTONIO MICHELE STANCA (UNIVERSITY OF MODENA E REGGIO EMILIA) ITALY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30-12:50</td>
<td>PX-04 CHARACTERIZATION OF ARABIDOPSIS INSERTIONAL MUTANTS FOR COPPER-CONTAINING AMINE OXIDASES.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALESSANDRA TISI (UNIVERSITY OF ROMATRE – ITALY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:50-13:10</td>
<td>PVII-11 THE HSP20-LIKE CHAPERONE P23 OF ARABIDOPSIS IS A SPECIFIC TARGET OF CK2, INVOLVED IN SALICYLIC ACID SIGNALLING PATHWAY.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>STEFANO D’ALESSANDRO (UNIVERSITY OF PADUA – ITALY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:10-13:30</td>
<td>PX-05 AUXIN AND GIBBERELLINS INTERACTION DURING FRUIT SET IN TWO TOMATO AUXIN MUTANTS: GIBBERELLIN BIOSYNTHESIS GENE EXPRESSION AND NUCLEAR DNA CONTENT.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCESCO MIGNOLLI (UNIVERSITY OF PISA – ITALY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>CLOSING REMARKS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Plenary Session: PLANT NUTRIENTS FOR HUMAN HEALTH

**Engineering phenylpropanoid production for healthy foods**

Cathie Martin¹, Lucilla Titta², Marco Giorgio², Chiara Tonelli³, Katia Petroni³, Marie Claire Toufektsian⁴, Michel de Lorgeril⁴, Maria Benedetta Donati⁵, Domenico Rotillo⁵, Jie Luo¹, Giuseppe Recupero Reforgiato⁶, Concetta Licciardello⁶ and Eugenio Butelli¹

¹John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom.
²Experimental Oncology Dept, European Institute of Oncology, Via Adamello 16, Milano, Italy.
³Dipartimento di Scienze Biomoleculari e Biotechnologie, 20133 Milan, Italy.
⁴Equipe PRETA-Coer et Nutrition, TIMC-IMAG Unite Mixte de Recherche, Universite Joseph Fourier CNRS 5525, F-38000, Grenoble, France.
⁵John Paul II Centre for High Technology Research and Education in Biomedical Sciences, Catholic University, 86100 Campobasso, Italy.
⁶Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee, Acireale, Sicily, Italy

Plants produce a very broad array of metabolites, which are not essential for growth, but are used to provide protection against stress and pathogens, to attract pollinators and dispersal agents and as signals for development. These are often referred to as 'secondary metabolites' but are known more generally as plant 'natural products'. Natural products, particularly phenylpropanoids, have recently been recognised as important components of the diet, offering protection against cardiovascular diseases, certain cancers and age-related degenerative diseases.

Plants often accumulate their natural products to relatively low levels, so there is interest in breeding or engineering plants that produce higher levels. The most effective way to increase the accumulation of secondary metabolites is to increase the activity of genes that regulate the activity of the biosynthetic pathways. This can be done using natural variants as in the case of corn and blood orange, or by metabolic engineering as in the case of tomato. Breeding of blood orange by selecting for regulatory gene expression in fruit has produced varieties which demonstrate effects against obesity and inhibition of adipocyte development, while varieties of corn which produce high levels of anthocyanin in their kernels show cardioprotective properties in preclinical studies. The effectiveness of metabolic engineering using genes encoding transcription factors has been demonstrated by the production of high-flavonol and high-anthocyanin tomatoes which have 3-4 fold higher antioxidant capacities. These have the potential to offer protection against a range of diseases when included as part of the diet and are currently being assessed on animal models.
Low phytic acid mutants: useful tools either for research in the field of seed physiology, or for food and feed biofortification.

Erik Nielsen

*Dipartimento di Genetica e Microbiologia, Università di Pavia, via Ferrata 1 27100 Pavia.*

Phytic acid is considered as one of the major antinutritional compounds in cereal and legume seeds. The development of *lpa* (low phytic acid) grains, resulting in increased phosphate and mineral cation availability, is a major goal in the improvement of the nutritional quality of seed crops such as maize, used to prepare feeds for monogastric animals, or as common bean, largely consumed by people of developing countries. We isolated a maize low-phytic-acid mutant (*lpa1-241*) which, due to negative pleiotropic effects verified on germination and seedling growth rates, did not result to be an exploitable means for feed biofortification with iron and phosphate. However, it revealed very useful to prove the role of phytic acid as an anti-oxidative stress molecule able to prevent ROS formation during seed storage. Moreover, genetic and molecular analyses of the *lpa1-241* mutation indicated an epigenetic origin of this trait, namely a paramutagenic interaction resulting in meiotically heritable changes in the expression of ZmMRP4 gene (encoding a vacuolar transporter), causing a strong pleiotropic effect on the whole plant.

An *lpa* mutation (*lpa 280-10*) was isolated also in common bean and, thanks to the collaborations with, dr. Pilu (Milan University), dr. Sparvoli (IBBA-CNR, Milan) and dr. Campion (CRA Institute of Montanaso Lombardo, Lodi) research groups, it was recently mapped on chromosome 1 and characterized at biochemical and molecular level. Although the genetic lesion appears to be in a gene orthologous to that mutated in the maize *lpa1-241* line, *lpa-280-10* mutant shows quite a different phenotype: agronomic trials performed during 2 growing seasons revealed that the mutant germination rate and seed yield are close to those of its parents, thus indicating it is the first *lpa* mutation devoid of visible macroscopic negative effects in plants, pods and seeds. Moreover, free iron content of *lpa 280-10* mutant grains is about seven fold higher as compared to parents, and preliminary data of experiments carried out in an “in vitro” system (caco-2 cells) indicate that their iron bioavailability is also increased, thus fulfilling the purposes of biofortification.

As to the basic aspects, we are planning experiments aimed to clarify the relationship between vacuolar transporter loss of function and phytic acid shortage as well as the reason for the difference in viability and yield of these two analogous mutants in maize and bean are underway.
PI01

**PHENOLS AND BIOLOGICAL ACTIVITY IN SWEET CHERRIES (CV. FERROVIA).**

C. NEGRO, S. PANZANARO, E. NUTRICATI, L. DE BELLIS, A. MICELI

Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Via per Monteroni, Università del Salento, 73100- LECCE.

*Keywords:* Prunus avium, polyphenols, antioxidant activity, anti-inflammatory activity.

Sweet cherries (Prunus avium L.) are one of the most popular fruits of the temperate zone, representing an important resource; they are consumed mainly non-processed as well as a component in fruit cocktails, yogurt, etc. Sweet cherries contain polyphenols which are responsible for different healthy effects. Different evidences suggest an inverse correlation between high intake of plant products, cardiovascular diseases and some cancers. Phenols and anthocyanins of the sweet cherries, contribute to the bioactivity of this fruit, principally for antioxidant (AA) and anti-inflammatory (AI) properties. In this work was determined the total phenols, qualitative and quantitative anthocyanins composition, AA and AI of cherries cv. Ferrovia extracts, cultivated in Apulia; AI was compared with synthetic drugs. Results showed that phenols was 142.9 mg/100 g of fresh weight (FW), while the anthocyanins was 36.4 mg/100 g FW and cyanidin-3-arabinoside was the pigment most abundant. AA was equal to 48 µmol Trolox/g FW and 70% was in relation to the anthocyanins content. AI, reported as percentage of inhibition of the COX, was comparable with the Ibuprofen 10 µM, a commercial anti-inflammatory.

PI02

**ROLE OF ANTHOCYANIN-RICH FOOD IN CARDIOPROTECTION AND OBESITY.**

TONELLI C.¹, PILU R.², PETRONI K.¹, CALVENZANI C.¹, TOUFEKTSIAN M.C.², DE LORGERIL M.³, REFORGIATO RECUPERO G.⁴, RAPISARDA P.⁵, TITTA L.⁵, GIORGIO M.⁵

¹ Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, 20133 Milano (Italy) ² Dipartimento di Produzione Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano (Italy) ³ Nutrition, Vieillisement et Maladie Cardiovasculaires, Université Joseph Fourier, Domain de la Merci, 38056 Grenoble (France) ⁴ Centro di Ricerca per l'Agrumicoltura e le Culture Mediterranee, Corso Savoia 190, 95024 Acireale (CT, Italy) ⁵ Dipartimento di Oncologia Sperimentale, Istituto Europeo Oncologico, Via Adamello 16, 20139 Milano (Italy).

*Keywords:* transcription factors, anthocyanins, Zea mays, Citrus sinensis, health.

In the frame of the EU-funded project FLORA, we developed maize isogenic lines with high levels or no anthocyanins by using specific alleles of flavonoid regulatory genes and we provided them as dietary supplements to test the impact of anthocyanins on cardiovascular and age-related diseases using animal model systems. These studies demonstrated that in rats fed anthocyanin-rich maize the amount of cardiac tissue that was damaged following ischemic conditions was reduced by approximately 30% compared to rats fed anthocyanin-free maize. Cardioprotection was associated with increased myocardial glutathione levels, suggesting that dietary anthocyanins modulate cardiac antioxidant defences. Furthermore, we investigated the effect of blood and common orange juice on fat accumulation in mice fed standard or high fat diet. Results revealed that providing blood orange juice, but not common orange juice, as a substitute of water, reduced significantly body weight gain and fat accumulation, thus impairing the high-fat induced obesity. The gene expression profile of fat tissue from mice eating high-fat diet and drinking blood orange juice strikingly resembled that of mice fed standard diet.
Plenary Session: PLANT NUTRIENTS FOR HUMAN HEALTH

PI03

CHARACTERIZATION OF RIBES NIGRUM AND ROSMARINUS OFFICINALIS FOOD SUPPLEMENTS.

GROSSO A.1, MARZOCHELLE L.2, ADUCCI P.1, BEI R.2, MARRA M.1.

1 Department of Biology, University of Tor Vergata, Rome, Italy. 2 Department of Experimental Medicine and Biochemical Sciences, University of Tor Vergata, Rome, Italy.

Keywords: Food supplement, HPLC, chemoprevention.

Ribes nigrum and Rosmarinus officinalis are widespread in the Mediterranean area, where they are commonly used as food spice, or odorant in fragrances, as well as traditional remedy in folk medicine. Despite the ever growing scientific and commercial interest in the characterization of the chemical composition and of the biological properties of officinal plant extracts and preparations, much work is still to be done to achieve a wider knowledge of their bioactive compound profiles and a better comprehension of their beneficial effects on human health. For instance, whereas antimicrobial, anti-inflammatory and antioxidant activities are often investigated, much less it is known about their effect in cancer prevention. Here, we report on the HPLC secondary metabolites profile characterization of commercially available Rosmarinus officinalis and Ribes nigrum ethanolic extracts. The effect of these extracts on the proliferation of two different head/neck cancer cells lines has also been investigated.

PI04

AN EMMER (TRITICUM DICOCCON SCHRANK) TETRAMERIC INHIBITOR OF ALPHA-AMYLASE.

FONTANINI D.1, CAPOCCHI A.1, GALLESCHI L.1, MUCCILLI V.2, CUNSOLO V.2, SALETTI R.2, FOTI S.2.

1 Department of Biology, University of Pisa, Via L. Ghini, 5 - 56126, Italy. 2 Department of Chemical Sciences, University of Catania, V.le A. Doria, 6 - 95125, Italy.

Keywords: emmer, tetrameric inhibitor, alpha-amylase.

Cereals seeds contain large amounts of starch, making them vulnerable to the attack of pests and herbivores feeding on it. Nonetheless, plants have developed a defence system involving the expression of a set of inhibitors acting on heterologous amylases of different origins. Plant alpha-amylase inhibitors have great potential as tools for engineering pest-resistance crops. They are also drug-design targets for treatment of diabetes and obesity and are well known as sensitizing agents in human. Wheat grains are rich in alpha-amylases inhibitors, which act as monomers, homodimers and heterotetramers. Investigating the soluble protein complement of the hulled wheat emmer with the purpose of profiling its antinutritional and allergenic factors of protein nature, we have isolated and characterized a tetrameric alpha-amylase inhibitor. Based on mass spectroscopy data, it is an assembly of proteins highly homologous to the CM2/CM3/CM16 found in durum wheat. Our data indicate that these proteins can also inhibit exogenous alpha-amylases in binary assemblies, suggesting that these proteins range of action, in vivo, may be wider than originally thought.
PI05

MAPPING AND MOLECULAR CHARACTERIZATION OF A COMMON BEAN LOW PHYTIC ACID MUTATION USABLE IN EFFORTS OF IMPROVING IRON AND ZINC BIOAVAILABILITY.

DARIO PANZERI¹, ELENA CASSANI², ENRICO DORIA³, GIOVANNI TAGLIABUE¹, ROBERTO BOLLINI¹, BRUNO CAMPION⁴, ERIK NIELSEN³, ROBERTO PILU², FRANCESCA SPARVOLI¹.

¹ Istituto di Biologia e Biotecnologia Agraria, CNR, Milano (Italy); ² Dipartimento di Produzioni Vegetali, Università degli Studi di Milano (Italy); ³ Dipartimento di Genetica e Microbiologia, Università di Pavia (Italy); ⁴ Istituto Sperimentale per l'Orticoltura, CRA, Montanaso Lombardo, Lodi (Italy).

Keywords: common bean, low phytic acid mutant, mapping, molecular characterization, MRP transporter.

Phytic acid (InsP6) is the main responsible for poor seed micronutrient bioavailability to humans. In fact, it chelates various mineral cations (i.e. calcium, iron, zinc etc.) so that they are poorly absorbed in the intestine and largely excreted, resulting in micronutrient deficiencies. In common bean, the legume most used for human nutrition, the phytic acid presence causes, particularly in developing countries, serious problems concerning iron and zinc bioavailability. We had previously isolated a lpa (low phytic acid) 280-10 common bean line which might be used for food biofortification displaying a 90% reduction of InsP6 content and a consequent seven-fold increase in the level of free iron. In the present work we performed a fine mapping of the lpa-280-10 mutation and found it is on bean chromosome 1 in a multi-drug resistance protein (MRP) ATP-binding cassette (ABC) membrane transporter gene (PvMRP-1). DNA sequencing data showed the occurrence of a single amino acid substitution (E to K) in this gene. We also provide data indicating the presence in both common bean and soybean of a second MRP gene, highly homologous to PvMRP-1.

PI06

TRANSCRIPTIONAL-METABOLIC NETWORKS IN BETA-CAROTENE-ENRICHED POTATO TUBERS: THE LONG AND WINDING ROAD TO THE “GOLDEN” PHENOTYPE.

GIANFRANCO DIRETTO¹, SALIM AL-BABILI², RAFFAELA TAVAZZA¹, FEDERICO SCOSSA¹, VELIA PAPACCHIOLI¹, MELANIA MIGLIORE¹, PETER BEYER² AND GIOVANNI GIULIANO¹.

¹ ENEA, Casaccia Research Center, Via Anguillarese 301, Roma 00123, Italy. ² Center for Applied Biosciences, Faculty of Biology, Universität Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany.

Keywords: potato, transcriptional-metabolic profiling.

Vitamin A deficiency (VAD) is a public health problem in a large number of countries. Biofortification of major staple crops (wheat, rice, maize or potato) with beta-carotene has the potential to alleviate this nutritional problem. Previously, we engineered transgenic “Golden” potato tubers accumulating the highest amount of beta-carotene in the four aforementioned crops. Here we report the systematic quantitation of carotenoid metabolites and transcripts in 24 lines carrying 6 different transgene combinations. Low levels of transgene expression are sufficient for interfering with leaf carotenogenesis, but not for β-carotene accumulation in tubers and calli, which requires high expression levels of three transgenes under the control of the Patatin promoter. We used hierarchical clustering pairwise correlation and network correlation analysis to assess the perturbations in transcript and metabolite levels in transgenic leaves and tubers. Through a “guilt-by-profiling” approach, we identified a series of endogenous genes likely to play a key regulatory role in “Golden” tubers, which are candidates for manipulations aimed at the further optimization of tuber carotenoid content.
Fructans are fructose polysaccharides occurring in species of Monocots and Dicots. They have prebiotic properties, since they are not digested by human gastro-intestinal enzymes and selectively promote the growth of beneficial bacteria of human gut such as *Lactobacilli* and *Bifidobacteria*. Among plant of nutritional interest wheat contains a significant amount of fructans. Kernels are particularly rich in graminan (the cereal fructans) during the first period of maturation (9-17 days from anthesis); whereas, their content decrease as the development proceeded. In this study the changes in the levels and polymerization degree of the graminan stored in different kernel tissues has been further investigated.

The enzymes responsible for fructans synthesis and hydrolysis have been also analysed at different phase of kernel maturation. During the first phase of wheat kernel maturation graminan biosynthesis is particular active, even if the expression of 6-SFT, the main enzyme involved in their biosynthesis, seem to be constant. Consistently with the change in the graminan level during kernel maturation, the activities of the enzymes involved in their hydrolysates show a peak corresponding to the phase of graminan decrease, while the expression of both 6FEH and 1FEH, the two enzymes responsible of graminan hydrolysis, does not change during the whole analysed period. The results here presented suggest that, during kernel maturation, fructan metabolism is not regulated at level of gene expression.
Production of reactive oxygen species at the plasma membrane and their role in germination and elongation growth

Anja Liszkay¹, Eiri Heyno¹, Kerstin Müller², Peter Schopfer²

¹Service de Bioenergetique, iBiTec-S, CEA Saclay, 91191 Gif-sur-Yvette, France
²Universität Freiburg, Institut für Botanik II, 79104 Freiburg, Germany

The plant plasma membrane exhibits enzyme activities for reducing O₂ to the superoxide anion radical (O₂⁻) utilizing NAD(P)H as a reductant. This activity is generally attributed to a transmembrane NADPH oxidase activity. In addition, a membrane-associated quinone reductase has been shown to produce O₂⁻ in vitro. Immunoblots show that both enzymes, the NADPH oxidase and the quinone reductase, are present in the growth zone of shoots and roots. The effect of inhibitors and activators of both enzymes were studied in isolated membranes. The physiological role of these enzymes during germination and elongation growth was investigated in vivo. A mutant defective in one of the NADPH oxidase genes (AtrbohC) produced significantly less 'OH radicals in vivo and was affected in growth. Abscisic acid inhibited the production of reactive oxygen species and germination. The presented results provide the experimental background for a hypothesis on the mechanism of plant cell growth in which 'OH, produced from O₂⁻ and H₂O₂ by cell wall peroxidase, acts as a wall-loosening agent. Backbone cleavage of cell wall polysaccharides can be accomplished by 'OH, produced from H₂O₂ in a Fenton reaction.
VIRAL K⁺ CHANNELS AS MINIMAL MODELS TO STUDY STRUCTURE AND FUNCTION OF CHANNEL GATING AND PERMEATION.

ANNA MORONI¹ AND GERHARD THIEL²

¹Dept. of Biology, University of Milan, Italy; ²Institute of Botany, TU-Darmstadt, Germany.

Keywords: channels, virus, potassium, transport.

Plant viruses belonging to the Phycodnaviridae family code for potassium (K⁺) channel proteins. Typical of these proteins is their miniature size due to the absence of cytosolic regulatory domains. Viral K⁺ channels expressed in heterologous systems show biophysical properties of more complex, plant and animal K⁺ channels: they are selective, sensitive to blockers and gated. Interesting for cell biology is that these channel proteins are sorted according to inherent structural information to distinct cellular membranes such as the plasma membrane and the mitochondria. Given their small size, their relative simplicity and the large variety of channel orthologs, these proteins offer a major advantage to pursue an understanding of structure/function correlates. Using a combination of functional assays, computational modeling and structural investigations we are able to uncover principles of structure/function, which are relevant to explain functional properties such as gating and block by inhibitors. Their similarity to all other K⁺ channels bears implications for the general understanding of K⁺ channel function.

SORTING AND TURNOVER OF TONOPLAST PROTEINS: THE ARABIDOPSIS POTASSIUM CHANNEL TPK1 AS A MODEL.

MARIE MAITREJEAN¹,², MICHAEL M. WUDICK²,³, CAMILLA VOELKER², EMANUELA PEDRAZZINI¹, KATRIN CZEMPINSKI², ALESSANDRO VITALE¹.

¹Istituto di Biologia e Biotecnologia Agraria, CNR, via Bassini 15, 20133 Milano, Italy, EU. ²Institut fur Biochemie und Biologie, Universitat Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm, Germany, EU. ³Biologie & Physiologie Molèculaire des Plantes, SupAgro/INRA/CNRS/UM2, 2 Place Viala, bat. 7, 34060 Montpellier, Cedex 2, France EU.

Keywords: endoplasmic reticulum, protein sorting, protein turnover, quality-control, tonoplast.

We are using the potassium channel AtTPK1 as a model to study how integral tonoplast proteins are sorted to their destination and eventually degraded. Starting from a TPK1-GFP fusion, domains were substituted by the corresponding ones of the plasma membrane homologue AtTPK4. Expression in Arabidopsis protoplasts indicated that the cytosolic C-terminus of AtTPK1 is necessary and sufficient for tonoplast sorting of the potassium channel. TPK1-GFP formed dimers both in transgenic Arabidopsis and in protoplasts, whereas a number of TPK1/TPK4 fusions that remained located in the endoplasmic reticulum (ER) were unable to assemble correctly and interacted extensively with the ER chaperone BiP. This indicated that chimeric fusions remained in the ER because of conformational defects leading to retention by ER quality control. Pulse-chase labelling of protoplasts indicated that TPK1-GFP has a half life time of at least 24 hours and that its slow turnover involves internalization into the vacuole, leading to the vacuolar accumulation of soluble, released GFP that was originally fused to the C-terminus. Supported by the EU Marie Curie RTN (MRTN-CT-2006–035833).
Parallel Session: MEMBRANE DYNAMICS AND FUNCTIONS

PII03

PLASMA MEMBRANE CA\textsuperscript{2+}-ATPASE FOR CRYSTALLISATION: PRODUCTION AND PURIFICATION OF THE DELETED MUTANT DELTA109ACA8.

LAURA LUONI, CLAUDIO OLIVARI, M. CRISTINA BONZA.

Department of Biology, University of Milano.

Keywords: Ca\textsuperscript{2+}-ATPase, heterologous expression, Arabidopsis thaliana, P-type ATPase.

The best characterised isoform of plant plasma membrane Ca\textsuperscript{2+}-ATPase is Arabidopsis ACA8. ACA8 is a P-type ATPase characterised by the presence of a N-terminal domain with autoinhibitory function that interacts with a region localised inside the actuator domain between the transmembrane elics 2 and 3. The autoinhibitory domain of ACA8 partially overlaps a CaM-binding site: CaM-binding suppress autoinhibition. To identify in detail the structural basis of the interaction between the autoinhibitory domain and the intramolecular site it is essential to obtain the three-dimensional structure of the protein. Unfortunately, crystallisation trials of wt ACA8 have been unsuccessful so far. Since N-terminus is predicted to be mostly disordered, we have produced a mutant deleted of the first 109 aa with a His-tag at the C-terminus. This mutant is successfully over-expressed in the yeast strain K616 and is fully functional. We have set the affinity chromatography purification procedure and we are arranging a size exclusion chromatography step to improve the purification level. We will try to co-crystallise this mutant with a peptide reproducing the CaM-binding domain.

PII04

DO DIFFERENT P-TYPE ATPASES SHARE A COMMON MECHANISM OF REGULATION?

M. CRISTINA BONZA\textsuperscript{1}, LAURA LUONI\textsuperscript{1}, SABINA VISCONTI\textsuperscript{2}.

\textsuperscript{1} Department of Biology, Uni Milano. \textsuperscript{2} Department of Biology, Uni Roma "Tor Vergata".

Keywords: chimeras, Ca\textsuperscript{2+}-ATPase, H\textsuperscript{+}-ATPase, plant, animal.

P-type ATPases actively pump ions across cell membranes. Various members have an autoinhibitory domain at the protein C- or N-terminus. ACA8 is a plasma membrane (PM) Ca\textsuperscript{2+}-ATPase of A. thaliana with a N-te autoinhibitory and CaM-binding domain: CaM binding suppresses autoinhibition. This regulatory mechanism is similar to that of animal PM Ca\textsuperscript{2+}-ATPase and plant PM H\textsuperscript{+}-ATPase, but in these proteins the regulatory site is located in the C-te. To test the hypothesis that different P-type ATPases may share a common mechanism of autoinhibition, a mutant in which the N-te of ACA8 is inserted at the C-te of the protein has been constructed using the CaM-insensitive mutant delta74ACA8. Moreover, chimeras in which delta74ACA8 is fused to the C-te of AHA1 (isoform 1 of A. thaliana PM H\textsuperscript{+} pump) or of PMCA4b (isoform 4b of human PM Ca\textsuperscript{2+} pump) have been produced in yeast. Results show that the regulatory function of the terminal domain is independent from its position in ACA8 and that regulatory domains belonging to related P-type ATPase are able to partially autoinhibit ACA8. This is the first experimental evidence of a general mechanism for pump autoinhibition of different P-type ATPases.
Mutations of phosphorylatable serine residues in the N-terminus of the plasma membrane Ca-ATPase ACA8.

SONIA GIACOMETTI, CLAUDIA ANNA MARRANO, MARGHERITA LIMONTA, MARIA CRISTINA BONZA, LAURA LUONI, MARIA IDA DE MICHELI.

Dip. Biologia, UNI Milano, Istituto di Biofisica del CNR, Sezione Milano, via Celoria 26, 20133 Milano, Italy.

Keywords: Ca-ATPase, Phosphorylation, Arabidopsis.

ACA8 is a type 2B Ca-ATPase with an autoinhibitory N-terminus whose action is suppressed by binding of calmodulin (CaM). Phospho-proteomic analysis of Arabidopsis plasma membrane has identified in ACA8 N-terminus 5 Ser phosphorylated in vivo. Since these Ser are not conserved in other isoforms of ACA, phosphorylation of any of them may represent an isoform specific mechanism of regulation of pump activity. We have produced ACA8 mutants in which each of the 5 Ser has been mutated either to Asp, to introduce a negative charge mimicking the phosphorylation event, or to Ala to eliminate a phosphorylation site. All these mutants are over-expressed in yeast and functional. Most of these mutants have a slightly higher basal activity than the WT enzyme, but the difference between the S/D and the S/A mutants is not highly significant: thus the effect of mutation cannot be ascribed to the introduction of the negative charge. Three mutants have a lower apparent affinity for CaM: fusion proteins between the first 116 aa of these mutants and a 6His-tag have been produced in E. coli and purified for a detailed analysis of their interaction with CaM by surface plasmon resonance (in progress).

Auxin-responsive genes AIR12 code for a new family of ascorbate-reducible plasma membrane b-type cytochromes.

MARIA RAFFAELLA BARBARO1, ALEX COSTA2, PAOLO PUPILLO1, PAOLO TROST1, VALERIA PREGER1.

1 Department of Experimental Evolutionary Biology, University of Bologna, Via Irnerio 42, Bologna 40126, Italy; 2 Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padua, Italy.

Keywords: redox, plasma membrane, cell wall, auxin.

AIR12 is the major plasma membrane cytochrome b belonging to a new family of ascorbate-reducible cytochromes b specific to flowering plants. It is bound in vivo to the external side of the plasma membrane by means of a GPI-anchor and has been found associated with lipid rafts both in Medicago and Arabidopsis. Arabidopsis AIR12, heterologously expressed for the first time, is shown to be a high-potential cytochrome b with a symmetrical İ=band at 560 nm, to be reduced by ascorbate and superoxide, and oxidized by monodehydroascorbate, NO and peroxynitrite. Arabidopsis lines transformed with AIR12 promoter fused to the GUS or GFP gene show localized expression at sites where cell separation events occur (e.g. lateral root caps, root epidermis at site of lateral root emergence, micropylar endosperm during germination, anthers and floral organ abscission zones, hydatodes) as well as in the vascular bundles of mature leaves and trichomes support cells. Exogenously applied auxins boost reporter gene expression in the entire roots while ABA enhances expression specifically at the root tip. From available data, a role of AIR12 in cell wall modification processes is proposed.
PII07

FIRST EVIDENCE FOR GLU/PRO ANTIPORT IN ISOLATED DURUM WHEAT CHLOROPLASTS.

CATELLO DI MARTINO.

Dipartimento SAVA Università degli studi del Molise.

Keywords: GLU/PRO exchange, reducing equivalents.

In the light of the chloroplast localization of the enzymes which metabolise glutamate in plant, we investigate how externally added glutamate enters the chloroplasts isolated from durum wheat, chosen as a model system since it grows naturally under stress conditions. As a result of glutamate addition, the appearance of proline was found outside chloroplasts as measured by HPLC measurement and spectroscopically. The rate of proline appearance outside chloroplasts proved to depend on the GLU/PRO exchange due to the novel chloroplast glutamate/proline antiporter (Vmax 4 nmol/min/mg protein and Km 3 mM). Moreover, the use of thiol reagent mersalyl, non-penetrant in chloroplasts, decreased significantly (about 50%) proline appearance rate in external medium. Because chloroplasts have been reported as the major site for the synthesis of proline that gets accumulated under stress, it is necessary to realize if over production of such compatible solutes in chloroplasts influence the bioenergetic processes in this organelle and if GLU/PRO exchange play a relevant role to export reducing equivalents from chloroplast to cytoplasm and/or other organelles across the chloroplast envelope.

PII08

COMMON PLAYERS IN PLANT ORGANELLE FISSION MECHANISMS: MORPHOLOGICAL AND MOLECULAR ANALYSES.

CRISTINA RUBERTI, ALEX COSTA, MICHELA ZOTTINI, FIORELLA LO SCHIAVO.

Dipartimento di Biologia, Università degli Studi di Padova, Italia.

Keywords: organelles dynamics, mitochondria, peroxisomes, fission machinery.

Mitochondria and peroxisomes have a dynamic plasticity in shape and morphology. Studies in mammals, fungi and plants indicate that mitochondria and peroxisomes partially share components of their division machinery. In Arabidopsis, dynamin-like proteins DRP3A and DRP3B and the fission-like protein BIGYIN have been implicated in mitochondria and peroxisomes division. In addition, another specific plant factor ELM1 has been reported to be involved in mitochondrial fission. In order to gain inside the molecular mechanisms involved in the organelles remodelling, we analyzed the expression pattern and the subcellular localization of BIGYIN and ELM1 during plant development. To this aim, Arabidopsis transgenic plants expressing the GUS reporter gene under the control of BIGYIN and ELM1 promoters, and plants transformed with pbBIGYIN-YFP:BIGYIN construct were produced and analyzed. The in vivo interaction between these two proteins was tested by means of BiFC. Our data indicate that ELM1 and BIGYIN show a strong tissue specificity and co-expression during plant development and that their subcellular localization is not restricted to the compartments previously described.
ER AND GOLGI PROTEOME DYNAMICS DURING TOMATO FRUIT RIPENING.

F. SPINELLI, B. MATTEI, D. PONTIGGIA, L. MARIOTTI, . FABBRI, F. CERVONE, G. DE LORENZO.

Dipartimento di Biologia Vegetale, Sapienza Università di Roma, P.le a. Moro 5, 00185 Roma, Italy.

Keywords: ER, Golgi, tomato, proteomics.

The endoplasmic reticulum (ER) and the Golgi apparatus are crucial for the secretory pathway and play a central role in fruit ripening. The Golgi complex functions to posttranslationally modify newly synthesized proteins and sort them, packaged in vesicles for transport to their sites of function. We aim to monitor the pattern of protein expression along the secretory pathway during tomato fruit ripening. ER and Golgi microsomal vesicles were separated by centrifugation through different types of density gradients. Assuming that an organelle has a unique distribution pattern it is possible to identify ER or Golgi components by comparing their enrichment using known markers of these compartments. Profiling of protein distribution was determined by DIGE analysis of gradient fractions, respectively enriched in the ER and Golgi microsomes, to allow quantitative evaluation of differentially expressed proteins. Moreover, to overcome technical limitations resulting in reduced identifications of transmembrane proteins and posttranslational modifications, we developed an alternative multidimensional identification strategy using a combination of 1-D electrophoresis and nanoLC-ESI-MS/MS.
**PIII01**

**IDENTIFICATION AND CHARACTERIZATION OF THE GRAPEVINE DEFENSIN-LIKE GENE FAMILY.**

GIACOMELLI LISA¹, NANNI VALENTINA², SILVERSTEIN KEVIN³, TOWN CHRISTOPHER⁴, ZANETTI MANUELA⁵, LENZI LUISA¹, BARALDI ELENA², DALLA SERRA MAURO⁵, AND MOSER CLAUDIO¹.

¹ Centro Ricerca e Innovazione, Fondazione Edmund Mach, via Mach 1, San Michele all'Adige, TN, Italy. ² DIPROVAL-Agraria, Universita' di Bologna, viale Fanin 46, 40127, Bologna Italy. ³ Dept. Biostatistics and Bioinformatics, Masonic Cancer Center, University of Minnesota, 425 Delaware Street, SE MMC 806, Minneapolis, MN 55455, USA. ⁴ The J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA. ⁵ Istituto di Biofisica-Fondazione Bruno Kessler, Via Sommarive, 18, I-38100, POVO (TN) Trento, Italy.

**Keywords:** defensin, grapevine, cysteine-rich, genome-wide.

Defensins are a diverse class of small cysteine-rich proteins sharing a common tertiary structure, which have been linked to the innate immunity in several organisms. By scanning the *Vitis vinifera* Pinot noir genome using a combination of HMM and BLAST searches we could identify 81 defensin-like sequences (DEFLs), eventually including allelic variants, pseudogenes or fragments. They share a common exon-intron-exon structure typical of other defensin genes, and their localization on the Pinot noir genome suggests a large extent of local duplications. We demonstrated the expression of 23 DEFLs or DEFL groups and further analyzed the transcript accumulation of 15 of them in seven different tissues and along berry ripening. The majority of DEFLs were predominantly expressed in reproductive tissues such as flowers and seeds. Interestingly, some DEFLs appeared to be induced in tissues infected by the fungal pathogen *Botrytis cinerea*. The corresponding recombinant proteins were indeed able to inhibit conidia germination *in vitro*. These results are consistent with a role of these DEFL sequences in the defense against pathogens in grapevine passed protecting cells from short-term fluctuations in water status.

**PIII02**

**PROFILING THE HYPOXIC RESPONSE OF ARABIDOPSIS THALIANA ROOTS FOR CHANGES IN TRANSCRIPTION FACTOR AND PRI-MIRNA EXPRESSION.**

FRANCESCO LICAUSI¹, DAAN A. WEITS¹, BIKRAM. D. PANT¹, WOLF-RUDIGER SCHEIBLE¹, PETER GEIGENBERGER², JOOST T. VAN DONGEN¹.

¹ Energy Metabolism and Molecular Genomics Research Groups, Max Planck Institute for Molecular Plant Physiology, Wissenschaftspark 1, 14476 Potsdam-Golm, Germany. ² Department Biologie I, Ludwig-Maximilians-Universitat Munchen, 82152 Martinsried, Germany.

**Keywords:** transcription factor, microRNA, hypoxia, Arabidopsis.

Plants do not only experience oxygen deficiency when its availability decreases externally, such as under flooding conditions, but hypoxia also occurs as part of the developmental program in bulky or scarcely gas-permeable tissues. With the aim of understanding the regulation of the molecular response to oxygen deprivation, we profiled the expression of genes coding for transcription factors (TFs) and microRNA precursors (pri-miRNAs) in *Arabidopsis thaliana* roots exposed to different hypoxic conditions, using a qRT-PCR platform encompassing over 1900 TFs and 180 pri-miRNAs. Subsequently, the promoter sequences of gene differentially regulated by hypoxia were searched for the presence of overrepresented DNA elements. The two dataset were finally combined to predict TF/DNA element interactions that might be relevant for the transcriptional response to hypoxia and the predicted interactions where tested *in vivo*. Members of selected TF families were also tested for their impact on the tolerance of Arabidopsis plants to low oxygen conditions, using stable over-expression and silencing strategies.
**PIII03**

**TRANSFORMIC APPROACHES FOR A STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF GRAPEVINE MIRNAS.**

E. MICA 1, E. BERTOLINI 1, V. PICCOLO 2, D. HORNER 2 AND M.E. PE' 1.

1 Agrobiodiversity Laboratory, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy; 2 Department of Biomolecular Sciences and Biotechnology, University of Milano, Via Celoria 26, 20133 Milano, Italy.

Keywords: miRNAs, grapevine, RNA-seq, introns.

MicroRNAs (miRNAs) are key post-transcriptional regulatory elements (18-21 nt long), coded by MIR genes and cleaved from hairpin precursors. miRNAs are one of the main players of RNAi regulating plant architecture, nutrient homeostasis and stress response. Their crucial role in fine tuning gene regulation clearly implies that a complete characterization of genomes cannot be attained without a deep analysis of these molecules. Here we present the characterization of miRNA genes in *Vitis vinifera* L., starting from the 140 conserved MIR genes, annotated in the grapevine genome (Jaillon et al., 2007). We used different transcriptomic approaches to provide the first indication that many of these miRNAs show differential expression patterns among tissues and during fruit maturation. Using RNA-seq data and deep-sequencing of small RNA fractions we determined the genomic structure and patterns of splicing for many primary miRNA transcripts. Finally, combining bioinformatic and molecular biology tools, putative targets were identified and validated for many conserved miRNAs.

**PIII04**

**COMPARATIVE ANALYSIS OF PROTEIN PATTERNS IN BARLEY CULTIVARS THAT DIFFER IN SALT SALINITY.**

STEFANIA MONTANARI1, NIKLAS HAUSMANN2, RUEDI GER HAMPP2, SERGIO ESPOSITO1.

1 Dipartimento di Biologia Funzionale, Universita' di Napoli Federico II, via Cinthia 80126 Naples, Italy. 2 University of Tubingen Botany Institute, Physiological, Ecology of Plants, Aus der Morgenstelle 1, D-7276, Tubingen, Germany.

Keywords: Salt Stress, 2D electrophoresis, Hordeum vulgare, Maldi-TOF.

Soil salinity and drought are the two most common abiotic stresses constraining crop growth and productivity. The effect of salinity on plant growth has been extensively studied. Data obtained so far indicate that responses to increased salinity involve physiological and biochemical changes. Among the cereal crops, barley (*Hordeum vulgare* L.) is a salt tolerant species, and is thus of considerable economic importance in salinity-affected arid and semiarid regions of the world. In this work, we compared the effects of salt-stress on the growth and protein pattern of two barley cultivars, Nure and Tidone. Salt stress was applied by hydroponic culture in a medium containing 50,150 and 300 mM NaCl, for different periods of time (8 and 12 hrs). Cultivar Tidone was more sensitive in comparison to Nure, and its growth was more reduced. For the analysis of salt-induced changes in protein patterns root tissue was used. After the extraction of total soluble protein, samples were subjected to two-dimensional SDS gel electrophoresis. Spots, differing in abundance are identified by mass spectrometry (Maldi-TOF). The data show differences in response-time and intensity between the two cultivars.
GENOMICS AND FUNCTIONAL GENOMICS OF SYMBIOTIC FUNGI.

RAFFAELLA BALESTRINI, FABIANO SILLO, LUISA LANFRANCO, VALENTINA FIORILLI, PAOLA BONFANTE.

Istituto per la Protezione delle Piante, UOS Torino - CNR and Dipartimento di Biologia Vegetale - UniTO Viale Mattioli, 25 - 10125 Torino, Italy.

Keywords: mycorrhizal fungi, genomics, functional genomics, laser microdissection, cellular specificity.

Mycorrhizal fungi, i.e., the microbes that live in symbiosis with the roots of many plants, are raising a special interest in agro-food context due to their role as biofertilizers. Sequencing projects of ectomycorrhizal fungi have been successfully launched, giving new vistas on the genetic bases of the symbiotic status. Starting from the sequenced genome of *Tuber melanosporum*, we performed an <in silico> analysis mostly focusing on cell wall related genes. Genes coding for fungal cell wall degrading enzymes resulted to be differentially expressed during the transition from mycelium to ectomycorrhizae, as well as those coding for plant cell wall degrading enzymes. The sequencing project of an AM fungus genome is also currently under progress, but the obligate biotrophic status of these fungi has raised unexpected difficulties. To solve the problem, GlomusDB database is integrating data from both *G. intraradices* EST and the JGI genome project. The current goal is to collect data on the expression patterns during the fungal life-cycle stages. The availability of a protocol for LMD applied to AM symbiosis is giving us information on cell specificity of transcriptional responses.

GLUTATHIONYLATION IN THE PHOTOSYNTHETIC MODEL-ORGANISMS CHLAMYDOMONAS REINHARDTII: A PROTEOMIC SURVEY.

ZAFFAGNINI M1,2, MARCHAND C3, AIAM G3, BEDHOMME M2, GAO XG2, LE-MARECHAL P3, DECOTTIGNIES P3, TROST P1 AND LEMAIRE SD2.

1 Laboratory of Molecular Plant Physiology, Department of Experimental and Evolutionary Biology, University of Bologna, Bologna, Italy. 2 Institut de Biologie des Plantes, UMR 8618, CNRS, Université Paris-Sud, Batiment 630, 91405 Orsay Cedex, France. 3 Institut de Biochimie et Biophysique Moléculaire et Cellulaire, UMR 8619, CNRS, Université Paris-Sud, Batiment 430, Orsay 91405, Cedex, France.

Keywords: glutathionylation, labeled glutathione, mass spectrometry, *Chlamydomonas reinhardtii*, redox signaling.

Glutathionylation is a ubiquitous redox-sensitive and reversible modification of protein cysteine residues promoted by oxidative stress. This modification can protect specific cysteine residues from irreversible oxidation but it can also modulate protein activities. While well established in mammals, little is known about the formation and function of these mixed disulfides in photosynthetic organisms. In order to get more insight into the importance and role of glutathionylation in *Chlamydomonas*, we developed several proteomic approaches based on the use of labeled glutathione by either 35S-cysteine or biotin. These methods allowed identification, *in vivo or in vitro*, of more than 100 glutathionylated proteins involved in numerous cell processes and metabolic pathways. Furthermore, we also identified the sites of glutathionylation facilitating further studies on the functional role of this modification of newly identified targets. Finally, we investigated the effect of glutathionylation on the activity of specific glutathionylated proteins. All these data indicate that glutathionylation likely constitutes an important mechanism of regulation in photosynthetic organisms.electrophoresis. Spots, differing in abundance are identified by mass spectrometry (Maldi-TOF). The data show differences in response-time and intensity between the two cultivars.
**BIOCHEMICAL, PROTEOMIC AND PHYSIOLOGICAL ANALYSIS OF CHLOROPLAST-CHROMOPLAST TRANSITION IN SOLANUM Lycopersicum.**

MATTEO BALLOTTARI 1, LINDA BIANCO 2, ALESSANDRO ALBORESI 1, GIOVANNI GIULIANO 3, GAETANO PERROTTA 2, ROBERTO BASSI 1.

1 Dipartimento di Biotecnologie Università di Verona, Strada le Grazie 15, 37134 Verona, Italy. 2 ENEA-Trisaia Research Center, S.S. 106 Ionica, 75026 Rotondella (Matera), Italy. 3 ENEA-Casaccia Research Centre, Via Anguillarese 301, 00123 Roma, Italy

**Keywords:** proteomics, photosynthesis, chloroplast, chromoplast, chlororespiration.

Plastid differentiation from chloroplast to chromoplast has been studied in *Solanum lycopersicum* fruits, applying a combined proteomic, biochemical and physiological approach. In particular MudPIT LC/MS proteomic analysis performed on plastids isolated from leaf tissues and from four different fruit ripening stages, allowed identifying 1218 tomato proteins, corresponding to 869 *Arabidopsis thaliana* genes. Our results elucidate the distribution during fruit ripening of gene products involved in different metabolic functions as sugars, lipids, amino acids, protein, nitrogen and sulphur metabolism, tetrapyrroles and carotenoids biosynthesis, photosynthesis and abiotic stress. In particular photosynthetic proteins are accumulated in all ripening stages, even if strongly reduced in mature red fruits. We thus compared by biochemical and physiologic approach the photosynthetic properties of leaf and fruit plastids demonstrating that green fruits are photosynthetically active, while "red" fruits are not. In particular reduction in green and orange fruits of linear electron flow compared to cyclic electron flow and chlororespiration is discussed.

**PECULIARITIES IN AMINO ACIDIC COMPOSITION OF PROTEINS ENCODED BY OVERLAPPING GENES: A PRELIMINARY STUDY IN VIRUSES.**

NICOLA CHIRICO, ANNA FEDELI, FRANCESCO LANDINI, ALBERTO VIANELLI.

Dipartimento di Biologia Strutturale e Funzionale, Università degli Studi dell’Insubria, via J.H.Dunant 3, 21100 Varese, Italy.

**Keywords:** frameshift, virus, intrinsic protein disorder, evolution.

Gene overlaps, which we define as having nucleotides coding for more than one protein by being read in multiple reading frames, are a common feature of viruses, but they are also present in prokaryotic and eukaryotic genomes, though systematic studies in plants are lacking. They are typically assumed to represent genome compression allowing the virus to increase its coding capability without increasing its genome length. It has been also proposed that such genes tend to have unusual protein structure and composition, which might be a key mechanism for de novo origins of proteins and/or protein domains. The purpose of our work is to expand previous analyses of the amino acid composition of viral overlapping coding sequences by including all available genome sequences. In broad agreement with previous studies, we show that proteins coded by overlapping genes are enriched in amino acids with the highest degree of codon degeneracy and in disorder-promoting amino acids. We note that their peculiar amino acid composition can be better understood taking also into account compositional constraints dictated by the necessity to avoid stop codons in both frames.
THE TOMATO GENOME REVEALS FUNCTIONAL SPECIALIZATION OF GENES CONTROLLING FRUIT NUTRITIONAL VALUE.

M PIETRELLA.

The International Tomato Sequencing Consortium

Keywords:

Tomato (Solanum lycopersicum) is an economically and nutritionally valuable crop and constitutes a model plant for the Solanaceae family. Sequencing of the whole genome has been completed through a Next Generation WGS approach, resulting in high quality assembly (ca 800 Mb) and annotation (ca 35,000 protein-coding genes). We have started a systematic investigation of gene families controlling fruit nutritional value in tomato. Gene families studied are those for carotenoid biosynthesis, ascorbate, and some photoregulatory genes, such as Cryptochrome, Phytochrome, Det and Cop genes. Comparison with Arabidopsis, Grape, and the recently available potato genome reveals Solanaceae- as well as tomato-specific gene duplication events. Deep RNAseq of several tomato tissues provides support for fruit-expressed genes.

PROTEOMIC PROFILING OF WHITE TRUFFLE (TUBER MAGNATUM PICO) NATURALLY GROWN IN DIFFERENT ITALIAN AREAS.

F. VITA 1, V. LUCAROTTI 1, E. ALPI 2, F. FANUCCHI 1 AND A. ALPI 1.

1 Department of Crop Plant Biology, Univ. of Pisa, Pisa, Italy. 2 Biomolecular Mass Spectrometry Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy.

Keywords:

The aim of this work is to verify the origin of the white truffle (Tuber magnatum Pico) from San Miniato (Pisa) through protein profiling in which it would be possible to identify one or more proteins specifically found in the San Miniato area samples. Truffles are considered an important economical and gastronomical resource with prices as high as 300 euros/100 gr. The Tuber magnatum variety is particularly appreciated and its demand is often outweighs the amount of truffles harvested in its natural habitat. Therefore, it would be crucial to set up a methodology capable of discriminating univocally not only the species analysed, but also its origin, in order to distinguish the more valuable varieties from the less expensive ones. Samples of Tuber magnatum Pico from selected areas in Tuscany and Piedmont were analysed through a proteomic approach. The analyses performed so far using bidimensional electrophoresis have shown a high reproducibility in the proteic pattern of Tuber. The analysis of differentially expressed proteins with mass spectrometry techniques has allowed us to identify specific proteins by using MALDI-TOF and LC-MS spectrometers.
Epialleles formation and inheritance in plants.
Carlo Soave

Dept. of Biology University of Milano, Italy

The Modern Synthesis (MS) is the current paradigm in evolutionary biology; it is an expansion of Darwin’s insights that all organisms share common descent, are adapted to the environment through natural selection and that the characteristics displayed by organisms are controlled — ultimately — by the nucleotide sequence of their genome. Another cornerstone of MS is that inherited information that is transmitted on the chromosomes changes only at random, without direction from the environment towards particular phenotypic outcomes. These basic tenets of MS have been repetitively challenged in the past (see for example the “Baldwin effect” and concepts like “reaction norm”, “phenotypic plasticity”, “canalization”, “genetic assimilation” etc.) by arguments based on the belief that the phenotypic modifications induced by the environment can potentially allow organisms to survive and reproduce in new environments until genetic variation for an appropriate obligate phenotype can be selected: that is phenotype first, genotype follows. Until the explosion of epigenetics, however, the molecular bases of phenotypic plasticity were largely unknown, as well as the mechanisms at the bases of epigenetic somatic inheritance and, more relevant for evolution, of germ-cell epigenetic states transmission. On the bases of the massive amount of new data and wealth of new ideas coming from epigenetics, the MS no longer provides an adequate general framework for 21st-century evolutionary thinking, so that M. Pigliucci, at the Evolution Meeting, Stony Brook University, June 2006, said: « Does all of this mean that a much-heralded new synthesis in evolutionary theory is around the corner? …..I think so, because new empirical and conceptual developments continue to enrich evolutionary biology far beyond the intellectual horizons delineated by the founding fathers of MS ».

Epigenetic Reprogramming in Maize.
Jose Gutierrez-Marcos*

School of Life Sciences University of Warwick Wellesbourne CV35 9EF, UK.

The two products of double fertilization in plants—the embryo and the endosperm—undergo distinct developmental fates despite being derived from the same genetic material. To understand the genetic and epigenetic basis for these differences, we are investigating the epigenetic machinery in the two female gametes (egg cell and central cell) and in male gametes (sperm cells). We will present data from our analyses of gametes and of pro-endosperms and zygotes, which suggest stark differences in the presence and abundance of components of the epigenetic machinery exhibited by the three gamete types, as well as differences in the genetic and epigenetic landscape between zygotes and pro-endosperms. Our data indicate that genome-wide epigenetic reprogramming occurs at stages when developmental potency of cells in the gametic lineage changes. Furthermore, we will discuss the plasticity of this epigenetic phenomenon and its impact on genome-wide gene expression.
Epialleles formation and inheritance in plants.

Serena Varotto

*Dipartimento di Agronomia ambientale e produzioni vegetali Università degli Studi di Padova*

The discovery of the importance of epigenetic mechanisms acting on chromatin to regulate global gene expression has revealed how heritable variation need not be sequence-based. Environmental factors can induce novel variation through the activation of specific epigenetic mechanisms that determine mutations of spatial and temporal pattern of gene expression. Plants have been shown to adapt to the changing environment by altering gene expression and by destabilizing the genome: the decrease in genome stability in response to environmental stress might be sequence independent. Moreover, there is sufficient evidence that environment can cause plants to grow differently and that the induced phenotypic changes are transmitted to the progeny. In fact, increasing data suggests that an important fraction of phenotypic variability is of epigenetic origin. This evidence also indicates that the epigenetic component of phenotypic variation might have played an important role in the microevolution of natural population. The concept of the epigenetic allele (epiallele), that is an allele showing a heritable difference in expression as a consequence of epigenetic modifications, it is of particular interest in the context of plant improvement. Although it is well known that environmental stresses can alter epigenetic states in plants, we lack information on stability throughout mitosis and meiosis of the newly formed epigenetic states.
**Parallel Session: PLANT AND ENVIRONMENT**

**Differential expression of saporin genes upon wounding, ABA treatment and leaf development.**
A. Tartarini\(^1\)*, G. Testone \(^2\), R.A. Rodrigues-Pousada \(^1\), D. Giannino\(^2\) and L. Spanò\(^1\)

\(^1\) Department of Basic and Applied Biology, University of L’Aquila, Via Vetoio Loc. Coppito, 67100 L’Aquila, Italy.

\(^2\) Institute of Biology and Agricultural Biotechnology, National Research Council of Italy (CNR), via Salaria km 29300, 00015 Monterotondo Scalo, Roma, Italy.

Ribosome-inactivating proteins (RIPs) are potent inhibitors of protein synthesis that accumulate in different tissues of many plant species. Saporins are type1 ribosome-inactivating proteins (RIPs: EC 3.2.2.22) produced in various organs of *Saponaria officinalis* L. Two distinct saporin types, saporin-L and saporin-S isoforms, were respectively purified from the intra- and extra-cellular fraction of soapwort leaves. The saporin-L were poorly identical, differed for toxicity, molecular mass and amino acid composition from saporin-S proteins forming a new monophyletic group. Genes encoding both L and S type isoforms were cloned from leaf specific cDNA library; the encoded products included the N-terminal diversity observed by protein sequencing and showed compatible weights with those from mass spectra. These genes were intron-less belonging to small gene families. RT-PCR/qRT-PCR experiments evidenced their differential expression during leaf development, wounding and ABA treatment. The expression of *saporin*-L genes augmented from young to adult leaves, whereas that of the *saporin*-S genes maintained a constant rate during leaf growth. In leaf wound assays, the transcription of *saporin*-L significantly augmented at six hours. On the contrary, *saporin*-S gene types were intensely triggered at one hour. The *saporin*-L and -S genes responded differentially to ABA treatment in short time (one hour) experiments, but showed overlapping responses in the long run. The results suggest that the saporin-L and -S proteins may play diversified roles during stress responses.

**Exploitation of plant diversity for adaptive-related traits**
A. Tondelli\(^1\)*, F. Rizza\(^1\), L. Cattivelli\(^1\), A.M. Stanca\(^2\)

\(^1\) CRA – Genomic Research Centre, Fiorenzuola d’Arda, Italy

\(^2\) Dept. of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy

The synchronization of the plant life cycle with the most favourable environmental conditions is a fundamental mechanism for the adaptation to different latitudes and climates. This in turn leads to the maximization of the growth through the best use of resources (e.g. water and radiant energy) and the avoidance of stressful circumstances. Frost tolerance, vernalization requirement, photoperiod sensitivity and earliness are the main traits for the synchronization of the life cycle with the environment. Evaluation of genetic resources has been shown to be crucial for the dissection of the physiological mechanisms involved in different adaptation strategies. As an example, by using the *Triticeae* model plant barley, we have shown that phenological adjustments are mainly driven by few well-known photoperiod (*Ppd*) and vernalization (*Vrn*) responsive genes, as well as earliness per se or early maturity genes (*Eps/Eam*) that affects life-cycle timing independently of these stimuli. In addition, key genes for low temperature stress response pathways identified by means of complementary family-based and population-based genetic approaches will be discussed.
Parallel Session: PLANT AND ENVIRONMENT

**PV01**

**ISOLATION AND MOLECULAR CHARACTERIZATION OF TDP1 (TYROSYL-DNA PHOSPHODIESTERASE) GENES FROM BARREL MEDIC INVOLVED IN REPAIR OF DNA TOPOISOMERASE I-INDUCED DAMAGE AND OTHER OXIDATIVE DNA LESIONS.**

ALMA BALESTRAZZI¹, ANCA MACOVEI¹, MASSIMO CONFALONIERI², DANIELA CARBONERA¹.

¹ Dipartimento di Genetica e Microbiologia, University of Pavia, via Ferrata 1, 27100-Pavia, Italy.
² CRA-FLC, Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, viale Piacenza 29, 26900-Lodi, Italy.

**Keywords:** DNA repair, Medicago, QRT-PCR, TDP1, TOPO I.

The Tdp1 gene encoding tyrosyl-DNA phosphodiesterase has been extensively investigated in animals, due to the role of the enzyme in the repair of topo I-DNA lesions and in view of its possible use as a target for anticancer therapy (Dexheimer et al. 2008). We report for the first time in plants on the cloning of MtTdp1a and MtTdp1b genes from Medicago truncatula Gaertn. QRT-PCR revealed that Tdp1 genes were constitutively expressed and up-regulated in response to stress agents (Macovei et al. 2010). During seed rehydration, when DNA repair is activated, the Tdp1 genes were also significantly up-regulated. The expression patterns of top1a and top1b genes, encoding distinct topo I isoforms, were also analysed and discussed, based on the recent findings concerning the involvement of this enzyme in DNA repair (Balestrazzi et al. 2010). Further investigation on the roles played in planta by the Tdp1 and top1 gene families, in relation to oxidative DNA damage are currently in progress. References: Balestrazzi et al. (2010) J. Exp. Bot. 61: 575-585; Dexheimer et al. (2008) Anticancer Agents Med. Chem. 8: 381-389; Macovei et al. (2010) Planta, doi: 10.1007/S00425-010-1179-9

**PV02**

**DIFFERENTIAL ACTIVATION OF DEFENCE GENES AND ENZYMES IN MAIZE GENOTYPES WITH CONTRASTING LEVELS OF RESISTANCE TO FUSARIUM VERTICILLIOIDES.**

LANUBILE ALESSANDRA¹, MASCHIETTO VALENTINA¹, BERNARDI JAMILA¹, PACIOLLA COSTANTINO², DE LEONARDIS SILVANA², MAROCCO ADRIANO¹.

¹ Istituto di Agronomia, Genetica e Coltivazioni erbacee, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza; ² Dipartimento di Biologia e Patologia Vegetale, Università degli Studi Aldo Moro, Via G. Amendola 165/A, 70126 Bari.

**Keywords:** PR genes, detoxification enzymes, Fusarium ear rot, Zea mays.

We attempt to identify genes involved in Fusarium ear rot resistance using resistant and susceptible maize genotypes. Gene expression data were obtained from microarray hybridizations using seeds inoculated at 15 days after silking. At 48 hours after infection, differentially expressed sequences were identified and classified into 11 functional categories, principally assigned to the category cell rescue, defence and virulence in both lines. These genes encode for PR proteins, detoxification enzymes and beta-glucosidases. The differentially expressed genes were validated in qPCR, also performed in silks 12, 24, 48 and 72 h after F. verticillioides infection. Parallel, we have studied the detoxifying enzymes ascorbate peroxidase (APX), catalase (CAT), total peroxidase (POD) and superoxide dismutase (SOD) in seedlings, before and at five days after F. verticillioides infection. In resistant seedlings, before infection, the defence genes, APX and SOD enzymatic activities were higher than in the susceptible ones while, after treatment, they remained unchanged. On the other hand, in susceptible seedlings, except for SOD, all enzymes and defence genes were activated by the pathogen.
Parallel Session: PLANT AND ENVIRONMENT

PV03

SURFACE NADH PEROXIDASES AND OXYLIPIN PATHWAY ARE INVOLVED IN THE RECOVERY FROM PHYTOPLASMA DISEASE IN APPLE.

BERTOLINI, A., PATUI, S., CLINCON, L., BRAIDOT, E., ZANCANI, M., PETRUSSA, E., ERMACORA, P., AND VIANELLO, A.

Sezione di Biologia Vegetale, Dipartimento Biologia e Protezione delle Piante, Università di Udine, Via delle Scienze 91, I-33100 Udine, Italy.

Keywords: hydrogen peroxide, jasmonate, lipoxygenase, phytoplasm.

Phytoplasmas are prokaryotic plant pathogens responsible for deterioration of several trees. In apple tree, the proliferation of the disease, caused by phytoplasmas, may undergo a spontaneous disappearance of symptoms, which are associated to hydrogen peroxide production in the phloem, a phenomenon called recovery. The aim of this work was to identify the biochemical pattern associated to the recovery by examining some enzymatic activities and metabolites in leaves from healthy, diseased and recovered plants. NAD(P)H oxidase/peroxidase, lipoxygenase, hydroperoxide lyase and phenylalanine ammonia lyase (PAL) activities were assayed. All these activities, except for PAL, were increased in leaves from recovered plants. Salicylate and jasmonate content was also determined, showing that the increase in recovered leaves was ascribed to jasmonate only. These results suggest that the activation of surface/plasma membrane redox systems is crucial in promoting the recovery process in apple tree, which then proceeds through the oxylipin pathway, leading to jasmonate. Conversely, salicylic acid appears to be involved only in the response to disease, but not in the subsequent recovery.

PV04

ION-MEDIATED COMPENSATION FOR DROUGHT-INDUCED LOSS OF XYLEM HYDRAULIC CONDUCTANCE IN FIELD-GROWING PLANTS OF LAURUS NOBILIS L.

PATRIZIA TRIFILÔ¹, ANDREA NARDINI², FABIO RAIMONDO¹, MARIA ASSUNTA LO GULLO¹ AND SEBASTIANO SALLEO².


Keywords: Xylem hydraulic conductance, xylem cavitation, ionic effect, water stress.

Adequate water flow rates through the xylem are of crucial importance for terrestrial plants. During transpiration, high water demand may cause leaf water potential to drop to critical levels triggering cavitation and, hence, reduction of xylem hydraulic conductance (Kx). Recent studies have suggested that diurnal Kx loss might be compensated by ion-mediated up-regulation of the hydraulic conductance of still functioning conduits. The present study reports experimental evidence for diurnal changes of xylem sap potassium concentration ([K⁺]) in field-growing plants of Laurus nobilis L. subjected to mild water stress. Our data show that the water stress applied caused substantial xylem conduit cavitation. Nonetheless, the increased [K⁺] induced an increase of the residual Kx possibly through modification of the volume of pit-membrane pectic matrix and consequent up-regulation of pit membrane water permeability. As a consequence, stressed plants appeared to maintain Kx and stomatal conductance at the same levels as recorded in control (well watered) plants. Our data suggest that the potential for fine regulation of Kx by plants is much larger than previously thought.
Parallel Session: PLANT AND ENVIRONMENT

**PV05**

ATP LEVEL IN PHRAGMITES AUSTRALIS AND BOLBOSCHOENUS MARITIMUS LEAVES IN RELATION WITH PHENOLOGY AND SALINITY.

VALENTINO CASOLO, CARLO PERESSON, ALBERTO BERTOLINI, FRANCESCO BOSCUTTI, ANGELO VIANELLO.

Department of Biology and Plant Protection, Section of Plant Biology, University of Udine, via delle Scienze, 91 I-33100 Udine, Italy.

**Keywords:** ATP, Bolboschoenus maritimus, Phragmites australis, saline stress.

The plant adaptation to salt is one of the most important topics in plant biology. In this work, the variations of ATP level were examined in *Phragmites australis* and *Bolboschoenus maritimus* leaves, collected during springtime, summertime and autumn, in different sites characterized by diverse substrate salinity. The results show that the concentration of ATP was strictly linked to the development of both species. In addition, *P. australis*, collected in fresh and transition salt water, showed an increased ATP level from autumn to springtime, while the plants growing in the salt water exhibited the higher value in summertime. In *B. maritimus*, whose distribution is linked to the low salinity of water, the ATP content was higher in springtime, to be reduced in summertime and to raise again in autumn. These results suggest that in *P. australis* the energetic state of leaves is maintained high during the annual cycle, being higher during full maturation of fruit and death of the annual shoot, except in plants grown in saline conditions, where the critical time is summertime. Conversely, in *B. maritimus*, anthesys corresponds to the maximal energy state for the plant.

**PV06**

2-CYS PEROXIREDOXIN IN EXTREME HALOPHYTES: DO THEY HAVE A ROLE IN PHOTOSYSTEM II PHOTOPROTECTION?

ANTONACCI A, TROTTE A, MARSANO F, REDONDO-GOMEZ S, FIGUEROA E, BARBATO R.

1 Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale, Alessandria, Italy. 2 Departamento de Biologia Vegetal y Ecologia, Universidad de Sevilla, Sevilla, Spain.

**Keywords:** 2-cys peroxiredoxin, Photosystem II, halophytes, photoprotection.

Here we report the identification of a 2-cys peroxiredoxin from thylakoids of the extreme halophyte *Arthrocnemum macrostachyum* by means of tetramethyl benzidine/hydrogen peroxide staining and de novo sequencing by mass spectrometry. The activity of the protein was fully inhibited by dithiothreitol but not by KCN or azide, suggesting that the peroxidase activity observed was sulphidril-dependent rather than heme-dependent. We also found that the level of 2-cys peroxiredoxin was strongly modulated by the concentration of salt in the growth medium (from 0 to 1 M NaCl, the optimal one being 0.51 M), the activity of the enzyme being increased when salt concentration was higher or lower than the optimal one. Moreover, at salt concentration higher or lower than 0.51M, plants displayed enhanced sensibility to light, as measured by chlorophyll fluorescence and protein biochemistry. The possibility that increased 2-cys peroxiredoxin activity upon salt stress is related to photoprotection should be considered.
Germination of barley under long-term cold conditions.

Caterina del Zoppo, Pierdomenico Perata, Lorenzo Guglielminetti.

1 Dipartimento di Biologia delle Piante Agrarie, Università di Pisa, via Mariscoglio 34 I-56124, Pisa, Italy; 2 PlantLab, Scuola Superiore di Studi e Perfezionamento Sant'Anna, via Mariscoglio 34 I-56124, Pisa, Italy.

Keywords: barley, cold, germination, amylase, sugar.

Barley caryopses are able to germinate under cold conditions (4°C in darkness) and to survive under this stressing treatment up to 120d. In this extreme conditions coleoptile protusion is appreciable after few weeks (few days under control condition, at 23°C in darkness). However, the cold-treated seedlings were morphologically identical to seedlings grown under control conditions. We present here data on carbohydrate metabolism and amylases pattern in endosperms and coleoptiles of barley caryopses germinated at 4°C or 23°C in darkness. These results indicate that it is possible to underline several differences concerning free sugars levels (glucose, fructose and sucrose) as well as reserve carbohydrates levels (starch). However, the most evident differences were the endospermic amylolytic enzymes pattern. A specific beta-amylase band was recorded in control (23°C) samples only, while two alpha-amylase bands were differentially expressed at 4°C. In addition (as recovery parameter) we have also analyzed levels of pigments in coleoptiles derived from treated or control seedlings recovered for 1 week under light at 23°C.

Improving genetic transformation protocols of Lemna minor, involved in phytoremediation applications.


Istituto di Biologia e Biotecnologia Agraria (IBBA) - CNR – Via Salaria, km 29,300, 00015 Monterotondo scalo (Roma - Italy).

Keywords: Lemna minor, heavy metals, biolistic transformation.

Lemna minor, a small aquatic floating angiosperm, has been well studied for its ability to remove metals from surface waters. This plant rarely flowers in nature and most often grows clonally, doubling every 2 to 3 days under optimal conditions. Lemna minor has been shown to accumulate as much as 1,300 times more Cd than concentrations present in the surrounding water, showing its ability to remove Cd from surface waters for phytoremediation. Nevertheless, Lemna minor offers an ideal plant-based gene expression system. A Lemna minor gene expression system provides technology that would be useful for a number of research and commercial applications. In our work, we are setting up and optimising protocols for Lemna minor genetic transformation through biolistic system to further study genes associated to metal-uptake and genes correlated to developmental processes in order to dissecting the molecular and morphological basis of metal hyperaccumulation and to improving the use of Lemna minor in phytoremediation applications.
EXPRESSED PECTIN METHYLESTERASE INHIBITOR (PMEI) GENES SHOW A DIFFERENT PATTERN OF ACCUMULATION IN WHEAT TISSUE AND FOLLOWING FUNGAL INFECTION.

VALENTINA ROCCHI¹, MICHELA JANNI¹, RAVIRAJ KALUNKE¹, CARLA CASTIGLIONI², FRANCESCO FAVARON², DANIELA BELLINCAMP², THIERRY GIARDINA², RENATO D’OVIDIO¹.

¹ Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via San Camillo de Lellis, s.n.c., Viterbo, Italy; ² Dipartimento Te.S.A.F. Università di Padova, Padova, Italy; ³ Dipartimento di Chimica, Università di Roma "La Sapienza", 00185 Rome, Italy; 4 ISM2/BiosCiences UMR CNRS 6263, case 342, Université Paul Cezanne (Aix-Marseille 3), 13397 Marseille cedex 20, France.

Keywords: pmei, wheat, gene expression, wheat resistance, fungal pathogens.

Cell wall pectin degradation represents an important step for a successful infection of the host tissue. An important feature of pectin is its degree and pattern of methyl esterification. Highly methyl esterified pectin or a random distribution of methyl ester can be associated with an increased host resistance response. Pectin methyl esterification is controlled by the activity of pectin methylesterase (PME), which de-methylate esterified pectin. Since the activity of PME can be controlled by its inhibitor protein (PMEI), we are characterizing Pmei genes in wheat to manipulate the methyl esterification of pectin and shed light on the involvement of this feature in wheat resistance. We report the characterization of the first Pmei gene (Tdpmei3) in wheat and the isolation of two additional Pmei-like genes (Tdpmei1 and Tdpmei2). qRT-PCR analysis showed that these genes are regulated during leaf development and Tdpmei1 and Tdpmei2 accumulate strongly in the ovary and stamen, whereas Tdpmei3 accumulate mainly in the stem. Tdpmei1 and Tdpmei3 are not induced following wheat leaf infection with the fungal pathogen Bipolaris sorokiniana, whereas Tdpmei2 transcript accumulate slightly.

FUNCTIONAL CHARACTERIZATION OF TAPGIP GENES IN WHEAT.

JANNI M.¹, BOZZINI T.¹, LUPI R.¹, DI GIOVANNI M.², MASCİ S.¹, D’OVIDIO R.¹.

¹ Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Viterbo, Italy. ² Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy.

Keywords: Pgip, wheat, transgenic plants, wounding, pathogen infection.

Polygalacturonase inhibiting proteins (PGIPs) are leucine-rich repeat proteins involved in plant defence. Based on sequence similarity, three Pgip genes, Tapgip1, Tapgip2 and Tapgip3 have been identified in the wheat genome. Tapgip1 and Tapgip2 are expressed in all tissues, whereas Tapgip3 is inactive. To verify whether Tapgip1 and Tapgip2 are involved in wheat defence and encode active PGIPs, we have analyzed their expression following stress conditions and in transgenic wheat. Tapgip1 and Tapgip2 are up-regulated by fungal infection and OG treatment, and strongly induced following wounding. This last result has been confirmed in transgenic wheat plants expressing the GUS gene under control of Tdpgip1 promoter. A strong and transient GUS staining was mainly detected close to the wounded sites. Gain of function experiments by the ectopic expression of Tapgip1 in a wheat genotype carrying all silent Pgip genes do not caused any improvement in the inhibition activity towards fungal PGs and any evidence of enhanced resistance against the fungal pathogen Bipolaris sorokiniana. Similar results have been obtained by overexpressing Tapgip2 in a wheat cv. having only an active Pgip1.
DECREASE OF CYTOSOLIC ATP CONTENT UNDER HYPEROSMOTIC STRESS IN DURUM WHEAT SEEDLINGS AS EVALUATED BY A NEW SIMPLE METHOD.

MARIO SOCCIO, MAURA NICOLETTA LAUS AND DONATO PASTORE.

Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Facoltà di Agraria, Università degli Studi di Foggia, Via Napoli 25, and Centro di Ricerca Interdipartimentale BIOAGROMED, Università degli Studi di Foggia, Via Napoli, 52 - 71122 Foggia - Italy.

Keywords: Cytosolic ATP assay, salt stress, osmotic stress, durum wheat.

ATP is an important physiological modulator of various cellular activities in plants. Recently, we reported that in durum wheat seedlings, submitted to either salt (NaCl) or osmotic (mannitol) stress, a decrease in the rate of mitochondrial ATP synthesis occurs. To test whether this determines a decrease of ATP content, in this study we optimized a new simple method in which cytosolic ATP was quickly extracted from durum wheat seedlings and immediately assayed by monitoring the NADPH fluorescence generated via hexokinase/glucose 6P dehydrogenase coupled reaction. By using this new method, we found that cytosolic ATP content is 1.99 ± 0.03 micromol g⁻¹ dry weight under control condition and 1.54 ± 0.09 (-23%) and 1.18 ± 0.08 micromol g⁻¹ dry weight (-41%) under severe salt and osmotic stress, respectively. The results are discussed in the light of the possible modulation of two mitochondrial energy dissipating systems, the uncoupling protein and the ATP-sensitive K⁺ channel, both able to control mitochondrial production of reactive oxygen species under hyperosmotic stress conditions and both inhibited by cytosolic ATP.

FLOODING TOLERANCE AND PHOTOPERIODIC RESPONSE IN DIFFERENT PRUNUS SPP ROOTSTOCK MUTANTS.

PISTELLI LAURA¹, IACONA CALOGERO², COLAO CHIARA³, LORETI FILIBERTO², MULEO ROSARIO³.

¹ Department of Crop Plant Biology, Pisa University (Pisa, Italy). ² Department of Fruit Science and Crop Protection, Pisa University (Pisa, Italy). ³ Department of Crop Production, Tuscia University (Viterbo, Italy).

Keywords: Rootstock Mutants, flooding tolerance, prunus spp.

In the last decades it has been observed in the Mediterranean areas that rainfall is becoming irregular and concentrated in short time leading to flooding and water stagnation in poorly drained soils. Among the aims of genetic improvement of stone-fruit trees, attention is focused on the increase in root tolerance of waterlogging. Two somaclonal lines were generated in vitro from leaf explants of rootstock Mr.S.2/5 of Prunus cerasifera L. Under in vivo conditions line S4 resulted tolerant to prolonged soil waterlogging, while line S1 (minus variant) showed a lower capacity to survival at waterlogging, similar to wild type The three lines exhibited different epinasty, gas exchange and assimilation and sugars content. Further investigations demonstrated that the rootstock lines are differently affected in the flooding tolerance among the variability of photoperiodism, with different alteration of some physiological parameters.
A FUNGAL TOXIN INDUCES AN OXIDATIVE BURST-INDEPENDENT PROGRAMMED CELL DEATH.

LOCATO V.1*, NOVO-UYAL E.1*, CIMINI S.1, AVOLIO F.2, DE PINTO M.C.3, EVIDENTE A.2, DE GARA L.1.

1 CIR - Università Campus Bio-Medico di Roma, Italy 2 Dipartimento di Scienze del Suolo della Pianta e dell'Ambiente, Università di Napoli Federico II, Italy 3 Dipartimento di Biologia e Patologia Vegetale, Università degli studi di Bari, Italy. * These authors have equally contributed to this work.

Keywords: Ophiobolin, Programmed Cell Death, Antioxidants.

Pathogen attack is one of the main causes of yield loss in crops. Hypersensitive response, a specific kind of programmed cell death (PCD) is one of the most studied plant defence response triggered against pathogen. Its activation requires specific molecules produced by pathogens or in the plant-pathogen interplay. Ophiobolins are secondary metabolites synthesized by pathogenic fungi that attack monocots. In order to evaluate whether ophiobolins were able to trigger defences in resistant plants, tobacco cells were inoculated with different concentrations of ophiobolin A. Low concentrations inhibited cell proliferation without affecting cell viability; while over a threshold concentration, ophiobolin A caused cell death. The analysis of specific apoptotic hallmarks revealed that ophiobolin A triggered PCD, since cytoplasm shrinkage, micro-nuclei formation and DNA laddering occurred. Diversely from other PCD processes, oxidative burst was not a precocious event in its activation, since \( \text{H}_2\text{O}_2 \) over-production only occurred in the cells already showing clear death symptoms. Consistently, pre-treatments with antioxidants were not able to recover cell viability.

TOXICITY OF PALLADIUM NANOPARTICLES TO FRESHWATER GREEN ALGA P.SUBCAPITATA.

CANDIDA VANNINI1, GUIDO DOMINGO1, MILENA MARSONI1, ALESSANDRO FUMAGALLI2, MASSIMO LABRA2, FABRIZIO DE MATTIA2, MARCELLA BRACALE1.

1 Dipartimento Ambiente, Salute, Sicurezza, Università degli Studi dell'Insubria, via G.B. Vico 46, 21100 Varese, Italy. 2 Dipartimento Biologia Strutturale e Funzionale, Università degli Studi dell'Insubria, via J.H. Dunant 3, 21100 Varese, Italy. 3 Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano Bicocca, Piazza della Scienza 2, 20126 Milano, Italy.

Keywords: palladium, nanoparticles, P.subcapitata, green algae.

Palladium (Pd) is the main component of the traffic-related pollution and its environmental concentrations is worldwide increasing. Despite to growing evidence of its toxicity, the knowledge of the molecular effects induced by Pd are still lacking. We analyzed the effect of K2PdCl4 (a Pd water soluble form) on cell growth and the molecular responses in the freshwater green alga Pseudokirchneriella subcapitata, a well known bio-indicator of water pollution. After 72h of exposure, Pd treatment affects algal growth until to a complete inhibition at 0.15 mg/l of Pd. The AFLP revealed that Pd is a powerful genotoxic agent, inducing genetic mutations, randomly distributed in the genome. 2-DE and MS/MS analysis allowed the identification of significant changes of proteins principally involved in photosynthesis, metabolism and cell detoxification. The abundance of a number of proteins involved in Calvin cycle, PSII complex and chloroplast stability were significantly decreased in Pd-treated algae indicating that the chloroplast is a major target of the deleterious effect of palladium. To confirm proteomic data we performed physiological and ultrastructural analysis.
PV15

EFFECT OF AQUEOUS EXTRACT FROM DRY OLIVE RESIDUE (ADOR) ON GROWTH AND CELLULAR REDOX STATE IN TOMATO PLANTS (SOLANUM LYCOPERSICUM L.).


1 Department of Soil Microbiology and Symbiotic System. Estacion Experimental del Zaidan, CSIC, C/ Profesor Albareda nº1, 18008 Granada (Spain). 2 Department of Plant Biology and Pathology, University of Bari, Via Orabona 4, I-70125, Bari (Italy). 3 Integrated Center of Research Univeristy Campus Bio-Medico of Rome, via Alvaro del Portillo 21, 00128 Rome (Italy). 4 Plant Science Department, University of Bari, via Amendola 165, Bari (Italy).

Keywords: Aqueous Dry Olive Residue (ADOR); Tomato; oxidative stress.

Dry olive mill residue (DOR), a solid by-product of olive oil production, has been proposed as fertilizer for its high organic content. However, DOR contains phenolic compounds potentially toxic for plants and microorganisms. In order to reduce their phenolic content, aqueous extracts of dry olive mill residue (ADOR) were treated with saprobe fungi Coriolopsis rigida and Penicillium chrysogenum, two species able to degrade toxic compound among which phenols. The effect of non treated and treated ADOR on growth of tomato plants has been studied. In particular root morphological changes and alteration in the cellular redox state after 4, 10 and 30 days from the treatment were analysed. We observed that in root, non treated ADOR reduced significantly growth and induced oxidative stress after 4 days of exposure, while ADOR treated with saprobe fungi induced a lower oxidative damage. In leaves, non treated ADOR affected antioxidant metabolism after 10 days of exposure, suggesting that phenolics compounds of ADOR could be absorbed by root and subsequently translocated to leaves.

PV16

IN VITRO CULTURE OF TUBER BORCHII VITTAD. MYCELIUM STRAINS GROWING IN SALENTO AREA TO ANALYZE DIFFERENT FORMS OF HEXOKINASE.

NUTRICATI E., SABELLA E., TOMAI PITUINCA M., PANZANARO S., APRILE A., DE BELLIS L.

Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, via Prov.le Lecce-Monteroni, 73100 Lecce.

Keywords: Tuber borchii, mycelium, in vitro culture, hexokinase.

Tuber borchii is a genus of ascomycete fungi that form ectomycorrhizae with shrubs and trees. Tuber spp. have been extensively studied because they form below-ground ascocarps (truffles), some of which are highly prized culinary commodities. The relative abundance of Tuber borchii Vittad. in the Salento area (Apulia, Italy) led us to try a characterization of the different strains of such truffle. Truffle are have a great dependence on carbohydrates supplied by their host plants and the catabolism of hexoses in the mycobiont is important for the production of energy. To investigate the presence of hexokinase activity, mycelia were growth in modified Melin-Norkrans nutrient solution (MMN) in a growth chamber at 24°C in the dark without agitation. A PCR analysis, carried out with T. borchii-specific THoI/THoII PCR primer pair (individuated in intragenic internal transcribed spacer (ITS) of the ribosomal genes), confirmed the identity of mycelia. Furthermore, two different hexokinase activities were detected in in vitro T. borchii mycelia, employing a non-denaturing electrophoresis followed by activity-staining.
DEFENCE RESPONSE OF TBY-2 CELLS TO SHORT AND LONG TERM HEAT STRESS.

SGOBB A¹, DIPIERRO N¹, PARADISO A¹, DIPIERRO S¹, DE GARA L², DE PINTO MC¹.

¹ Department of Plant Biology and Pathology, University of Bari, Via Orabona 4, 75100 Bari, Italy.
² Interdisciplinary Center for Biomedical Research, Campus Biomedico, Via A. del Portillo 21, I-00128 Roma, Italy.

Keywords: Heat stress, reactive oxygen species, TBY-2 cells, cell cycle, defence protein.

Heat stress is an adverse environmental condition that plants commonly experience in our latitudes for the occurrence of progressively hotter and longer summers. Resistance to heat stress becomes a worldwide challenge for plant survival and agricultural yield. It is known that during heat stress, Reactive Oxygen Species (ROS) levels increase resulting in significant damage to plant macromolecules. On the other hand, ROS can act as messengers that play a pivotal role in stress responses. However, the precise molecular mechanism of ROS in response to stress remains to be explored. It has been reported that plant cells exposed to ROS show temporary cell cycle arrest. Interestingly up-regulation of defence genes during oxidative stress seems to be correlated with a down-regulation of cell cycle genes. In order to clarify the relationship between cell growth and defence mechanisms the effect of short and long term heat stress has been studied in TBY-2 cells. In both stress conditions the level of expression of different cyclins, HSPs and antioxidant enzymes has been studied. The different responses of TBY-2 cells to short and long term exposure to higher temperature will be discussed.

RESPONSES OF RESISTANT AND SUSCEPTIBLE TOMATO PLANTS TO OIDIUM NEOLYCOPERSICUM.

CALABRESE IT, PARADISO A, DIPIERRO S, CICCARESE F, DE PINTO MC.

Department of Plant Biology and Pathology, University of Bari, Via Orabona 4, I-70125, Bari (Italia).

Keywords: Oidium neolycopersicum, reactive oxygen species, tomato plants, callose.

Powder mildew (Oidium neolycopersicum) is a common fungal disease of tomato plants, which is world widespread and cause great damages to plant growth and yield. These damages are especially severe in glasshouse conditions under high humidity and low temperature. A resistant tomato line (R-28), characterized by incomplete resistance to powdery mildew, had been previously selected from the LA-1230 accession of Licopersicum esculentum var cerasiforme (Ciccarese et al., 1998- Plant Patol. 47, 417-419). The resistance was due to a single recessive gene, named ol-2. In this work, susceptible (Super Marmande) and resistant (R-28) tomato plants were grown in greenhouse and inoculated with Oidium neolycopersicum conidia. In order to compare the different responses of resistant and susceptible tomato plants to powder mildew, some defence mechanisms were studied. In particular callose deposition, reactive oxygen species (ROS) production and antioxidant metabolism were analyzed.
CELL DEATH PROCESSES INDUCED BY OZONE STRESS IN SENSITIVE POPLAR LEAVES: A MENACE OR AN USEFUL EVENT FOR PLANT SURVIVING?

G. BARTOLI ¹,², L.M.C. FORINO ², A.M. TAGLIASACCHI ², M. DURANTE ¹.

¹ Department of Agricultural Plant Biology, Genetics Section, University of Pisa (Italy). ² Department of Biology, University of Pisa (Italy).

Keywords: ozone stress, Populus deltoides x maximowiczii, Eridano clone, cell death.

When subjected to episodic O₃ peaks, the leaves of the more sensitive trees frequently experience cell death events. In order to well understand these processes and to assign them a biological significance, we exposed to an acute O₃ stress the sensitive Populus deltoides x maximowiczii, Eridano clone. Within 48 hrs after fumigation end, injured areas reached the 60% of the whole leaf surface. The lesions were preceded, at cellular level, by some events referable to a programmed cell death (PCD) process evoked by the apoplastic O₃ dissociation (i.e. cell membrane asymmetry and permeability, nuclear shrinkage and DNA fragmentation). Concurrently, a biphasic oxidative burst and NO overproduction took place around the dying cells. We suggest that an acute O₃ stress may elicit PCD processes in Eridano clone leaves, aimed to limit the spreading of the oxidative burst triggered by the intercellular O₃ dissociation and consequently to preserve the integrity of tissues. We propose also a key role of PCD in cell and tissues development promotion: the nutrients from cell dismantling could be remobilized toward developing organs, which can conclude their ontogenetic program after the stress.

EFFECT OF PHOSPHORUS AVAILABILITY ON NITROGEN ASSIMILATION IN DURUM WHEAT MYCORRHIZED PLANTS.

CATELLO DI MARTINO¹, MARIA LUIGLIA PALLOTTA² AND GIUSEPPE PALUMBO¹.

¹ Dipartimento SAVA Università del Molise Via De Sanctis 86100 Campobasso. ² Dipartimento S.pe S. Università del Molise Via De Sanctis 86100 Campobasso.

Keywords: mycorrhized plant, nitrogen metabolism.

The purpose of this study was to examine the influence of phosphorus soil level on the development and on the activities of key enzymes (i.e. nitrate reductase (NR) and glutamine synthetase (GS)) involved in nitrogen metabolism in mycorrhized plant. NR and GS activities in leaf and root tissues were correlated with the P soil availability under the optimal soil nitrogen conditions in mycorrhized plants (MP) and non-mycorrhized plants (NMP). The micorrhizal treatment increased nitrate reductase activity in roots more markedly than in leaf under the different soil phosphorus conditions tested (0-10-20 and 40 ppm). In particular under phosphorus limitation the efficiency of mycorrhizal-colonization on NR activity in plant was widely increased. Our data showed that also GS activity in same conditions above reported, was higher in MP than NMP mainly in the leaf tissue to indicate that the fundamental leaf role in the nitrogen organization was particularly pronounced in micorrhizal plant condition. In apparent contrast the few important amino acid found in MP root showed an N/C ratio higher than MP leaf where was found a more large content of amino acid pool.
Parallel Session: PLANT AND ENVIRONMENT

PV21

PECTIN METHYLESTERASE IS REQUIRED FOR SUSCEPTIBILITY OF PLANTS TO NECROTROPHIC PATHOGENS AND FOR TURNIP VEIN CLEARING VIRUS SPREADING IN A. THALIANA.

VINCENZO LIONETTI¹, ALESSANDRO RAIOLA², IBRAHIM ELMAGHRABY², LUCIA SALINARO¹, FELICE CERVONE¹ AND DANIELA BELLINCAMPI¹.

¹ Department of Plant Biology University of Rome "La Sapienza", Rome. ² Department of Land and Agro-forest Environments, University of Padua, Legnaro (PD).

Abstract:
The majority of fungi and bacteria have evolved the ability to cleave the polysaccharides of the cell wall to release nutrients that sustain their growth and tissue colonization. The production of pectic enzymes, such as pectin methylesterases (PMEs) and polygalacturonases (PGs), is the pre-requisite for subsequent cell wall degradation by other hydrolytic enzymes. Evidence indicates that variation of methylesterification of pectin affects plant defense. PME knockout and over-expression of pectin methylesterase inhibitors (PMEIs) have been used to reduce PME activity in planta. We show here that growth of two necrotrophic pathogens is affected by host PME activity which is induced by the pathogens and play a role as a susceptibility factor. PME is also involved in virus infection. Interaction between the tobacco mosaic virus (TMV) movement protein (MP) and host PME is required for viral cell-to-cell and systemic movement. Our results indicate that PMEIs control the function of PME as host-receptor of viral MP and can be used as a tool for improving resistance of A. thaliana to the Turnip vein clearing virus.

PV22

IMPACT OF IRRADIANCE ON THE C ALLOCATION IN THE COASTAL MARINE DIATOM SKELETONEMA MARINOI SARNO & ZINGONE.

ALESSANDRA NORICI¹, ANNA MARIA BAZZONI², ALESSANDRA PUGNETTI², JOHN A. RAVEN³, MARIO GIORDANO¹.

¹ Laboratorio di Fisiologia delle Alghe, Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy; ² Istituto di Scienze Marine, Consiglio Nazionale delle Ricerche, Castello,1364/a, 30122 Venezia, Italy; ³ Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, United Kingdom.

Abstract:
Growth in the presence of very different photon flux densities may profoundly alter C allocation in algal cells. The need to alleviate the effect of excess radiation may lead to an unbalance in nutrient assimilation, favoring the accumulation of C (as the primary electron sink) relative to other nutrients, especially those whose assimilation requires modest proportion of reducing powers, such as P; this may be especially true in environments, such as the Northern Adriatic, where the strain of Skeletonema marinoi used for this work was isolated and where P is often the limiting nutrient for phytoplankton growth. This work is based on the hypothesis that light availability affects C allocation in S. marinoi with respect to the partitioning of C between storage lipids and carbohydrates, since the energy demand for the biosynthesis of lipids is much higher than that of carbohydrates. Our data show that, when light is in excess to the growth requirement, lipids accumulate and may serve as an effective energy and C storage. Different C allocation patterns may have profound ecological relevance, as the cell lipid to carbohydrate ratio may affect buoyancy and palatability for grazers.
PV23

PHYSIOLOGICAL CHARACTERISATION OF THE PARENTS OF A DURUM WHEAT MAPPING POPULATION UNDER DEVELOPMENT.

FULVIA RIZZA1, ELISABETTA MAZZUCOTTI1, CATERINA MARÈ2, ERNESTO BUCCIANTE1 JALEH GHASHGHAI2, HAKIM MESSAS3, SYLVIE MEYER3, ANNA MARIA MASTRANGELO4, FRANZ-W. BADECK2.

1 CRA-GPG, Centro di Ricerca per la Genomica e Postgenomica Animale e Vegetale, Via San Protaso, 302 – 29017 Fiorenzuola d’Arda (PC), Italy. 2 Potsdam Institute for Climate Impact Research (PIK), PF 60 12 03, D-14412 Potsdam, Germany. 3 Laboratoire d'Ecologie, Systematique et Evolution (ESE), CNRS AgroParisTech-UMR 8079, Batiment 362, Université de Paris-Sud (XI), F-91405 Orsay Cedex, France. 4 CRA-CER Centro di Ricerca per la Cerealicolture, SS 16 Km 675 - 71100 Foggia, Italy.

Keywords: durum wheat, drought, stomatal conductance, water use efficiency, osmotic adjustment.

The durum wheat varieties Cappelli and Ofanto are employed as parents of a mapping population, currently under development. Physiological traits related to performance under drought were studied with several growth chamber and field experiments under well watered and drought conditions. Here, we report results on Water Use Efficiency (WUE), osmotic adjustment and traits related to growth and biomass allocation. WUE was higher at leaf and whole plant level for Cappelli studied during vegetative and reproductive phases. Integrated WUE, as recorded by grain delta13C studied for 3 years of field trials confirmed this difference. The higher WUE Cappelli was associated with lower stomatal conductance over a significant range of relative soil water contents. Thus, different varietal WUE turned out to be constitutive. Lower WUE of Ofanto together with sustained higher photosynthetic rates under incipient drought were correlated with higher osmotic adjustment. Several methods employed to analyse traits related to growth and biomass allocation in the map parents showed a contrasting behaviour that can be associated to different adaptive strategies for resource use of Cappelli and Ofanto.

PV24

IN VITRO EVOLUTION HIGHLIGHTS THE RESIDUE 224 OF PGIPS (POLYGALACTURONASE-INHIBITING PROTEINS) AS A PRIMARY SPOT OF VARIABILITY FOR IMPROVEMENT OF INHIBITION CAPABILITY.

MANUEL BENEDETTI1, ELISA BASTIANELLI1, GIULIA DE LORENZO1, GIANNI SALVI1, FELICE CERVONE1, CLAUDIO CAPRAR12.

1 Dipartimento di Biologia Vegetale, Università di Roma La Sapienza, Rome, Italy. 2 Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio - S.T.A.T., Università degli Studi del Molise, Pesche (Is), Italy.

Keywords: PGIP, Pichia pastoris, epPCR, PG.

Polygalacturonase-inhibiting proteins (PGIPs) are extracellular proteins that specifically inhibit fungal endopolygalacturonases produced by fungi during the infection process. PGIPs play an important role in plant protection by favouring the accumulation of oligogalacturonides (OGs), which are elicitors of plant defence responses. We have subjected the gene encoding PGIP2 of P. vulgaris to error prone PCR (epPCR) with the aim of obtaining mutated inhibitors with novel and improved recognition capabilities. By using an expression library in Pichia pastoris and a high-throughput screening method, two mutated PvPGIP2 active against PG produced by the phytopathogenic fungus F. phyllophilum (FpPG) were isolated. Both mutants were affected by a mutation in the residue 224 indicating that this is a primary spot of variability of the protein. Both mutants were better inhibitors of FpPG.
RESPONSES OF *KOLIELLA ANTARCTICA* TO LIGHT CHANGES.

N. LA ROCCA, T. MOROSINOTTO, I. MORO, K. SCIUTO, C. ANDREOLI, N. RASCIO.

Department of Biology, University of Padova, Via U. Bassi 58/b, Italy.

*Keywords*: Koliella antarctica, light changes, photosynthesis.

The Antarctic green microalga *Koliella antarctica* (Trebuoxiophyceae), usually growing at temperature of 4°C and at continuous light intensity of 15 umol photons m⁻² s⁻¹ (low light=LL), was exposed at 150 umol photons m⁻² s⁻¹ (high light=HL) for four weeks to study its acclimation to higher light irradiance. In comparison with the LL, the HL led to a slight decrease of the cell growth and induced morphological and ultrastructural changes. At LL, cylindrical cells formed pseudo-filaments of 2-5 cells, while during the HL exposure the filaments became longer with smaller cells which contained large storage bodies. The chloroplasts, reduced in size, accumulated osmiophilic globules in the stroma. Photosynthetic pigment analyses showed a decrease of total chlorophyll contents, and an increase of carotenoids/chlorophylls values. No changes in the chl a/b ratios occurred, instead. HL acclimated cells quickly acquired an enhanced capability to dissipate the energy excess as heat, through the activation of a strong Non Photochemical Quenching. The involvement of xanthophylls and/or Li 818 protein in this last process will be discussed.

GENETIC SELECTION FOR FLOWERING TIME GENES DURING SPECIATION OF TOMATO.

FANTINI E., FALCONE G., PIETRELLA, M, AND GIULIANO G..

ENEA, Casaccia Research Center, Via Anguillarese 301, 00123 Roma, Italy.

*Keywords*: wild tomato species, flowering photoperiodic response.

The tomato clade is evolutionarily a young group of species that have diversified to occupy a great variety of habitats of the western coast of South America, from central Ecuador to northern Chile, including the Galapagos Islands. We are studying one of the most important characters in terms of fitness and adaptation: flowering. Some wild tomato species, that grow between 0 and -25 degrees of latitude and between 0 and 3700 m of elevation, show different photoperiodic responses. We have focused our efforts in the study of genes involved in the photoperiodic regulatory pathway. These genes are members of three gene families, whose orthologs in Arabidopsis and rice play a key role in the regulation of flowering in dependence of day length: *COL*, *CRYPTOCHROME* and *GIGANTEA* gene families. We provide information on the organization and expression of these families in cultivated tomato, of the microsynteny with Arabidopsis and Potato and we investigate the sequence diversification of the three gene families during speciation in the tomato clade, using both sequencing and expression profiling approaches.
PROTEOMIC ANALYSIS OF COLD STRESSED ARABIDOPSIS THALIANA CHLOROPLASTS.

V. LUCAROTTI, F. VITA AND A. ALPI.

Department of Crop Plant Biology, Univ. of Pisa, Pisa, Italy.

Keywords:

A proteomic approach was used to study the dynamics of chloroplast proteome of Arabidopsis thaliana during freezing stress. Low temperatures lead to severe damage in most plants, due to the cellular dehydration and to inhibition of photosynthesis. Arabidopsis thaliana was chosen because of its importance in molecular biology and its ability to tolerate cold. The success of this study depends on an efficient protocol for chloroplast isolation. We used Deutscher’s protocol and introduced same modifications to increase chloroplasts yield. The best results (75% of intact chloroplasts) were obtained when all steps were carried out at 4 °C with 60 g of 4-week-old Arabidopsis leaves. To estimate the integrity of the Percoll-purified chloroplasts the ferricyanide test and an SDS-PAGE analysis was used. Comparative proteomic analysis of chloroplast by 2-DE has received significant attention; we developed an efficient method to extract chloroplast proteins and produced 2-DE profiles from Arabidopsis thaliana with the aim of finding different proteins in various conditions that would allow us to spot mechanisms involved in plants stress response and tolerance.
The dynamics of photosynthesis electron transport: molecular details of regulatory mechanisms

Paolo Pesaresi\textsuperscript{1}, Mathias Pribil\textsuperscript{2,3}, Giovanni DalCorso\textsuperscript{2}, Vera Bonardi\textsuperscript{2}, Roberto Barbato\textsuperscript{4} and Dario Leister\textsuperscript{2}

\textsuperscript{1}Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli studi di Milano, Via Celoria 26, 20133 Milano, Italy
\textsuperscript{2}Lehrstuhl für Botanik, Department Biologie I, Ludwig-Maximilians-Universität Munich, Großhaderner Str. 2, D-82152 Planegg-Martinsried, Germany
\textsuperscript{3}Mass Spectrometry Unit, Department Biologie I, Ludwig-Maximilians-Universität Munich, Großhaderner Str. 2, D-82152 Planegg-Martinsried, Germany
\textsuperscript{4}Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale, 'Amedeo Avogadro', Corso Borsalino 54, I-15100 Alessandria, Italy

Proteomics studies have lead to the identification of 370 proteins associated to the chloroplast thylakoids, several of them with an as yet unknown function. Most of the unknown proteins are characterized by very low abundance with respect to the major protein complexes of the thylakoid electron transport chain, thus hampering the elucidation of their role. During the last few years, we have focused our efforts on the functional characterization of few of those proteins with unknown function by using the reverse genetics approach. Candidate genes have been selected according to aminoacid sequence comparisons and transcription patterns. Based on these studies we have been able to attribute a function to the PGRL1 protein that together with PGR5 is part of the long sought protein complex responsible of Cyclic Electron Flow (CEF) around Photosystem I (PSI). Similarly, the STN7 kinase has been identified as responsible of phosphorylation of the light harvesting complex of Photosystem II (LHCII). Plants devoid of the STN7 kinase are unable to induce the lateral migration of phosphorylated LHCII to PSI, thus preventing the redistribution of excitation energy among photosystems, during State Transitions. More recently, the TAP38 protein has been identified as the Thylakoid Associated Phosphatase responsible to dephosphorylate LHCII during State Transitions, thus promoting its migration from PSI back to Photosystem II (PSII). Interestingly, tap38 mutant plants show better phosynthetic performance under stable green-house condition, as shown by the marked increase in biomass production, indicating that the elucidation of electron transport regulatory mechanisms represents an essential step toward the improvement of plant photosynthesis and production.
SIMPLE ONE-STEP ISOLATION OF A PURE ACTIVE FORM OF PHOTOSYSTEM II DIRECTLY FROM PEA THYLAKOIDS.

CRISTINA PAGLIANO 1, FABIANA CHIMIRRI 1, GUIDO SARACCO 1, JAMES BARBER 1,2

1 Politecnico di Torino, Dipartimento di Scienza dei Materiali e Ingegneria Chimica, BioSolar Lab, viale T. Michel 5, 15121 Alessandria, Italy. – 2 Wolfsons Laboratories, Imperial College, London, SW7 2AZ, United Kingdom.

Keywords: Photosystem II, extrinsic polypeptides, PsbP.

The tight stacking of thylakoid membranes in the grana of higher plant chloroplasts selectively protects photosystem II from solubilization by detergents, presumably because most of PSII is associated with the light-harvesting Chl a/b protein LHCII, thought to be responsible for the stacking. Until today, membrane sheets enriched in LHCII and PSII (BBY), isolated by incubation of thylakoids with Triton X100, have been extensively used as starting material for further solubilizations in order to obtain purified PSII cores, after removal of their large component of LHCII. During this step of purification, a total disappearance of the extrinsic subunits PsbP and PsbQ from the reaction center of PSII was usually detected. We have optimized a simple method of purification of an active PSII reaction center based on a single direct solubilization of stacked pea thylakoids with 20 mM n-dodecyl-beta-D-maltoside, followed by a sucrose density gradient centrifugation in the presence of betaine. We found that this PSII core, depleted of the Lhcb proteins, besides the presence of PsbO, contained also a certain amount of PsbP, contributing to its integrity and high oxygen evolution rate.

REGULATION OF LIGHT HARVESTING IN PLANTS: A STRUCTURAL BASIS FOR THE PH-DEPENDENT XANTHOPHYLL CYCLE.

GIORGIA SAGA 1, ALEJANDRO GIORGETTI 2, CHRISTIAN FUFEZAN 3, GIORGIO M. GIACOMETTI 1, ROBERTO BASSI 2 AND TOMAS MOROSINOTTO 1.

1 Department of Biology – University of Padova, via U. Bassi 58B – 37121 Padova, Italy.
2 Dipartimento Biotecnologie, Università di Verona, strada le Grazie 15, Verona, Italy.
3 Westfalische Wilhelms-Universität Münster, Institut für Biochemie und Biotechnologie der Pflanzen, Hindenburgplatz 55, 48143 Münster, Germany.

Keywords: photosynthesis, photoprotection, carotenoid, conformational change, ascorbate.

Plants deal with variable environmental conditions: when exposed to strong illumination, they dissipate excess energy as heat and increase the scavenging of reacting oxygen species. Both these protection mechanisms involve activation of the xanthophyll cycle, where the carotenoid violaxanthin is converted to zeaxanthin by Violaxanthin De-Epoxidase (VDE), using ascorbate as reducing power. The inactive enzyme is soluble in the lumen and binds to the thylakoids membrane upon activation by low pH. Our structural data obtained at neutral pH shows that VDE is monomeric and its active site is occluded. At acidic pH there is a dimerization of the enzyme with the opening of a hydrophobic cavity. By in silico docking we identified within this cavity violaxanthin and ascorbate binding sites. Amino acid residues lying in close contact with the two substrates were analysed for their involvement in the catalytic mechanism allowing for the identification of two residues fundamental for activity, D177 and Y198. We also identified by in silico analysis the residues involved in the pH dependent conformational change which were after confirmed by site directed mutagenesis.
HEAT DISSIPATION IN THE MOSS PHYSCOMITRELLA PATENS: INSIGHTS ON THE EVOLUTION OF PROTECTION MECHANISMS UPON LAND COLONIZATION.

ALESSANDRO ALBORESI 1, GHAZI AZZABI 1, JULIEN GIRARDON 1, CATERINA GEROTTO 2, TOMAS MOROSINOTTO 2, ROBERTO BASSI 1.

1 Università degli Studi di Verona, Dipartimento di Biotecnologie, Strada le Grazie, 15-I, 37134 Verona. 2 Università degli Studi di Padova, Dipartimento di Biologia, Via Ugo Bassi, 35121 Padova.

Keywords: NPQ, evolution, photoprotection, Physcomitrella, LHC.

In the 16th century, Paracelsus stated "All things are poisons… only the dose makes something not poisonous". This is even more true for oxygenic photosynthetic organisms. They all need light, but it becomes toxic if in excess. A precise regulation system for light harvesting has arisen during evolution of photosynthesis. We adopted the moss Physcomitrella patens to shed light on the role of the photoprotective mechanism called Non Photochemical Quenching (NPQ) during land colonization by plants. LHCSR and PSBS chloroplast proteins are key elicitors of NPQ respectively in algae and vascular plants. Surprisingly, knock-out mutants obtained by homologous recombination showed that both NPQ mechanisms are active in P. p. In order to detail evolutionary steps we undertook analysis of three mechanistic components of NPQ: a) its regulation under the stresses typical of subaerial habitats (eg drought); b) the dependence of NPQ on zeaxanthin; c) the molecular architecture of the energy-dissipation catalytic site(s). Deciphering NPQ regulation by the key dissipater of light LHSCR will be important for biofuels research and for the industrial use of P. p. in biofarming.

IN VITRO CELL CULTURES FROM JATROPHA CURCAS L., A POTENTIAL SOURCE OF VEGETABLE OIL.

BISOGNO STEFANO 1, NICOLI JENNIFER 1, PURELLI MARINA 2, BARBI TOMMASO 2, FABBRI ANDREA 2, BALDAN BARBARA 1.

1 Dipartimento di Biologia, Università degli Studi di Padova, via U. Bassi 58/B 35131, Padova, Italy. 2 Geneticlab S.r.l., via Corte Ferrighi, 16/B Noventa Vicentina (VI), Italy.

Keywords: plant biomass, plant cell cultures, organogenesis, regeneration, Jatropha curcas.

Jatropha curcas L. is a drought-resistant, photo-insensitive perennial tree plant belonging to the Euphorbiaceae family. This species probably originated in Mexico and was introduced all over the world where is now naturalized throughout the tropical and subtropical areas. J. curcas is becoming an increasingly useful oleaginous crop in several countries for its value in many application fields. Mainly it is known as a source of oil-rich seeds (i.e. fat content of whole seeds varies up to 45%) traditionally used as a feedstock for biodiesel production. The aim of our project is to obtain, from this species, cell cultures that could be grown in bioreactors, where metabolites could be produced and isolated avoiding the restrictions imposed by cultivation in fields, by seasons and climatic zones. Furthermore, in vitro cell culture could be a starting point for crop improvement through biotechnological tools. Different culture conditions were set modulating media composition, phytohormone combinations and concentrations. We obtained calli, regeneration via organogenesis and cell suspension cultures starting from different explants of some toxic and edible J. curcas varieties.
**PVI05**

**CHARACTERIZATION OF AN ABC1-LIKE GENE OF ARABIDOPSIS THALIANA.**

**ANNA MANARA, GIOVANNI DALCORSO, ANTONELLA FURINI**

Department of Biotechnology; University of Verona; Strada Le Grazie 15, Verona Italy.

*Keywords: Abc1-like, chlorophyll fluorescence.*

The Abc1 protein family was first characterized in *S. cerevisiae* in which the ABC1 gene is required for the function of the mitochondrial bc1 complex. Our work is focusing on the characterization of an *A. thaliana* Abc1-like protein containing the Abc1 motif, a kinase domain and two transmembrane regions. The closest homolog to *AtAbc1*-like is *AtOSA1*, a protein involved in response to oxidative stress. *AtABC1L* and *AtOSA1* are localized in chloroplasts and their putative functional redundancy has been analyzed in the double mutant *atosa1/atabc1L*. Single and double mutants show no morphological or developmental abnormalities; interestingly *atosa1* and double mutant have a paler phenotype. Chlorophyll content in the *atosa1* mutant is decreased and a chl a/b ratio change is observed also in the double mutant. On MS without sucrose wild type shows the shortest roots while the double mutant the longest. Moreover, H$_2$O$_2$ causes a reduction of roots elongation in *atabc1L* and in *atosa1*. Photosynthetic electron transport was characterized by comparing chlorophyll fluorescence parameters. NPQ is induced by increasing light in all genotypes but in *atosa1* this induction is amplified.

---

**PVI06**

**REVISED ASSIGNMENT OF ROOM-TEMPERATURE FLUORESCENCE EMISSION BANDS IN SINGLE LIVING CELLS OF CHLAMYDOMONAS REINHARDTII.**

**L. FERRONI$^1$, C. BALDISSEROTTO$^1$, M. GIOVANARDI$^1$, L. PANTALEONI$^2$, T. MOROSINOTTO$^3$, S. PANCALDI$^1$.**

$^1$ Dept. of Biology and Evolution, University of Ferrara, C.so Ercole d'Este 32, 44121 Ferrara, Italy.

$^2$ Dept. of Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy.

$^3$ Dept. of Biology, University of Padova, Via Ugo Bassi 58/B, 35121 Padova, Italy.

*Keywords: Chlamydomonas, room-temperature fluorescence, photosystem II.*

In order to get new information on RT fluorescence bands, especially in the region attributed to PSII (675-695 nm), we analysed some mutants of *Chlamydomonas reinhardtii* lacking fundamental thylakoid proteins by comparison with a WT strain. Spectra were recorded from single living cells with a microspectrofluorimeter (exc. 436 nm) and characterised thorough derivative analyses and Gaussian deconvolution. The spectra of mutants defective in PSI, PSII core, or LHCs, compared with WT spectra, suggested that the dynamism in LHCII assembly could be sufficient to explain the variations in amplitudes of F680 (free LHCII), F694 (LHCII-PSII supercomplexes) and F702 (LHCII aggregates); F686 was assigned to PSII core. The variations in band amplitudes were further tested in WT cells exposed to high light. The meaning of two fluorescence emission ratios has been discussed. Under moderate photoinhibition F680/(F686+F694) mainly reflects the assembly of LHCII-PSII, while F702/(F686+F694) ratio allows to highlight the occurrence of aggregated LHCII. In the most photoinhibited samples, the RT spectra tend to degenerate, showing characteristics of mutants that are partly depleted in PSII.
**PVI07**

**MIXOTROPHIC GROWTH OF *NEOCHLORIS OLEOABUNDANS* AND LIPID SYNTHESIS INDUCTION UNDER NUTRIENT STARVATION.**

C. BALDISSEROTTO\(^1\), L. FERRONI\(^1\), M. GIOVANARDI\(^1\), L. PANTALEONI\(^2\), S. PANCALDI\(^1\).

\(^1\) Dept. of Biology and Evolution, University of Ferrara, C.so Ercole 1 d'Este 32, 44121 Ferrara, Italy. 
\(^2\) Dept. of Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy.

*Keywords: Neochloris oleoabundans, mixotrophy, starvation, lipids.*

*Neochloris oleoabundans* (Chlorophyta) can accumulate lipids, especially under N starvation, and could be proposed for biofuel production. The growth of the alga in a brackish medium under autotrophic or mixotrophic conditions was compared. For mixotrophy, organic carbon from apple processing was used. After 7 d cultivation, the lipid production was tested under starvation. Mixotrophy highly promoted growth during the first week of cultivation. Starch accumulation, increased chlorophyll content and higher PSII efficiency were observed in mixotrophic compared to autotrophic cultures, while the PSII-LHCII assembly was unaffected. Conversely, starvation tests showed increasing lipid droplets inside cells coming from both autotrophic and mixotrophic cultures. The pigment content decreased in both conditions, but a more evident decrease occurred in PSII yield for autotrophic samples. On the whole, a two-step culturing system could be proposed for *N. oleoabundans* with a 7-d mixotrophic growth in brackish medium for biomass production followed by starvation to induce lipids. The use of a waste product as organic carbon source represents an added value for the entire process.

---

**PVI08**

**THE STRUCTURE OF HOMOGALACTURONAN IS CRITICAL FOR PLANT BIOMASS PROCESSING.**

VINCENTO LIONETTI, FEDRA FRANCOCCI, SIMONE FERRARI, DANIELA BELLINCAMPI, DANIELA PONTIGGIA, LORENZO MARIOTTI, ELISA BASTIANELLI, DANIELA PONTIGGIA, GIULIA DE LORENZO AND FELICE CERVONE.

Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza", Rome, Italy, 00185.

*Keywords: plant cell wall, saccharification, bioprocessing, biofuel.*

The cell wall recalcitrance to enzymatic hydrolysis is the main bottleneck for the industrial scale-up of biomass processing and bioconversion to fermentable sugars. Pectin stabilizes the cell wall by calcium-mediated cross-links formed by stretches of acidic homogalacturonan (HGA). We have demonstrated the length reduction of HGA in Arabidopsis and tobacco through the expression of a fungal polygalacturonase (PG) and the reduction of the de-methylated form of HGA in Arabidopsis and wheat through the expression of an inhibitor of pectin methylesterase enhance the tissue digestibility and reduce the need of acid pre-treatment used before biomass processing. We show here that a better digestibility of the plant tissue is consistently correlated with a lower content of de-methylated stretches of HGA in the cell wall. This can be achieved by transforming plants not only with fungal PGs and inhibitors of pectin methylesterases but also by other pectic enzymes of different origin. Plants with reduced quantities of the acidic form of HGA may also be genetically selected.
**A hitchhiker’s guide of Ca$^{2+}$ handling: basic principles of Ca$^{2+}$ measurement and handling in mammalian cells.**

Ilaria Drago$^1$ and Tullio Pozzan$^{1,2,3}$

$^1$ Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, Padova, Italy
$^2$ CNR Neuroscience Institute, Viale G. Colombo 3, Padova, Italy;
$^3$ Venetian Institute of Molecular Medicine, via Orus 2 Padova, Italy

Cells have evolved a complex protein toolkit that allow them to use Ca$^{2+}$ as a second messenger to modulate processes ranging from ATP production to muscle contraction and apoptosis. The fine regulation of such a number of processes is achieved by a strict control of [Ca$^{2+}$] variations inside the cell from both a temporal and spatial point of view. The use of synthetic and genetically encoded Ca$^{2+}$ probes has greatly contributed to unravel the role of different subcellular compartment in Ca$^{2+}$ signaling and nowadays information on mitochondrial, endoplasmic reticulum, Golgi apparatus, peroxisomal and secretory vesicles Ca$^{2+}$ homeostasis are available. The role played by intracellular compartments in mammalian cells Ca$^{2+}$ handling and the tools available to measure it will be discussed and linked to recent finding on Ca$^{2+}$ handling abilities of intracellular organelles (e.g. peroxisomes and mitochondria) in plants.

**A calcium sensor / protein kinase network for decoding calcium signals in plants.**

J. Kudla

Universität Münster, Institut für Botanik und Botanischer Garten, Schloßplatz 4, 48149 Münster, Germany.

Intracellular release of calcium ions belongs to the earliest events in signal perception. Calcium-binding proteins are involved in sensing and relaying these signals to downstream signalling and adaptation responses. Calcineurin B-like proteins (CBLs) represent a group of calcium sensor proteins that are closely related to Calcineurin B and Neuronal Calcium Sensors (NCS). CBLs exclusively interact with a group of serine-threonine kinases designated as CBL-interacting protein kinases (CIPKs). In Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 26 CIPKs. Preferential complex formation of individual CBLs with defined subsets of CIPKs appears to be one of the mechanisms generating the temporal and spatial specificity of calcium signals in plant cells.

Reverse genetics and cell biological approaches have begun to unravel the functional principles of this signalling network. I will present results of our characterization of cbl and cipk loss-of-function mutants and of our investigation of the sub-cellular localization of all CBLs from Arabidopsis. These studies suggest that CBL/CIPK complexes function predominantly at cellular membranes and can decode Ca$^{2+}$ signals in different compartments. In this context, dual lipid modification by myristoylation and palmitoylation appears to play an important role in determining the membrane targeting of CBL/CIPK complexes. Our reverse genetics analyses indicate that alternative complex formation of CIPK-type kinases with different CBLs enables the simultaneous regulation of ion transport processes in different compartments of the plant cell. In this way CBL/CIPK complexes contribute to regulating the extrusion of Na$^+$ ions in root tissues and in addition regulate the sequestration of Na$^+$ in the vacuole in green tissues. Moreover, several CBL/CIPK complexes appear to Ca$^{2+}$-dependently modulate K$^+$ channels.
Plenary Session: PLANT CELL SIGNALLING

**Plastid-to-nucleus signalling – involvement of dual-targeted proteins?**

Karin Krupinska

*Institute of Botany, Christian-Albrechts University of Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany.*

The effects of plastid originating molecules acting in retrograde signaling such as reactive oxygen species and intermediates of tetrapyrrole biosynthesis on nuclear gene expression are well characterized. The mechanisms of transducing information from the plastid to the nucleus are however still unknown. Proteins dually located in plastids and the nucleus might be involved in such plastid-to-nucleus signalling. One rationale of their dual location could be storage or sequestration inside the plastid until specific conditions require the activity of such proteins in the nucleus (Krause and Krupinska 2009, Trends Plant Sci 14: 194). One such protein is Whirly1 which recently was shown to be located in the nucleus and plastids of the same cell (Grabowski et al. 2008, Plant Phys 147: 1800). It is hypothesized that Whirly1 is involved in transduction of information on plastid development and photosynthetic function to the nucleus. Evidence for a control of leaf senescence by Whirly1 will be presented.
**PVII01 - PLANT CELL SIGNALLING**

**CLONING AND CHARACTERIZATION OF TWO SERK-LIKE GENES HOMOLOGOUS TO ATSERK1 AND ATSERK2 AND DIFFERENTIALLY EXPRESSED DURING SOMATIC EMBRYOGENESIS IN CYCLAMEN PERSICUM.**


1 "Sapienza" University of Rome – Dept. of Genetics and Molecular Biology – Rome (Italy). 2 "Sapienza" University of Rome – Dept. of Plant Biology – Rome (Italy). 3 C.R.A.-Research Unit for Floriculture and Ornamental Species-Sanremo (Italy).

Keywords: somatic embryogenesis, SERK genes, Cyclamen persicum.

Somatic embryogenesis is regarded as a powerful tool to study zygotic embryogenesis in higher plants, as well as to propagate plants of commercial significance. This is particularly true for plants, such as Cyclamen, which can be only propagated through somatic embryos. To study the molecular mechanism of somatic embryogenesis we searched Cyclamen persicum for homologues of AtSERK1, a gene involved in somatic embryogenesis, but also in organogenesis, brassinosteroid signaling, programmed cell death, and innate immunity. By using a combination of different techniques, we eventually identified the full cDNA and genomic sequences of CpSERK2 and CpSERK1, as well as partial cDNAs similar to SERK3, and SERK4. We are currently investigating, by means of qRT-PCR and in situ hybridization from callus lines with different morphogenic potentials, the relative roles of these genes in the process of somatic embryogenesis, with the ultimate goal to create transgenic callus lines with high embryogenic potential. The analysis of expression of these genes represents a first step towards the determination of the redundant or specialized functions of the different members of the SERK family in Cyclamen.

**PVII02 - PLANT CELL SIGNALLING**

**LEAF SAPERIN ISOFORMS: CELLULAR LOCALIZATION BY GFP-FUSION ANALYSIS.**

RENAITO RODRIGUES POUSADA 1, ANDREA TARTARINI 1, M. ADELAIDE IANNELLI 2 & LAURA SPANO 1.

1 Dipt Biologia di Base e Applicata, Università dell'Aquila, Aquila. 2 UOS Roma IBBA-CNR, Monterotondo (RM).

Keywords: Saporins, GFP fusions, Subcellular localization.

Ribosome-inactivating proteins (RIPs) are a large group of plant enzymes present in a great variety of species, which inhibit protein synthesis through a site-specific deadenylation of the large ribosomal RNA at level of the conserved alpha-sarcin/ricin loop. Saporins are highly basic single chain RIPs present in different organs of the plant *Saponaria officinalis* (Caryophyllaceae). Previously in our laboratory, two leaf isoforms, saporin L3 and saporin S6-like, characterized by different toxicity profiles were isolated. These isoforms were isolated from intra and extracellular fractions, respectively. We constructed GFP fusions to obtain more detailed information on the localization of these two saporins. Transformation of *Arabidopsis thaliana* seedling roots using Agrobacterium and of onion epidermal tissues using biolistics were performed. In roots, our preliminary data on the fusions with the NH-terminus portion of two saporins (first 100 aa of the immature protein with signal peptide) suggests this region is sufficient for intra- and extracellular targeting. A potential tissue dependent targeting of saporin S6-like derived from onion epidermal data is presented.
THE RATE OF CELL DIFFERENTIATION CONTROLS THE ARABIDOPSIS ROOT MERISTEM GROWTH PHASE.

SERENA PERILLI 1, LAILA MOUBAYIDIN 1, RAFFAELE DELLO IOIO 1,2, RICCARDO DI MAMBRO 1, PAOLO COSTANTINO 1, SABRINA SABATINI 1.

1 Dipartimento di Genetica e Biologia Molecolare, Laboratory of Functional Genomics and Proteomics of Model Systems, Sapienza UniversitÃ di Roma - P.le Aldo Moro, 5 - 00185 Rome, Italy.
2 Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.

Keywords: root meristem, cell differentiation, cytokinin, gibberellin.

Upon seed germination, apical meristems grow as cell division prevails over differentiation and reach their final size when division and differentiation reach a balance. In the Arabidopsis root meristem, this balance results from the interaction between cytokinin (promoting differentiation) and auxin (promoting division) through a regulatory circuit whereby the ARR1 cytokinin-responsive transcription factor activates the gene SHY2, which negatively regulates the PIN genes encoding auxin transport facilitators. However, it remains unknown how the final meristem size is set. Here, we show that during meristem growth, expression of SHY2 is driven by another cytokinin-response factor, ARR12, and that completion of growth is brought about by the upregulation of SHY2 caused by both ARR12 and ARR1: this leads to an increase in cell differentiation rate that balances it with division, thus setting root meristem size. We also show that gibberellins selectively repress expression of ARR1 at early stages of meristem development, and that the DELLA protein REPRESSOR OF GA 1-3 (RGA) mediates this negative control.

INHIBITION OF GLUCURONIDASE ACTIVITY (GUS) IN PLANT EXTRACTS IN RELATION TO THE USE OF GUS AS REPORTER GENE.

FIOR SIMONE 1 AND GEROLA PAOLO 2.

1 Istituto Agrario di San Michele all'Adige, via E.Mach 1, 38010 San Michele all'Adige (TN), Italy
2 Dipartimento Biologia Strutturale e Funzionale, Via H. Dunant 3, 21100 Varese, Italy.

Keywords: Reporter gene, GUS assay, Arabidopsis, Rice, Tobacco.

The glucuronidase (GUS) gene reporter system is one of the most effective and employed techniques in the study of gene regulation in plant molecular biology. We report that inhibitors of GUS activity are ubiquitous in organ tissues of Arabidopsis, tobacco and rice, and significantly bias quantitative assessment of GUS activity in plant transformation experiments. Combined with previous literature reports on non-model species, our findings suggest that inhibitors may be common components of plant cells, with variable affinity towards the E. coli enzyme. The reduced inhibitory capacity towards the plant endogenous GUS discredits the hypothesis of a regulatory role of these compounds in plant cells, and their effect on the bacterial enzyme is better interpreted as a side effect due to their interaction with GUS during the assay. This is likely to have a bearing also on histochemical analyses, leading to inaccurate evaluations of GUS expression. In order to achieve reliable results, inhibitor activity should be routinely tested during quantitative GUS assays. Two separate methods to correct the measured activity of the transgenic and endogenous GUS are presented.
FROM PLANT TO HUMAN PHYSIOLOGY: ROLE OF THE PHYTOTOXIN FUSICOCCIN IN THE AGGREGATION PROCESS.

DI LUCENTE CRISTINA, CAMONI LORENZO, VISCONTI SABINA, ADUCCI PATRIZIA.

Department of Biology, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133 Rome, Italy.

Keywords: Plant H^+-ATPase, fusicoccin, 14-3-3 proteins, glicoprotein Ib-alpha, aggregation process.

The fungal toxin Fusicoccin (FC) affects a variety of physiological processes in plants. Its effects have been attributed to its ability to stimulate the plasma membrane H^+-ATPase. FC promotes the permanent association of 14-3-3 proteins to the C-terminal autoinhibitory domain of the H^+-ATPase, thereby leading to proton pump activation. 14-3-3s are conserved dimeric proteins involved in several cellular processes. 14-3-3s associate to their targets in a sequence-specific and phosphorylation dependent manner. The H^+-ATPase binds 14-3-3 via an unusual motif (–YpTV), located at the very end of the C terminus. A similar C-terminal binding motif is present in a human protein, the glycoprotein Ib-alpha (GPIb-alpha). This protein is part of a platelet membrane complex, which mediates the initial adhesion of circulating platelets to vessel wall matrix. We explored the possibility that FC action could not be restricted to the H^+-ATPase/14-3-3 interaction, but it may be extensible to other 14-3-3 targets with a C-terminal motif. Therefore, we investigated the FC effect in the 14-3-3/GPIb-alpha interaction and the functional consequence of FC action on the platelet aggregation process.

CLONING AND CHARACTERIZATION OF SERK GENES FROM ORCHIDS.

AUGUSTA YADIRA CUEVA AGILA ¹, IVAN GUACHIZACA ², LORENZO CONCIA ¹, RINO CELLA ¹.

¹ Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy. ² Institute of Ecology, Universidad Tecnica Particular de Loja, Loja. CP 11-01-608, Ecuador.

Keywords: biodiversity, orchids, propagation, somatic embryogenesis RLKs, SERK genes.

Conservation of endangered species can rely on germplasm banks or in vitro propagation. Cyrtochilum loxense (Lindl.) Kraenzl, is an endemic orchid widely distributed in the southern province of Loja (Ecuador). Its conservation is at risk of extinction due to natural habitat reduction. For this reason an in vitro regeneration strategy based on somatic embryogenesis was implemented. The latter is a convenient plant regeneration method as well as a tool for studying the processes of de-differentiation and re-differentiation in plants. Somatic Embryogenesis Receptor like Kinase (SERK) genes were initially identified and studied for their role during embryogenesis in several model species (carrot, Arabidopsis and rice) even though they now appear to have additional roles. Although Orchids are not model plants, it is important to know the factors that stimulate somatic embryogenesis finalized to their propagation and active conservation. One of the aim of this study was the cloning and the characterization of orchid SERK genes. To this end we have used a PCR cloning strategy. The obtained sequences were used for evaluating their expression during somatic embryogenesis in orchids.
INTERACTION STUDIES OF THE ARABIDOPSIS 14-3-3 ISOFORMS GF14-OMEGA AND GF14-EPSILON WITH THE PLASMA MEMBRANE H⁺-ATPASE.

PALLUCCA ROBERTA¹, VISCONTI SABINA¹, CAMONI LORENZO¹, MELINO SONIA² AND ADUCCI PATRIZIA¹.

¹ Dipartimento di Biologia, Università degli Studi di Roma “Torvergata”. ² Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma "Torvergata".

Keywords: 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. So far it is not fully clarified whether different isoforms may accomplish different functions. In several systems 14-3-3 proteins are experimentally interchangeable, however a specific target preference has also been reported both in vivo and in vitro.

In order 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 expressed in Escherichia coli and 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, thus suggesting that the H⁺-ATPase may be regulated by 14-3-3 proteins in an isoform-specific manner.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF WHIRLY2: A MITOCHONDRIAL PROTEIN INVOLVED IN PLANT PCD/SENESCENCE REGULATION.

ALEX COSTA¹, MARTINA BODNER¹, CRISTINA RUBERTI¹, YING MIAO², RENA ISEMER², FIORELLA LO SCHIAVO¹, KARIN KRUPINSKA², MICHELA ZOTTINI¹.

¹ Department of Biology, University of Padova – Via U. Bassi 58/b, 35131 Padova – Italy. ² Institute of Botany, University of Kiel - Olshausenstr. 40, D-24098 Kiel, Germany.

Keywords: Whirly proteins, mitochondria, programmed cell death, confocal in vivo analyses, subcellular localization.

Programmed cell death (PCD) is a genetically regulated process of cell suicide, essential for plant growth and development. Mitochondria play a central role in controlling PCD induced during plant development and in response to various stresses. Recently a role for chloroplasts has been proposed in cell death induction events. At present, the relationship between mitochondria and plastids in PCD regulation remains unknown and needs to be addressed. Whirly proteins are good candidates to be considered inter-organelar signals since they are a small gene family coding for proteins targeted to the nucleus, chloroplasts and mitochondria. The focus of the present work is on Whirly2. We analysed the sub-cellular localization and expression pattern of Why2-YFP, under control of the p2X35S or pWhy2, in stably transformed Arabidopsis plants. To better understand the role of Why2 in PCD regulation, RT-PCR analyses and GUS assays were performed during PCD. Mitochondria morphology and dynamics have been investigated, by confocal in vivo analyses, in over-expressing- and knock out- Why2 plants.
A CHIMERIC RECEPTOR APPROACH FOR THE MANIPULATION OF DANGER SENSING APPARATUS OF PLANTS.

FRANCESCA SICILIA¹, ALEXANDRE BRUTUS¹, VANESSA MODESTI¹, GIOVANNA GRAMEGNA¹, FEDERICO ANDREANI¹, ALBERTO MACONE², FELICE CERVONE¹, GIULIA DE LORENZO¹.

¹ Istituto Pasteur Fondazione Cenci Bolognetti, Dipartimento Biologia Vegetale and ² Dipartimento di Scienze Biochimiche "A. Rossi-Fanelli" Università di Roma "Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy.

Keywords: damage-associated molecular patterns, pectin-mediated signaling, plant immunity, chimeric receptors, wall associated kinases.

Oligogalacturonides (OGs) released from the plant cell wall pectin are active as both damage-associated molecular patterns (DAMP) and regulators of plant growth. Members of the Wall-Associated Kinase (WAK) family bind these oligosaccharides in vitro and are candidate receptors. A domain swap approach was used to define to role of WAK1. We first defined the appropriate chimeric design and demonstrated that the Arabidopsis pattern recognition receptor EFR is amenable to the construction of functional chimeric receptors, carrying the ectodomain of another Arabidopsis PRR, FLS2. After, we analyzed chimeras derived from EFR and WAK1. Our results show that, upon stimulation with OGs, the WAK1 ectodomain is capable of activating the EFR kinase domain. On the other hand, upon stimulation with the cognate ligand elf18, the EFR ectodomain activates the WAK1 kinase, triggering defense responses that mirror those normally activated by OGs and are effective against fungal and bacterial pathogens. Finally, we show that transgenic plants overexpressing WAK1 are more resistant to Botrytis cinerea. Intercatomic approaches are in progress to identify WAK1 signaling partners.

EXPRESSION STUDY OF MTN5, A MEDICAGO TRUNCATULA GENE REQUIRED FOR NODULATION, DURING THE EARLY PHASES OF RHIZOBLIA INFECTION.

YOURY PII, BARBARA MOLESINI AND TIZIANA PANDOLFINI.

Biotechnology Department, University of Verona, Verona, Italy.

Keywords: MtN5, Medicago truncatula, Root Nodule, Rhizobia, early nodulin.

MtN5 is a nodulin expressed both in the initial stages of infection and in mature nodules. The suppression of MtN5 resulted in a marked reduction of root nodules, suggesting that MtN5 is required for nodulation. In order to investigate the role of MtN5 in root nodules induction pathway, its expression profile during the early stages of infection was studied. MtN5 showed to be co-expressed with two early marker of rhizobia infection, RIP1 and ENOD11, and resulted to be more precocious than ENOD20 and MtN6. These results confirm that MtN5 is an early nodulin and suggest that it might be involved in the very initial events following the NFs perception. In transgenic roots carrying the MtN5promoter::GUS fusion, GUS expression was detected in epidermis and root hairs a few hours after inoculation, whereas 24 hpi it was observed in close proximity of the central stele. In root nodules, GUS was detected in the distal zone, corresponding to the nodule meristem. MtN5 expression was also observed in both the apical meristem of young roots and lateral roots primordia, suggesting an involvement in cell division/differentiation and/or organogenesis.
**Plenary Session: PLANT CELL SIGNALLING**

**PVII11**

**THE HSP20-LIKE CHAPERONE P23 OF ARABIDOPSIS IS A SPECIFIC TARGET OF CK2, INVOLVED IN SALICYLIC ACID SIGNALLING PATHWAY.**

STEFANO D’ALESSANDRO¹, ALEX COSTA¹, KENDRA TOSONI², GIORGIO ARRIGONI², FIORELLA LO SCHIAVO¹, MARIA RUZZENE² AND MICHELA ZOTTINI¹.

¹ Dipartimento di Biologia, Università degli Studi di Padova. ² Dipartimento di Chimica Biologica, Università degli Studi di Padova, Viale G. Colombo 3, 35131 Padova, Italy.

*Keywords: Casein Kinase 2, Salicylic acid signalling, p23.*

Salicylic acid (SA) plays a key role in various physio-pathological processes in plants. We demonstrated that the SA-induced nitric oxide (NO) production in Arabidopsis seedlings is mediated by the activity of casein kinase 2 (CK2). Comparing the phosphorylation pattern of proteins obtained from Arabidopsis seedlings, treated with SA and/or with a specific inhibitor of CK2, we identified a 23KDa protein (p23) that was further investigated. Recombinant p23 was obtained and it showed to be highly phosphorylated, in vitro, by the human and maize recombinant CK2 and by cytoplasmic protein extracts of Arabidopsis. Moreover, by in gel kinase assay we demonstrated that CK2 is the major protein kinase responsible for p23 phosphorylation. Transient transformation experiments, performed in Arabidopsis mesophyll protoplasts, showed that both p23 and the Arabidopsis CK2alpha-C co-localize at nuclear and cytoplasmic level. Bimolecular Fluorescence Complementation (BiFC) and "Surface Plasmon Resonance" analyses show that interaction occurs between p23 and CK2. In order to gain inside the physiological role of p23 we then analysed the in planta expression pattern by promoter analysis.

**PVII12**

**SUPRAMOLECULAR COMPLEXES IN REDOX SIGNALLING: A CASE STUDY ABOUT INTRINSICALLY DISORDERED PROTEINS AND STRUCTURED PARTNERS.**

MARRI L¹, SPARLA F¹, TRIVELLI X², THUMIGER A¹, FERMANI S³, CALVARESI M³, ZERBETTO F², FALINI G², PUPILLO P¹, TROST P¹.

¹ Department of Experimental Evolutionary Biology - University of Bologna. ² Unité de Glycobiologie Structurale et Fonctionnelle - Science and Technology University of Lille. ³ Department of Chemistry - University of Bologna.

*Keywords: redox signalling, intrinsically disordered protein, supramolecular complex.*

About one third of eukaryotic proteins are predicted to contain long regions of intrinsic disorder. Within protein interacting networks, intrinsically disordered proteins (IDPs) are often hubs and play a role in signalling pathways. Potential of IDPs in protein-protein interactions may be amplified by post-translational modifications, though binding to structured partners is limited by the entropic cost associated with IDP folding within complexes. Here we report on the interaction between a plant IDP, CP12, and one of its structured partners, GAPDH, studied by a combination of protein crystallography, NMR and molecular dynamics. Though largely unstructured in solution, a small structural motif in CP12 is stabilized upon formation of a disulfide under thioredoxin control (e.g. in the dark in chloroplasts). Such local structure is selected by GAPDH for a preliminary interaction which favours the folding of the disordered C-terminal tail of CP12 into the catalytic site of GAPDH, the large entropic cost of this process being compensated by a series of hydrogen bonds. Such synergistic mechanism of binding may be generally advantageous for complex assembly by disordered proteins.
EARLY TRANSDUCTION ELEMENTS INVOLVED IN OLIGOGALACTURONIDE SIGNALLING.

DANIEL V. SAVATIN, ROBERTA GALLETTI, SIMONE FERRARI, NORA GIGLI, FELICE CERVONE, GIULIA DE LORENZO.

Università di Roma "Sapienza", Dipartimento "Charles Darwin", Roma - Italy.

Keywords: plant defence, OG signalling.

Pectin-derived fragments (oligogalacturonides, OGs) function as damage-associated molecular patterns (DAMPs) and activate the plant immune response. So far, little is known about the intracellular elements involved in the early events triggered by OGs. We are currently studying the MAPKs modules that positively regulate OG signalling and the phosphatases that switch off elicitor-activated immunity. OGs also regulate developmental responses, likely due to their ability to antagonize auxin. The molecular basis of OG/auxin antagonism is still unknown. Here we show that this process does not require the signal molecules ethylene, jasmonic acid and salicylic acid and is independent of the NADPH oxidase AtrbohD. Moreover, the auxin antagonism exerted by OGs does not require microRNAs activity, unlike that exerted by the pathogen-associated molecular pattern flg22.

ROLE OF PECTIN IN PLANT DEFENSE AND DEVELOPMENT.

SIMONE FERRARI¹, ROBERTA GALLETTI¹, FEDRA FRANCOCCI¹, ALBERTO FERRARINI², FELICE CERVONE³, GIULIA DE LORENZO⁴.

¹ Dipartimento "Charles Darwin", Sapienza Università di Roma, Rome, Italy. ² Dipartimento di Biotecnologie, Università degli Studi di Verona, Verona, Italy.

Keywords: pectin, defense, sugar, cell wall, plant-pathogen interactions.

The cell wall has a major role in plant development and in defense against pathogens. In particular, pectin is important because it mediates cell adhesion and is the first target for hydrolytic enzymes secreted by pathogens. Plant expressing pgIa, a fungal polygalacturonase that degrades the homogalacturonan component of pectin (PG plants), have reduced growth, constitutive production of hydrogen peroxide and enhanced resistance to Botrytis cinerea. Similar phenotypes were also observed in quasimodo2-1 (qua2-1), a mutant affected in homogalacturonan biosynthesis. Microarray analysis revealed that defense proteins involved in resistance against B. cinerea are constitutively expressed in both PG plants and qua2-1. Furthermore, PG and qua2-1 seedlings show sugar hypersensitivity, determined as hypocotyl growth inhibition and beta-amylase expression. The relationship between pectin modification, sugar sensing and activation of defense responses will be discussed.
THE ARABIDOPSIS HOMEODOMAIN-LEUCINE ZIPPER II GENE FAMILY: DIVERSITY AND REDUNDANCY.

MONICA CARABELLI 1, LUANA TURCHI 1, MASSIMILIANO SASSI 1, VALENTINO RUZZA 1, MARCO POSSENTI 2, GIORGIO MORELLI 2 AND IDA RUBERTI 1.

1 Institute of Molecular Biology and Pathology, National Research Council, Italy. 2 National Research Institute for Food and Nutrition, Italy.

Keywords:
The Arabidopsis genome contains 10 genes belonging to the HD-Zip II family including ATHB2 and HAT2. Previous work has shown that ATHB2 is rapidly and strongly induced by light quality changes that provoke the shade avoidance response whereas HAT2 expression responds to auxin. Recently, a genome-wide analysis of the HD-Zip II family has been undertaken. Phylogeny reconstruction revealed that almost all of the HD-Zip II genes can be subdivided into 4 clades (alpha - delta), each clade comprising 2–3 paralogs. Gene expression studies demonstrated that all the gamma and delta genes are regulated by light quality changes. Kinetics of induction, low R/FR/high R/FR reversibility and auxin response analyses strongly suggested that HAT1, HAT3 and ATHB4, as ATHB2, are under the control of the phytochrome system whereas HAT2 is up-regulated by low R/FR as a consequence of the induction of the auxin signaling pathway provoked by FR-rich light. Whole mount in situ expression analysis revealed that gamma and delta genes are also tightly regulated during plant development with both distinct and overlapping patterns. Assessment of the role of each of the HD-Zip II gamma and delta genes is presently in progress, by means of phenotypic and molecular analysis of single, double and triple loss-of-function mutants in high and low R/FR light. Together, the results provide evidence for a complex pattern of expression and regulation of this gene family, and they strongly suggest that HD-Zip II genes act as members of highly integrated networks in controlling organ development as well as plant responses to light quality changes.
Sensing and Signaling in the Abscisic Acid Pathway; Molecular Mechanisms.
José A. Márquez

The High Throughput Crystallization Laboratory EMBL Grenoble France.

The plant hormone Abscisic Acid (ABA) plays a central role in the regulation of plant growth and development as well as the coordination of the adaptive response to abiotic stress. Recently, a 14-member family of intracellular ABA-receptors, named PYR1/PYL/RCAR, has been identified. These proteins inhibit in an ABA-dependent manner the activity of a family of key negative regulators of the ABA signaling pathway; the clade A protein phosphatases type 2C (PP2Cs). The analysis of the crystallographic structures of the PYR1 receptor and receptor-PP2C complexes, shows that the hormone binds to a large cavity in the receptor which favors the binding of the (+) ABA stereoisomer. In the ligand-bound form, a series of loops surrounding hormone binding cavity seem to act as gates, folding over the ABA molecule and stabilizing it inside the cavity. Structural and biochemical data confirm that conformational changes in these “gating loops” also play a critical role in the formation of the receptor-PP2C complex and the inhibition of the phosphatase activity. The implications for the mechanism of hormone perception and the activation of the stress response pathway will be discussed.

Temporal and spatial regulation of cell cycle genes by the SHR/SCR network links patterning and growth.
Sozzani R.¹, Moreno-Risueno M.A.¹, Busch W.¹, Van Norman J.M.¹, Murray J.A.², Benfey P.N.¹

¹ Department of Biology and IGSP Center for Systems Biology, Duke University, Durham, North Carolina, USA;
² Cardiff School of Biosciences, Cardiff CF10 AX, Wales, UK

The development of multicellular organisms relies on the coordinated control of cell divisions that lead to proper organ patterning and growth. The molecular mechanisms underlying pattern formation are still poorly understood, in particular how developmental pathways regulate genes involved in formative divisions. In the Arabidopsis root, the formative cell divisions that give rise to the cortex and endodermis are controlled by the transcription factors, SHORTROOT (SHR) and SCARECROW (SCR). In this study, the cell-type specific transcriptional effects of SHR and SCR induction combined with ChIP-chip data revealed that SHR regulates the spatial and temporal activation of specific genes involved in cell division. Coincident with the onset of a specific formative division, SHR and SCR directly activate a D-type cyclin. Altering its expression resulted in formative division defects in both loss-of-function and gain-of-function plants. Our results indicate that proper pattern formation is achieved through transcriptional regulation of specific cell cycle genes in a cell-type and developmental stage-specific context. Taken together we provide evidence for a direct link between developmental regulators, specific components of the cell cycle machinery and organ patterning.
Danger sensing in plants.

Giulia De Lorenzo

Department of Plant Biology, University of Rome "Sapienza"

Oligogalacturonides (OGs) released from the plant cell wall pectin are active as both damage-associated molecular patterns (DAMP) and regulators of plant growth. Members of the Wall-Associated Kinase (WAK) family bind these oligosaccharides in vitro and are candidate receptors. A domain swap approach was used to define to role of WAK1. We first defined the appropriate chimeric design and demonstrated that the Arabidopsis pattern recognition receptor EFR is amenable to the construction of functional chimeric receptors, carrying the ectodomain of another Arabidopsis PRR, FLS2. After, we analyzed chimeras derived from EFR and WAK1. Our results show that, upon stimulation with OGs, the WAK1 ectodomain is capable of activating the EFR kinase domain. On the other hand, upon stimulation with the cognate ligand elf18, the EFR ectodomain activates the WAK1 kinase, triggering defense responses that mirror those normally activated by OGs and are effective against fungal and bacterial pathogens. Transgenic plants overexpressing WAK1 are more resistant to Botrytis cinerea.
Marine Plant Metabolites in the Light of Ecological and Physiological Considerations.

Angelo Fontana

Marine Bio-Organic Chemistry Group, Institute of Biomolecular Chemistry - Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy.

This presentation is a report of our studies on marine plant organisms, in relation to the established context of the world-wide identification of bioactive marine natural products as promising drug leads and cell biology tools. The contribution, which is focused on the description of the chemical structures and biological activities of compounds isolated from marine eukaryotes, will also discuss the main methodologies used to pursue bioactive molecules, starting from recognition of the natural function as eco-physiological mediators. Ultimately, understanding of the factors that modulate production of these compounds, together with knowledge of the biochemical pathways responsible for their biosynthesis, is a crucial step to enable the production in the scaled-up quantities that are necessary to transform a marine natural product into a commercial entity.
THE ACTIVITY OF *ARABIDOPSIS THALIANA Δ¹-PYRROLINE-5-CARBOXYLATE REDUCTASE IS MODULATED BY GLUTAMATE AVAILABILITY, AND BY BOTH THE RATIO AND THE REDOX STATUS OF PYRIDINE NUCLEOTIDE COFACTORS.

SAMUELE GIBERTI¹, DIETMAR FUNCK², DAVIDE PETROLLINO¹ AND GIUSEPPE FORLANI¹.

¹ Department of Biology and Evolution, University of Ferrara, Italy. ² Department of Plant Physiology and Biochemistry, University of Konstanz, Konstanz, Germany.

Keywords: glutamate, ornithine, proline, NAD(H)/NADP(H) ratio, redox status.

Δ¹-pyrroline-5-carboxylate reductase (P5CR, EC 1.5.1.2) catalyses the last step in both the two pathways, from glutamate or ornithine, that have been hypothesized to occur in plants and lead to proline biosynthesis. In response to several stress conditions, proline is accumulated inside the cell to highest levels, even though the physiological role of such an accumulation is still being debated. P5CR transcripts were found to be induced under hyperosmotic stress, yet the carbon flow in the glutamate pathway is believed to be regulated at the level of the first enzyme, P5C synthetase. Moreover, in many instances transcript levels were consistent with neither activity levels, nor the resulting free proline content. The occurrence of post-translational regulative mechanisms has been hypothesized, but poorly investigated to date. We previously isolated P5CR from *A. thaliana cultured cells. Here we report a thorough characterization pointing out several allosteric regulative mechanisms. Among them an enzyme modulation based upon NADP/NAD ratio, that has been described to change following the exposure to stress conditions, could play a main regulative role in vivo.

AMINO ACID CONTENT IN RAPESEED NECTARS: DO NECTARIES MODIFY PHLOEM SAP?

MICHELE BERTAZZINI AND GIUSEPPE FORLANI

Department of Biology and Evolution, University of Ferrara, Italy.

Keywords: phloem sap, nectar, rapeseed, amino acid content, attractiveness to honeybees.

Many plants require pollinators to obtain efficient seed set. Dicotyledonous species often attract pollinating insects by offerings them a reward represented by floral nectar, a nutrient-rich aqueous solution derived from the phloem sap and produced by a group of specialized cells, called nectaries. Sugar content ranges from 5 to 80%, and in several cases sucrose, glucose and fructose are present in equal amounts. Since phloem sap contains mostly sucrose, chemical reactions must occur that are catalyzed by nectary-localized enzymes. Nectar contains also amino acids, whose biological significance is still being debated. Increasing evidence supports a preference of bees and butterflies for proline-containing nectars, and a co-evolutionary strategy enhancing fitness of plants that produce proline-rich nectar by visiting insects that perceive its presence has been hypothesized. However, our comprehension is hampered by a substantial lack of information concerning proline metabolism in the nectaries. Here we report on amino acid content of nectar and phloem sap collected from various rapeseed cultivars, whose comparison sheds some light on nectary contribution to nectar composition.
Parallel Session: PLANT METABOLISM

PIX03

P5C REDUCTASE, THE ENZYME AT THE CONVERGING POINT OF PROLINE PATHWAYS, AS A NEW TARGET FOR BIOLOGICALLY ACTIVE COMPOUNDS.

DAVIDE PETROLLINO, SAMUELE GIBERTI, MASSIMO FUSETTI AND GIUSEPPE FORLANI.

Department of Biology and Evolution, University of Ferrara, Italy.

Keywords: proline metabolism; weed control; P5C reductase; new herbicide targets; bisphosphonates.

Similarly to infectious agents, for which new antibiotics are urgently needed to combat multidrug-resistant strains, the rapid diffusion of herbicide-tolerant weed biotypes represents an increasing threat for crop yield and agricultural sustainability. Despite the efforts, the few active principles developed during the last two decades seem unable to effectively control their emergence. The identification of alternative targets in cell metabolism would greatly help in searching new compounds endowed with biological activity. From this point of view, inhibitors of amino acid synthesis represent promising lead structures. To date, proline metabolism has not been exhaustively investigated with this aim, possibly because multiple biosynthetic pathways do occur. However, the two main routes share the last step, catalyzed by δ1-pyrroline-5-carboxylate reductase (P5CR, EC 1.5.1.2). We previously identified a derivative of aminomethylenebisphosphonic acid able to interfere with P5CR activity in the micromolar level. Here we report the screening of several analogues designed on the basis of an in silico docking analysis with the crystal structure of the enzyme from Streptococcus pyogenes.

PIX04

ANALYSIS OF THE REGULATORY SITES DIRECTING THE MERISTEMATIC EXPRESSION OF A DHFR/TS PROMOTER OF ARABIDOPSIS THALIANA.

MARIA GIOVANNA MARCHE1, STEFANIA GHISAURA1, RINO CELLA2, ROBERTA GIORDO1, DIEGO ALBANI1

1 Dept. of Botanical, Ecological and Geological Sciences, University of Sassari, Via Piandanna 4, 07100 Sassari, Italy. 2 Dept. of Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy.

Keywords: Folate metabolism, bifunctional DHFR/TS enzymes, gene regulation.

Plants, like protozoa, possess bifunctional dihydrofolate reductase/thymidylate synthase enzymes allowing a concerted regulation of these essential activities. Arabidopsis has three DHFR-TS genes which, according to their promoter activity, show distinct patterns of expression. AtDRTS1 is mostly expressed in vascular tissues, whereas AtDRTS2 shows a strong proliferation-specific expression in both root and shoot apical meristems. On the contrary, the AtDRTS3 promoter drives the expression of a truncated isoform, lacking most of the C-terminal TS domain, in parts of the shoot meristem as well as in the root columella and central cylinder but not in root meristems. Functional studies on the meristematic AtDRTS2 promoter have revealed the presence of various regulatory regions. The inactivation of Up1 and Up2 sites decreased promoter activity in meristematic cells whereas the inactivation of an E2F cis element, shown to be functional by chromatin immunoprecipitation, increased the activity of the AtDRTS2 promoter. Moreover, a novel regulatory site, identified in the first intron of the gene, appears to be necessary for the meristematic expression of the AtDRTS2 gene.
GREEN COFFEE (COFFEA ARABICA L.) BEANS HARVESTED IN ETHIOPIA, INDIA, KENYA AND TANZANIA SHOW DIFFERENT LIPOLYTIC ACTIVITIES.

PATUI, S.¹, PERESSON, C.¹, CLINCON, L.¹, BRAIDOT, E.¹, ZANCANI, M.¹, NAVARINI, L.², COLUSSI, A.², DEL TERRA, L.², AND VIANELLO, A.¹.

¹ Sezione di Biologia Vegetale, Dipartimento Biologia e Protezione delle Piante, Università di Udine, Via delle Scienze, 91, I-33100 Udine, Italy. ² Research and Innovation, Illycaffè, Biolab, via Flavia 110, I-34147 Trieste, Italy.

Keywords: green coffee, lipolytic acitvity, oil body, seed.

Oil bodies are specialized organelles in oilseeds that accumulate triacylglycerols (TAGs), which are enclosed by a phospholipid monolayer. The mobilization of TAGs during early stages of germination occurs through a process involving phospholipases and lipases. During seed storage, lipolytic degradation could lead to the production of metabolites that could be potentially harmful for seed viability. The aim of this work was to investigate the involvement of phospholipase and lipase activities in oil body degradation during storage of green coffee (Coffea arabica L.) beans harvested in Ethiopia, India, Kenya and Tanzania. Phospholipase A1 and A2 activities were detected by fluorimetric methods in extracts from green coffee beans. Lipase activity was assayed by a colorimetric assay. PLA2 activity was monitored in a wide range of pHs, evidencing two peaks of pH optimum and a limited stimulation by free Ca²⁺. These results indicate that green coffee beans possess at least two isoforms of PLA2. The profiles of phospholipases (PLA2 and total) and lipase showed a higher activity in beans harvested in Kenya, while the lower was associated to those from India.

L-CYSTEINE DESULFHYDRASE ACTIVITY REGULATES CYSTEINE HOMEOSTASIS IN CHLORELLA SOROKINIANA.

SALBITANI G.¹, VONA V.¹, BRANCACCIO A.¹, DI MARTINO RIGANO V.², CARFAGNA S.¹.

¹ Dipartimento delle Scienze Biologiche, Sez. Biologia Vegetale, Università di Napoli Federico II, Via Foria 223, I-80139 Napoli, Italy. ² Dipartimento di Farmacologia Sperimentale, Università di Napoli Federico II, via Montesano 49 I-80131 Napoli, Italy.

Keywords: Chlorella sorokiniana; cysteine metabolism; sulfur starvation; O-acetyl-L-serine metabolism; O-acetylserine(thio)lyase.

Plants, algae and bacteria absorb sulphate and assimilate it in L-cysteine, that represents the first S-aminoacid coming from sulphur assimilation. Cysteine synthesis in higher plants takes place in plastids, cytosol, and mitochondria, in algae it is probably realized only into chloplasts. Cys formation is catalyzed by the enzyme O-acetylserine(thio)lyase (OASTL) using O-acetylserine and sulfide as substrates. For many time this protein was reputed capable to degradate cysteine too, but a recent paper shows that it is wrong, in fact another enzyme, L-cysteine desulfhydrase (DES), is involved in this activity. In Arabidopsis thaliana, OASTL enzyme is completely not able to act cys degradation but DES enzyme catalyzed the desulfuration of L-cysteine to sulphide plus ammonia and pyruvate. In this work we investigated about metabolism and catabolism of cys in the unicellular green alga Chlorella sorokiniana in conditions of S-sufficiency and S-deprivation. We found that the degradation of cysteine is realized from L-cysteine desulfhydrase enzyme, in spite of OASTL, also if in a very little proportion, is able to contribute to this degradation.
**PARALLEL SESSION: PLANT METABOLISM**

**PIX07**

**PROTEOLYTIC ENZYMES INVOLVED IN STORAGE PROTEIN MOBILIZATION AND CELL DEATH OF THE MEGAGAMETOPHYTE OF ARAUCARIA BIDWILLII HOOK. POST-GERMINATED SEEDS.**

**CAPOCCHI A.**1, **FONTANINI D.**1, **CASANI S.**1, **GALLESCHI L.**1 **MUCCILLI V.**2, **CUNSOLO V.**2, **SALETTI R.**2, **FOTI S.**2.

1 Department of Biology, University of Pisa, Va L.Ghini, 5 - 56126, Pisa – Italy.
2 Department of Chemical Sciences, University of Catania, V.le A. Doria, 6 - 95125 Catania - Italy.

*Keywords*: megagametophyte, araucaria, cell death, protease.

*Araucaria bidwillii* megagametophyte is the major storage tissue of the bunya pine seed containing the bulk of reserves for the growing embryo. Soon after seed germination the megagametophyte undergoes storage protein mobilization and by the late germinative stages it degenerates as a no longer needed tissue. In the current work we report a study on the proteolytic enzymes acting in the megagametophyte throughout seed germination. Early stages of germination were characterized by the activity of aminopeptidases and proteinases belonging to the aspartic, metallo and cysteine classes, while carboxypeptidases and serine proteinases became highly active by the late stages. Mass spectrometric analyses of a protein spot responsible for the late stage serine peptidase activity (sensitive to the caspase-6 inhibitor) showed a set of amino acid sequences homologous to those belonging to various plant subtilisin-like serine proteinases. The participation of the early stage proteases in the storage protein mobilization and the involvement of the late stage proteases in the megagametophyte cell death are proposed and discussed.

**PIX08**

**OVEREXPRESSION, REFOLDING AND CHARACTERISATION OF P2-G6PDH FROM BARLEY ROOTS.**

**MANUELA CARDI**1,2, **KAMEL CHIBANI**2, **NICOLAS ROUHIER**2, **DANIELA CASTIGLIA**1, **DONATA CAFASSO**1, **JEAN-PIERRE JACQUOT**2, **SERGIO ESPOSITO**1.

1 Dipartimento di Biologia Strutturale e Funzionale – Università di Napoli Federico II – Via Cinthia ia - 80126 Naples – Italy.
2 Unité Mixte de Recherche INRA-UHP 1136, Interactions Arbres/Micro-organismes, Université Henri Poincaré, IFR 110, Faculté des Sciences, BP 239 54506 Vandoeuvre Cedex France.

*Keywords*: Plastidic glucose-6-phosphate dehydrogenase, G6PDH, OPPP, Hordeum vulgare.

Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49) represents the regulatory enzyme of the oxidative pentose phosphate pathway (OPPP). The main function of OPPP in plants is the production of NADPH for biosyntheses and stress tolerance. To study the role of the barley P2-G6PDH isoform, we investigate the biochemical properties of the protein. This isoform is localized in non photosynthetic plastids in the roots the cDNA sequence encodes for a 490 AA enzyme with a molecular weight of 56KDa. The sequence without the N-terminus extension where cloned in the pET3d vector and overexpressed in E.coli BL21. The bulk of the enzyme was present in inclusion bodies, and using an appropriate strategy of denaturation/ renaturation we succeeded in obtaining a functional soluble protein to study its biochemical properties, giving a Km G6P = 0.93 mM; a Km NADP+ = 8.2 µM and Ki NADPH = 76 µM. Moreover a His-tagged version of P2-G6PDH has also been produced and purified by affinity chromatography, then analyzed by mass spectrometry confirming that this purified protein is indeed the P2-G6PDH from barley roots.
PIX09

CLONING AND EXPRESSION OF CYT-G6PDH ENZYME FROM HORDEUM VULGARE.

DANIELA CASTIGLIA, MANUELA CARDI, DONATA CAFASSO, AND SERGIO ESPOSITO.

Dipartimento di Biologia Strutturale e Funzionale – Università di Napoli Federico III – Via Cintia - 80126 Naples – Italy.

Keywords: cytosolic glucose-6-phosphate dehydrogenase, G6PDH, OPPP, Hordeum vulgare.

Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49) regulates the oxidative pentose phosphate pathway (OPPP), which main function in plants is the production of NADPH for various biosynthetic processes. In this study, we report the cloning of a cytosolic isoform of G6PDH from roots of barley (Hordeum vulgare cv Nure). The complete sequence of the cytosolic enzyme was obtained after PCR amplification of roots cDNA, the amplified product was cloned into “pGEM-T easy” vector and the ligation product used for the transformation of E.coli DH cells. The recombinant plasmid was sequenced to determine the nucleotide sequence. The enzyme presents, as other plant G6PDHs, a NADP$^+$ binding domain, a conserved active site sequence and a Rossman fold. Hence, to express the recombinant protein in its mature form, the sequence was sub-clonated into pET3d vector and expressed in E. coli BL21 cells. The protein was produced as confirmed by SDS page and by western blotting with antibodies directed against potato cyt-G6PDH.

PIX10

THIOREDOXIN-REGULATED BETA-AMYLASE (BAM1) TRIGGERS DIURNAL STARCH DEGRADATION IN GUARD CELLS, AND IN MESOPHYLL CELLS UNDER OSMOTIC STRESS.

CONCETTA VALERIO$^1$, ALEX COSTA$^{1,2}$, LUCIA MARRI$^1$, EMMANUELLE ISSAKIDIS-BOURGUET$^2$, PAOLO PUPILLO$^1$, PAOLO TROST$^1$, FRANCESCA SPARLA$^1$.

$^1$ Department of Experimental Evolutionary Biology, University of Bologna, Via Irnerio 42, Bologna 40126, Italy. $^2$ Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padua, Italy. $^3$ Institut de Biotechnologie des Plantes, Unité Mixte de Recherche 8618, Centre National de la Recherche Scientifique, Université Paris-Sud, 91405 Orsay cedex, France.

Keywords: starch, redox, disulfide, guard cell, osmoregulation.

BAM1 is the only plastid-targeted beta-amylase of Arabidopsis thaliana specifically activated by reducing conditions. Among eight different chloroplast thioredoxins (trxs), trx f1 was the most efficient redox mediator, followed by trxs m1, m2, y1, y2 and 4. Promoter activity of BAM1 showed that in leaves of young plants BAM1 was specifically expressed in guard cells, where starch is degraded during the day, thus nicely fitting with BAM1 being reductively activated by thioredoxins. In comparison to wild type plants, bam1 knockout mutants were characterized by having more starch in illuminated guard cells and reduced stomata opening, suggesting that BAM1 plays a role in diurnal starch degradation. Under osmotic stress BAM1 expression appears also in mesophyll cells and a parallel increase of total beta-amylase activity together with its redox-sensitive fraction was measured in wild type plants but not in bam1 knockout mutants. It is concluded that trx-regulated BAM1 activates a starch degradation pathway in illuminated mesophyll cells upon osmotic stress, similar to the diurnal pathway of starch degradation which normally occurs in guard cells.
EFFECT OF STORAGE TEMPERATURE ON VITAMIN E CONTENT (TOCOPHEROLS) IN BROCCOLI BRASSICA RAPA L. SUBSP. SYLVESTRIS.

M.G. ANNUNZIATA, G. MASSARO, F. NACCA, F. IANNUZZI, P. CARILLO, A. FUGGI.

Dip. di Scienze della Vita, Seconda Università degli Studi di Napoli (Caserta, Italy).

Keywords: alpha and gamma tocopherols, Brassica rapa, storage conditions.

To control and prevent the oxidative stress plants synthesize many antioxidant metabolites among which tocopherols are the most important lipophilic ones, playing a key role in preserving membrane structures. They are synthesized exclusively by photosynthetic organisms and are acquired by animals through the diet. Vegetables, during storage, suffer qualitative and quantitative changes that can affect tocopherols as well as other nutritional, nutraceutic and organoleptic characteristics. In this work tocopherols have been studied in broccoli from Brassica rapa cv. sylvestris kept in different conditions (4°C, 9°C, 20°C) and stored for different time intervals from the harvest. Sample materials (in triplicate) were extracted in methanol and used to determine alpha and gamma tocopherols. Such compounds were separated by reverse phase HPLC, detected fluorimetrically and quantified by comparison with pure standard compounds. The results show that the storage at 4°C better preserves the tocopherol contents. Other results were also discussed. The work was financially supported by "Seconda Università degli Studi di Napoli" and "MIUR" (PRIN2006077008_005;2008S9T3KK_003).

GLUTATHIONE AND OTHER SULPHUR METABOLITE CHANGES INTO BRASSICA RAPA L. CV. SYLVESTRIS DURING POSTHARVEST STORAGE.

NACCA F., ANNUNZIATA M.G., MASSARO G., IANNUZZI F., CARILLO P., FUGGI A.

Department of Life Sciences, Second University of Naples, Via Vivaldi 43, 81100 Caserta, Italy.

Keywords: Brassica rapa L. cv. Sylvestris, glutathione, metabolism.

Biotic and abiotic stresses results in an increased reactive oxygen species production (oxidative stress) that can cause changes of metabolism until to cell death. Glutathione appears as the major thiol compound involved in the control of oxidative stress and has been involved in the prevention of age-related degenerative diseases. Glutathione occurs mainly in leafy vegetables that have an important role in the Mediterranean diet. Leafy vegetables, due to cutting and manipulation processes at the harvest, apart the stop of the water, nutrient and hormone flow, suffer an oxidative stress that activate repair or senescence processes. In this study glutathione and other sulfur metabolites were determined during postharvest storage in an ecotype of Brassica rapa L cv. Sylvestris. An innovate pre-column monobromobimane based derivatization of the sulphur compounds producing highly fluorescent and stable compounds was developed. Their separation and quantification was performed by fluorimeter equipped HPLC. The results were analyzed and discussed. The work was financially supported by "Seconda Università degli Studi di Napoli" and "MIUR" (PRIN2006077008_005;2008S9T3KK_003).
THE CP12-MEDIATED COMPLEX OF PHOTOSYNTHETIC ENZYMES ISOLATED FROM NICOTIANA TABACUM AND ARABIDOPSIS THALIANA.

LUCIA MARRI¹, A. ELIZABETE CARMO-SILVA², PAOLO TROST¹, MICHAEL E. SALVUCCI¹, FRANCESCA SPARLA¹.

¹ Laboratory of Molecular Plant Physiology, Department of Biology, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy. ² US Department of Agriculture, Agricultural Research Service, Arid-Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, 85138, Arizona, USA.

Keywords: Calvin Cycle, CP12, GAPDH, multiprotein complex, PRK.

The requirement for coordinate regulation of Calvin Cycle activity has led to much speculation and some evidence for an association of the enzymes in supramolecular complexes. Most convincing is the reversible association between glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK), mediated by the small disordered CP12. Despite strong evidence for its presence, an intact complex was not yet been purified. To examine its composition and the possibility of additional partners, a high molecular weight CP12-complex was isolated by size fractionation of tobacco stromal proteins, verifying that GAPDH and PRK were associated with CP12 in dark- but not light-adapted chloroplasts. Nano-LC-MS/MS of the intact complex only identified CP12, GAPDH and PRK, indicating that tight association with CP12 under oxidizing conditions is probably specific for GAPDH and PRK. To clarify which of the three CP12 isoforms (CP12-1, -2 and -3) participates in the Arabidopsis complex, CP12 was purified from dark-adapted leaves and analyzed by nano-LC-MS/MS. CP12-1 and CP12-2, but not CP12-3, were unambiguously identified as components of the native Arabidopsis.

EVOLUTION OF FRUIT QUALITY TRAITS IN TOMATO.

FALCONE G., FANTINI E., PIETRELLA M., AND GIULIANO G.

ENEA, Casaccia Research Center, Via Anguillaresi 301, 00123 Roma, Italy.

Keywords: wild tomato species, carotenoid biosynthetic pathway.

The study of the genetic diversity between tomato and related wild species can provide useful tools for the breeding of agronomically useful characters. We took a candidate gene approach to identify genetic differences responsible for the variability of the colour of ripe berries, studying the carotenoid biosynthetic pathway genes in S. lycopersicum ecotypes and in closely related wild tomato species with different berry colour. Sequencing of genes from PSY down to LCY-e (alpha-branch) and CHY1/CHY2 (beta-branch) is complete. Sequence analysis has highlighted the presence of numerous mutations that differentiate the red-fruited species from the green-fruited ones. Some non-synonymous substitutions are candidate to be hypomorphic or hypermorphic alleles and preliminary proof for one of them has been obtained through expression in E. coli. Carotenoid content and gene expression profiles of the red-fruited species are similar. The orange-fruited species has the lowest carotenoid content among all species and has carotenoid and transcriptional profiles similar to the green-fruited species.
Parallel Session: PLANT SECONDARY METABOLITES

PIX15

A PHYSIOLOGICAL ROLE FOR THE PHENYLALANINE ANALOGUE β-PYRAZOLE-L-ALANINE IN CUCUMIS SATIVUS: A CASE OF ALLELOPATHY?

GIUSEPPE FORLANI, DAVIDE MILAN AND MICHELE BERTAZZINI.

Department of Biology and Evolution, University of Ferrara, Italy.

Keywords: β-pyrazol-yl-L-alanine, cucumber, allelopathy, reversal experiments, aromatic amino acid metabolism.

Starting from the middle of the last century, a plethora of non-proteinaceous amino acids were discovered and characterized in plants. The routes leading to their synthesis, their metabolic fate and acceptable human intakes were quite extensively investigated, yet little is known concerning their physiological role. β-pyrazol-yl-L-alanine (pyrAla) was identified in seeds of many members of the Cucurbitaceae, where it represents the major free amino acid and accounts for up to 1‰ of total dry weight. Because it progressively decreases during germination, and it is barely detectable in 2 week-old seedlings, pyrAla was early hypothesized as an unusual nitrogen storage compound. Consistently, its presence did not act as antifeedant against aphids. As an alternative, it could represent an allelochemical. Here we report on pyrAla phytotoxicity. At millimolar concentrations it was indeed found to inhibit plant growth at both the undifferentiated cell and seedling level. Reversal studies suggested that pyrAla may act by interfering with either the uptake or the metabolism of aromatic amino acids. In vivo experiments strengthened a possible allelopathic role for this amino acid analogue.

PIX16

TRANSCRIPT PROFILING OF TOMATO MUTANTS PRODUCING ANTHOCYANINS IN THE FRUIT.

LAURA BASSOLINO¹, GIOVANNI POVERO¹, SILVIA GONZALI¹, ANDREA MAZZUCATO², PIERDOMENICO PERATA¹.

¹ Plant Lab, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy.
² Dipartimento di Agrobiologia e Agrochimica, Università degli Studi della Tuscia, 01100 Viterbo, Italy.

Keywords: anthocyanins, tomato, RT-PCR, GeneChip® Tomato Genome Array, agronomic traits.

Anthocyanins are high value plant antioxidants which are not present in the fruits of the cultivated tomato. However, both the dominant gene Anthocyanin fruit (Aft) and the recessive gene atroviolacea (atv) stimulate a limited anthocyanin pigmentation. Surprisingly, the double mutant atv/atv Aft/Aft tomatoes can be distinguished by the presence of intensely pigmented fruit. A transcript profiling analysis was carried out using quantitative RT-PCR and GeneChip® Tomato Genome Array to identify differentially expressed genes among wild type, Aft/Aft, atm/atm, and Aft/Aft atm/atm fruits. Anthocyanin levels and the expression of the genes involved in the anthocyanin pathway were higher in the peel of double mutant fruits than in the individual parental lines. Among the differentially expressed transcripts, genes involved in the phenylpropanoid pathway, biotic and abiotic stress responses, cell wall and hormone metabolism were overrepresented in Aft/Aft atm/atm fruit peel. Transcriptomic analysis thus revealed that the activation of anthocyanin synthesis in the peel of tomato fruit was accompanied by a complex remodulation of gene expression, likely affecting important agronomic traits.
GENETIC ALTERATIONS RESPONSIBLE FOR DIFFERENT PIGMENTATION PATTERNS IN PLANTS.

GIOVANNI POVERO 1, CORNELIS SPELT 2, PIERDOMENICO PERATA 1, RONALD KOES 2, FRANCESCA QUATTROCCHI 2.

1 Plant Lab, Scuola Superiore Sant’Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy. 2 Genetics, Vrije Universiteit, de Boelelaan 1087, 1081HV Amsterdam, The Netherlands.

Keywords: Anthocyanin, Arabidopsis, petunia, pigmentation, tomato.

The plant kingdom shows a variety of pigmentation patterns even within the same family. A class of MYB transcription factors play a key role in modulating anthocyanin biosynthesis and are possibly involved in patterns formation. Swapping of MYBs from different species result in pigmentation of nearly any plant part in all hosts. Differences in the proteins are therefore not responsible for patterns formation, and we propose a role of divergence in regulatory elements in the promoter of MYB genes. To test this hypothesis, we are studying the expression of the MYB genes AtPAP1, SlAN2, SlANT1, PhAN2 and PhAN4 (complete of own regulatory regions and fused to the GUS reporter) in swapping experiments in tomato, petunia, Arabidopsis. The result will show e.g. whether PhAN2 can reproduce in Arabidopsis the Petunia pigmentation pattern, proving that regulatory sequences of these genes, rather than the coding sequences (or differences in more upstream regulators) are responsible for the pigmentation pattern observed in the original species. We use also a GUS assays in order to follow the behaviour of the promoters driving these AN2 hortologues in the different species.

ARTEMISININ PRODUCTION IN ARTEMISIA ANNUA SUSPENSION CELL CULTURES.

S. CARETTO, M. DURANTE, A. QUARTA, R. NISI, A. DE PAOLIS, F. BLANDO, G. MITA.

CNR, Istituto di Scienze delle Produzioni Alimentari – Via Monnteroni, 73100 Lecce, Italy.

Keywords: Artemisia annua, artemisinin, malaria, in vitro production, elicitation.

Artemisinin (AN) is an antimalarial compound obtained by the herbal plant Artemisia annua L. Unfortunately, the production of AN in plants is very low (0.1-1% on a dry weight basis) and its chemical synthesis is difficult. Due to the great importance of this compound, many efforts have been made to improve knowledge on AN production in plants and in cell cultures. We have recently established A. annua cell cultures able to biosynthesize AN. To improve AN production in these cell cultures, elicitation experiments were carried out to investigate the effects of various compounds on AN biosynthesis. Twenty-two microM methyljasmonate (MeJA) induced a 3-fold increase of AN production in around 30 min. Miconazole (200 microM) induced a 2.5-fold increase of AN production after 24h, but had severe effects on cell viability. Fifty mM 2,6 dimethyl-beta-cyclodextrins (DIMEB) induced a 300-fold increase of AN levels in the spent culture medium after a three-day-treatment. The influence of these treatments on the expression of AN biosynthetic genes was also investigated by RealTime PCR, revealing that MeJA, miconazole and DIMEB differently affected gene expression in A. annua cell cultures.
SYNERGISTIC INTERACTIONS OF ANTIOXIDANT COMPOUNDS EXTRACTED FROM PLANT-BASED FOODS AS EVALUATED BY DIFFERENT ANTIOXIDANT ACTIVITY ASSAYS.

MAURA NICOLETTA LAUS, MARIO SOCCIO, GAETANO PIO SPERA, ZINA FLAGELLA AND DONATO PASTORE.

Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Facoltà di Agraria, Università degli Studi di Foggia, Via Napoli 25, and Centro di Ricerca Interdipartimentale BIOAGROMED, Università degli Studi di Foggia, Via Napoli, 52 - 71122 Foggia - Italy.

Keywords: Antioxidant activity, antioxidant synergism, durum wheat grain, LOX/RNO, ORAC and TEAC methods.

The reduced risk of chronic diseases associated with regular consumption of fruits and vegetables has been reported; health benefits of these foods have been attributed to high content in antioxidants and their synergistic effects. In this study, three antioxidant activity (AA) assays were compared with respect to the evaluation of antioxidant synergism: the LOX/RNO method, that measures the scavenging capacity against some biological radicals together with several important antioxidant functions of food extracts, and the ORAC and TEAC assays, based on the peroxyl radical and ABTS radical cation scavenging capacity, respectively. AA of mixture of antioxidants extracted from durum wheat whole flour and other vegetable products was assayed: in all the cases, higher synergistic effects were found with the LOX/RNO method with respect to the other AA assays. The results are discussed in the light of the ability of the LOX/RNO method to simultaneously detect different mechanisms of antioxidant protection, thus better highlighting synergistic interactions of food antioxidants. This is an important goal to assess an AA possibly reflecting an actual health promoting potential of foods.
Proton pump interactor 1: a regulatory protein in search of a physiological function.

M.I. De Michelis

Dip. Biologia, UNI Milano, Istituto di Biofisica del CNR, Sezione Milano, via Celoria 26, 20133 Milano, Italy.

In the late nineties P. Morandini, searching for interactors of the plasma membrane H⁺-ATPase C-terminal domain by the two-hybrid technique, identified a protein which he named proton pump interactor 1 (PPI1). PPI1 did not resemble any known protein, but came up to be a member of a small gene family (5 members in Arabidopsis, of which PPI1 is probably the most expressed) present in different plants.

PPI1, expressed in soluble form (deleted of the C-terminal hydrophobic portion) in E. coli, binds the H⁺-ATPase C-terminus in vitro with high affinity and stimulates its activity: the PPI1 binding site is upstream the 14-3-3 binding site of the H⁺-ATPase and the effect of PPI1 on enzyme activity is clearly distinct from that of 14-3-3 proteins.

To identify the role played by PPI1 – and other members of the PPI family – and other members of the PPI family, single and double Arabidopsis mutants knock out for PPI1 and PPI2 were isolated and analyzed: no reproducible phenotypic alteration could be observed either in standard growth conditions or under acid load or salt stress, which demand higher plasma membrane H⁺-ATPase activity. Moreover, the bulk of PPI1 is associated to the endoplasmic reticulum and only by immunogold electron microscopy a light signal associated to the plasma membrane can be detected.

Current hypothesis on the possible physiological role of PPI1 – and other members of the PPI family – will be discussed.
Parallel Session: PLANT GROWTH REGULATORS

**PX01**

**THE DEVELOPMENTAL MUTANTS OF BARLEY: GENETIC AND PHYSIOLOGICAL ANALYSIS TO DESIGN THE PLANT FOR THE FUTURE.**

ANTONIO MICHELE STANCA¹, ALESSANDRO TONDELLI², DONATA PAGANI², RENZO ALBERICI², FULVIA RIZZA², UDDA LUNDQUIST³, CATERINA MORCIA², VALERIA TERZI².

¹Department of Agricultural and Food Science, University of Modena e Reggio Emilia, Via A. Allegri, 9 - 42121 Reggio Emilia, Italy. ²CRA – GPG, Genomic Research Centre, Via San Protaso 302, 29017-Fiorenzuola d’Arda (PC), Italy. ³Nord Gen,P.O. Box 41, SE-230 52 Alnarp, Sweden.

Keywords: Hordeum vulgare, genetic stocks, development.

During the last century thousands of different barley mutants were brought together world-wide and designed as Barley Genetic Stocks (BGS). In particular, the collection of morphological barley mutants developed in Fiorenzuola in the last decades, is continuously implemented by including new mutants and by developing double mutants by intercrossing simple mutants. Some of them are NILs (Near Isogenic Lines) and have been obtained and evaluated under field conditions to study the effect of mutation for agronomic performance. The mutants spontaneously or artificially induced, are grouped on the basis of plant morphology. Different mutant groups are described as mutant of spike, leaves, stem, grain. Many stocks have been studied and characterized in detail, both genetically and physiologically, and provide the most efficient entries to analyze individual genes, to understand regulation and interactions with other genes, to sequence and clone them. Hooded, awned vs awnless, naked vs covered grain, leafy lemma, lodicules size of cleistogamous vs non cleistogamous cultivars etc. genes are cloned and their function established.

**PX02**

**NEW INSIGHTS INTO THE ROLE OF KNOXI TRANSCRIPTION FACTORS IN LEGUME COMPOUND LEAF DEVELOPMENT.**

DI GIACOMO E. ¹,², IAFRATE S. ¹, IANNELLI M.A. ¹, RODRIGUES-POUSADA R.A. ³, AND FRUGIS G. ¹.

¹Istituto di Biologia e Biotecnologia Agraria (IBBA), Operative Unit of Rome, Consiglio Nazionale delle Ricerche (CNR), Via Salaria Km. 29,300 – 00015, Monterotondo Scalo (Roma), Italy. ²Present address: CRA - Centro di Ricerca per la Frutticoltura, Via di Fioranello, 52 - 00134 Roma. ³Dipartimento di Biologia di Base ed Applicata, University of L’Aquila, Via Vetoio 67010 Coppito (L’Aquila), Italy.

Keywords: KNOX transcription factors, Medicago truncatula, leaf development, alternative splicing.

KNOX1 homeobox transcription factors act through the modification of hormonal pathways to integrate developmental signals at the shoot apical meristem (SAM). Besides their role in SAM formation and maintenance, KNOX1 are important in the development of compound leaves. In <Medicago truncatula>, a model species for forage legumes, three KNOX1 genes have been shown to expressed both in the SAM and at early stages of leaf development, differently from what observed in other IRLC clade legume compound-leaved species. MtKNOX6 was further characterized and showed a complex pattern of expression during leaf and inflorescence development. MtKNOX6 mRNA associated with the marginal blastozone that gives rise to leaf primary morphogenesis events. Later during leaf development, MtKNOX6 expression restricted to few parenchyma cells that surround the leaf secondary veins. These findings suggest a role of KNOX1 in M. truncatula leaf lamina dissection and development. MtKNOX6 is post-transcriptionally regulated. Four alternatively spliced transcripts for MtKNOX6 were identified and shown to be regulated in a tissue-specific manner and differentially induced in response to hormones.
THE DEVELOPMENTAL MUTANTS OF BARLEY: GENETIC AND UNRAVELING THE MOLECULAR MECHANISM GUIDING VASCULAR CELL FATE DETERMINATION DURING PRIMARY STEM DEVELOPMENT.

SILVIA IAFRATÉ1, CARLA TICCONI2, SIMONE D'ANGELI3, ELISABETTA DI GIACOMO1, DONATO GIANNINO1, GIOVANNA FRUGIS1, FRANCESCO LORETO3, MARIA MADDALENA ALTAMURA2, GIOVANNI MELE1.

1 Institute of Agricultural Biology and Biotechnology, National Council of Research. Via Salaria Km. 29.300, 00015 Monterotondo Scalo, Rome, Italy. 2 Department of Plant Sciences, University of California, Davis. One Shields Ave. Davis, CA 95616, USA. 3 Department of Plant Biology, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy. 4 Institute of Plant Protection, National Council of Research. Via Madonna del piano 10, 50019 Sesto Fiorentino, Florence, Italy.

Keywords: Arabidopsis thaliana, stem, vascular development, brassinosteroid.

The appearance of the plant vascular system was a crucial event for the evolution of terrestrial vegetation and the colonization of land by animals. However, the molecular mechanism of intra-fascicular xylem and phloem determination during primary stem development remains an open question in plant development. Our data demonstrate that the extensive regulation of the brassinosteroid hormone cascade by the BREVIPEDICELLUS (BP) Arabidopsis homeobox gene is the central mechanism guiding the establishment of intra-fascicular vascular cell identity. The model was validated molecularly and biochemically as well as genetically through the rescue of the bp phenotype by exogenous brassinosteroid application. The role of BP-dependent molecular pathways in regulating vascular cell fate suggests that BP is one of the main players in dictyostele to eustele evolution interactions with other genes, to sequence and clone them. Hooded, awned vs awnless, naked vs covered grain, leafy lemma, lodicules size of cleistogamous vs non cleistogamous cultivars etc. genes are cloned and their function established.

CHARACTERIZATION OF ARABIDOPSIS INSERTIONAL MUTANTS FOR COPPER-CONTAINING AMINE OXIDASES.

ALESSANDRA TISI1, PARASKEVI TAVLADORAKI1, RENATO RODRIGUES POUSADA2, RODOLFO FEDERICO1, RICCARDO ANGELINI1, ALESSANDRA CONA1.

1 Dipartimento di Biologia, Università Roma Tre, Viale Marconi 446 – 00146 Roma, Italy. 2 Dipartimento di Biologia di Base e Applicata, Università dell' Aquila, Via Vetoio snc, Coppito – 67010 L'Aquila, Italy.

Keywords: plant amine oxidases, CuAO, methyl jasmonate.

In plants, polyamines are catabolised by amine oxidases, which are present in different sub-cellular compartments. The chemical identity of AO-catalyzed reaction products depends on mode of polyamine oxidation, hydrogen peroxide representing a shared compound in all the AO-mediated reactions. It has been suggested that AO-mediated hydrogen peroxide production in the apoplasm is involved in signalling pathways leading to stress and defence responses. Herein, a study on the physiological roles of cell wall-localized copper-containing (Cu)-AOs has been carried out. In Arabidopsis, ten putative CuAO genes have been identified, four of which (AtCuAO 1-4) encoding for proteins with a deduced extracellular localization. Insertional mutant lines for these genes were genotypically characterised by PCR, RT-PCR and Southern-blot analysis in order to identify the homozygous plants for single-locus T-DNA insertion and to confirm the absence of the full-length gene transcripts. Plant phenotype analysis revealed that methyl jasmonate (MeJA) treatments differently affects root growth of mutant and wild type plants, i.e. root growth of atcuao1 plants is less inhibited than wild type plants.
Parallel Session: PLANT GROWTH REGULATORS

PX05

AUXIN AND GIBBERELLINS INTERACTION DURING FRUIT SET IN TWO TOMATO AUXIN MUTANTS: GIBBERELLIN BIOSYNTHESIS GENE EXPRESSION AND NUCLEAR DNA CONTENT.

F. MIGNOLLI, L. MARIOTTI, P. PICCIARELLI, N. CECCARELLI.

Dept. of Crop Plant Biology, University of Pisa via Mariscoglio, 34 56124 (Pisa).

Keywords: tomato mutants, auxin/gibberellin interaction, flow cytometry.

Various evidences suggest the involvement of different hormones during tomato fruit development. Although auxin and gibberellins cross-talk may play an important role during fruit set, the precise mechanism of hormones interaction and the regulation of this process is still uncertain. The auxin insensitive diageotropaica (dgt) mutant and auxin hypersensitive entire (e) mutant were used as tools to dissect this interaction. To this purpose, we initially measured ovary growth upon GA3 and 4-chlorophenoxyacetic acid (4-CPA) application in wild type and mutant plants. Subsequently, we monitored gibberellin biosynthesis gene expression throughout the first stage of fruit growth. Amongst the possible mechanisms through which auxin and gibberellins could affect fruit organogenesis the regulation of cell division and cell expansion is generally considered to play a main role. However, the correlation between phytohormones and cell cycle has not been clearly established. Information about a possible significance of auxin perception on mitotic cycle and endoreduplication events has been provided estimating nuclear DNA content of e and dgt fruit tissues by flow cytometry.