Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.06

CRYPTOCHROMES MODULATE HORMONE-PHOTORECEPTOR CROSS-TALK IN TOMATO

FACELLA P.*, DADDIEGO L.*, GIULIANO G.**, PERROTTA G.*

*) ENEA, Trisaia Research Center, Rotondella (MT, Italy)
**) ENEA, Casaccia Research Center, Rome (Italy)

Cryptochromes, phytochromes, hormones, diurnal cycle

Photoreceptors, phytochromes and cryptochromes, regulate many aspects of plant development and growth, such as seed germination, stem elongation, seedling de-etiolation, cotyledon opening, flower induction and circadian rhythms. There are several evidences of interaction between photoreceptors and hormones in all of these physiological processes, but little is known about molecular and genetic mechanisms underlying hormone-photoreceptor crosstalk. In this work, we investigated the molecular effects of exogenous phyto-hormones to the photoreceptorial system of tomato *wt* and transgenic or mutant lines with altered crypthochromes, by monitoring day/night transcript oscillations.

We demonstrated that exogenous GA and auxin modify diurnal expression patterns of tomato photoreceptor genes. Transcript changes result amplified in genotypes with altered cryptochrome content. Our data highlight the presence of molecular relationships among cryptochrome proteins, hormones, and photoreceptor genes in tomato, showing that manipulation of cryptochromes represent a good strategy to understand more in deep the role of phyto-hormones in the plant photoperceptive mechanism.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.07

CAROTENOID PROFILING AND BIOSYNTHETIC GENE EXPRESSION IN FLESH AND PEEL OF TOMATO FRUIT UNDER UV-B DEPLETION

CALVENZANI V.*, LAZZERI V.**, , TONELLI C.*, RANIERI A.**, PETRONI K.*

*) Department of Biomolecular Sciences and Biotechnology, Via Celoria 26, 20133 Milano (Italy)
**) Department of Agricultural Chemistry and Biotechnology, University of Pisa, Via del Borghetto 80, 56124 Pisa (Italy)

Carotenoid, UV-B radiation, RT-PCR, tomato

Carotenoids are health-protecting molecules as a result of their high antioxidant properties and their involvement in physiological functions, such as vision. The tomato fruit is particularly rich in carotenoids, especially lycopene which is the most abundant, but also b-carotene and lutein.

Our previous analyses showed that the UV-B component of solar radiation is important to determine the final content of carotenoids in entire tomato fruits of commercial cultivars grown in open field and that UV-B depletion has a detrimental or enhancing effect on carotenoid accumulation depending on the cultivar considered (Giuntini et al., 2005). However, what may happen at the molecular level between the UV-B stimulus perception and the resulting modulation of the carotenoid biosynthetic pathway is still unknown. Therefore, we evaluated more in detail the response of the tomato fruit flesh and peel in terms of carotenoid accumulation under the deprivation of UV-B during the ripening process and the analysis of the expression of genes involved in carotenoid biosynthesis.

References

Giuntini *et al.* (2005) *J Agr Food Chem* 53:3174-3181.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.08

PROTEOMICS FOR THE ELUCIDATION OF HEAT STRESS RESPONSE MECHANISMS IN ANTHERS OF TOMATO PLANTS

MAZZEO M.F.*, CACACE G.*, MASSARELLI I.**, LEONE A.***, GRILLO S.**, SICILIANO R.A.*

*) Proteomic and Biomolecular Mass Spectrometry Center- Institute of Food Science-CNR, Via Roma 64, Avellino (Italy)
**) Institute of Plant Genetics - CNR, Via Università 133, 80055 Portici (Naples, Italy)
***) DIFARMA, University of Salerno, Via Ponte don Melillo, 84084 Fisciano (Salerno, Italy)

Abiotic stress, tomato, proteomics

Global warming represents a key problem in agriculture as it reduces plant growth and productivity. For tomato plants, suboptimal temperature conditions mainly affect fruit set due to the sensitivity of flower developmental processes to temperatures changes; as matter of fact, anthers are the most sensitive male organs to temperatures changes (1, 2). At molecular level, heat stress causes protein aggregation and/or denaturation, inhibition of protein synthesis, inactivation of enzymes in chloroplasts and mitochondria, increased fluidity of membrane lipids (3). To counteract that, plants activate defence mechanisms involving alterations of gene expression patterns and consequently changes in protein expression levels. In order to investigate stress response mechanisms in tomato plants, we carried out proteomic studies on anthers collected from flowers of thermo-tolerant (cv Saladette) and thermo-sensitive (cv M82) genotypes grown under control (26°C/20°C day/night) and high temperature (36°C/25°C day/night) conditions. A classical bottom-up proteomic approach, integrating two dimensional electrophoresis and mass spectrometry (MALDI-TOF-MS and nano-HPLC ESI-MS/MS), has been applied to identify proteins whose expression was influenced by the heat stress conditions. Protein identification was achieved using mass spectrometric data for searches against the NCBInr DB by means of the Mascot algorithm (http://www.matrixscience.com/). As tomato genome complete sequence was not already publicly available, tandem mass spectrometric data have been also used for database searches against the Plants_Est database and protein functions have been inferred to the identified EST_sequences using BLAST (4).

The proteomic analysis revealed that, under heat-stress conditions, 22/23 proteins were up-regulated and 19/18 proteins were down regulated in the Saladette and M82 genotypes, respectively. Proteins were also classified on the basis of their biological functions, using the KEGG tool (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/), to define the metabolic pathways mainly affected by heat stress and the activated defence mechanisms. Results clearly showed that, in both genotypes, heat stress mainly affected carbohydrate metabolism (glycolysis, TCA Cycle), amino acid and energy (such as oxidative phosphorylation) metabolisms and induced an enhanced expression of chaperones and folding catalysts (such as high and low molecular weight heat shock proteins) to prevent protein dysfunction due to denaturation and aggregation phenomena.

Moreover, results evidenced the increased expression of proteins involved in redox stress response (such as ascorbate peroxidases, peroxiredoxin) which ensure the cellular protection from oxidative damage caused by the rapid production of ROS, usually associated with heat stress events.

1. Peet M.M., Sato S., Gardner R.G. *Plant, Cell and Environ*. 1998, 21: 225-231.
2. Sato S., Peet M.M., Thomas J.F. *Plant, Cell and Environ*. 2000, 23: 719-726.
3. Leone A., Perrotta C., Maresca B. Plant Tolerance to Heat Stress: Current Strategies and New Emergent Insights In: "Abiotic Stresses in Plants" L. Sanità di Toppi and B. Pawlik-Skowronska Eds., Kluwer Academic Publishers, 2003, 1-22.
4. Liska A.J., Shevcenko A. *Trends Anal. Chem.* 2003 22: 291-298.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.09

ANALYSIS OF THE mRNA AND miRNA MAIZE ROOT TRANSCRIPTOME DURING SULPHATE STARVATION AND COLD STRESS

ALTANA A.*, STEVANATO P.**, TONONI P.***, MICHELOTTI V.*, FERRARINI A.***, CACCO G.**, ROSSI V.*

*) Unità di Ricerca per la Maiscoltura, Consiglio per la Ricerca e la Sperimentazione in Agricoltura (CRA), Via Stezzano 24, 24126 Bergamo (Italy)
**) Dipartimento di Biotecnologie Agrarie, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD, Italy)
***) Functional Genomics Center, Dipartimento di Biotecnologie, Università degli Studi di Verona, Strada le Grazie 15, 37134 Verona (Italy)

Transcriptome, small RNA, Zea mays, sulphate starvation, cold stress

The maize root system plays an essential role in mediating plant interaction with environmental stimuli. Recently, it has been shown that epigenetic mechanisms are involved in mediating transcriptome changes induced by various environmental stresses. In particular, miRNAs act in negatively regulating the mRNA level of target genes.

In this study we have analyzed the effect of sulphate starvation and cold stress in inducing transcriptome changes in maize primary roots. Changes in mRNA as well as miRNA expression levels were assessed using a Combimatrix-microarray platform, containing 2X45.000 oligonucleotides representing 30.190 protein-encoding maize genes and 51.109 transcripts and a 4X2000 platform, representing 200 maize miRNAs.

Preliminary data allowed to identify a differential profile of miRNA expression between treated and control samples suggesting a role for miRNAs in the response to sulphate starvation and cold stress. Moreover, a negative relationship of the expression pattern between microRNAs and their computationally predicted targets was identified in several case, supporting their potential interaction and the relevance of these miRNA–target pairs in the.plant response to these stresses.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.10

TRANSCRIPTOMIC AND METABOLOMIC CHARACTERIZATION OF *BETA VULGARIS* COLD RESPONSE

PACIFICO D.*, ONOFRI C.*, VALENTINI M.**, CASCIANI L.**, MANDOLINO G.*, MOLITERNI V.M.C.***

*) CRA - Industrial Crop Research Centre, Via di Corticella 133, 40128 Bologna (Italy)
**) CRA - Research Centre for the Soil-Plant System, Rome (Italy)
***) CRA - Genomics Research Centre, Fiorenzuola D'Arda (Italy)

Beta vulgaris, cold stress, cDNA-AFLP, NMR, metabolome

The response of hydroponically-grown plantlets of a sugar beet spring cultivated variety to cold stress was studied, using molecular and metabolic approaches, and focusing specifically on metabolic pathways involved in the control of sucrose biosynthesis/cleavage and distribution in the different tissues of the plant. The identification of sugar beet cold-modulated sequences was carried out by cDNA-AFLP analysis. Cold-modulated cDNA fragments from either leaves and roots exposed to low-temperatures (0°C and -2°C) were gel-recovered, and 257 unigenes putatively involved in the response to low temperatures were identified and functionally annotated, through a bioinformatic research based on sequence similarity. This functional classification has allowed to estimate which biological and molecular processes were mainly involved in the response to low temperatures both in leaf and root tissues. Fortyseven per cent of the analyzed sequences either did not find any similarity in the databases interrogated, or found a similarity with sequences coding for proteins with unknown function. A putative correlation between the gene expression profiling and the changes of metabolites level was carried after analysis of the same plant organs by ^{1}H-HRMAS-NMR analysis. The results of this study showed that sugar beet plants grown at low temperatures underwent deep changes at the genetic expression level, involving mainly photosynthesis, carbohydrate and aminoacid metabolic processes but only few of these changes in transcriptome implied a congruent variation in the pertinent metabolite contents.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.11

METABOLIC RESPONSE TO COLD AND FREEZING OF *OSTEOSPERMUM ECKLONIS* OVEREXPRESSING *OSMYB4*

LAURA M.*#, CONSONNI R.**#, LOCATELLI F.***, FUMAGALLI E.**, ALLAVENA A.*, CORAGGIO I.***, MATTANA M.***

*) Unità di Ricerca per la Floricoltura e le Specie Ornamentali, Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Corso Degli Inglesi 508, 18038 Sanremo (IM, Italy)
**) Istituto per lo Studio delle Macromolecole, Laboratorio NMR, Consiglio Nazionale delle Ricerche, Via Bassini 15, 20133 Milano (Italy)
***) Istituto di Biologia e Biotecnologia Agraria, CNR, Via Bassini 15, 20133 Milano (Italy)

# the two authors contributed equally to this work

Cold tolerance, freezing tolerance, NMR spectroscopy, transcription factor

The constitutive expression of the rice *Osmyb4* gene in Arabidopsis plants gives rise to enhanced abiotic and biotic stress tolerance, probably by activating several stress-inducible pathways. However, the effect of *Osmyb4* on stress tolerance likely depends on the genetic background of the transformed species.

In this study, we explored the potential of *Osmyb4* to enhance the cold and freezing tolerance of *Osteospermum ecklonis*, an ornamental and perennial plant native to South Africa, because of an increasing interest in growing this species in Europe under field condition where winter temperatures are low.

Transgenic *Osteospermum ecklonis* plants were obtained through *Agrobacterium* transformation with the *Osmyb4* rice gene under the control of the *CaMV35S* promoter and *Nos* terminator.

We examined the phenotypic adaptation of transgenic plants to cold and freezing stress. We also analysed the ability of wild-type and transgenic *Osteospermum t*o accumulate several solutes, such as proline, amino acids and sugars. Using nuclear magnetic resonance, we outlined the metabolic profile of this species under normal growth conditions and under stress for the first time. Indeed, we found that overexpression of *Osmyb4* improved the cold and freezing tolerance and produced changes in metabolite accumulation, especially of sugars and proline. Based on our data, it could be of agronomic and economic interest to use this gene to produce *Osteospermum* plants capable of growing in open field, even during the winter season in climatic zone Z9.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.12

COMPARATIVE PROFILING OF TRANSCRIPTOME IN EVERBEARING AND SD WILD STRAWBERRY GENOTYPES

SANTARCANGELO M.**, CARBONE F.*, FACELLA P.*, MARTELLI G.**, MILELLA L.**, PERROTTA G.*

*) ENEA Trisaia, S.S. 106, Km 419+500, 75026 Rotondella (MT, Italy)
**) Università degli Studi della Basilicata, Viale Dell'Ateneo Lucano 10, Macchia Romana, 85100 Potenza (Italy)

Wild strawberry, flowering, diurnal gene expression, microarray, everbearing

Flowering is a crucial event in the life cycle of seed propagated plants and is affected by environmental stimuli. The flowering plants go through a phase of vegetative growth and a flowering phase where they produce the organs for sexual reproduction. In annual plants, like Arabidopsis, the vegetative phase begins with germination of the seed. Flowering follows and ends with the senescence and death of the plant. In perennials plants, as strawberry (*Fragaria* sp.) flowering typically occurs year after year when conditions are appropriate. Flowering of wild strawberry (*Fragaria vesca*) is accelerated by short-days (SD) and low temperature. However, because of a pronounced interaction of photoperiod and temperature, floral initiation also takes place in many cultivars even in 24-h long days (LD) if the temperature is below about 15 °C.

Identification of genes playing a key role in flowering time could be an important resource to better understand the processes affecting flowering and consequently to accelerate breeding programs of strawberry.

In this study we monitored the diurnal gene expression in short-day (SD) and ever-bearing (EB) genotypes of wild strawberry (*Fragaria vesca*) using both DNA microarray and quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) approaches. Moreover, in order to valuate both light and temperature influences, the plants were chamber- and field-grown in LD and SD conditions. By both approaches, we identified a number of transcripts showing altered diurnal expression patterns in EB plants with respect to SD ones. These transcripts could be directly or indirectly involved in flowering, representing candidate genes for further molecular characterization of this intricate process in strawberry.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.13

ANALYSIS OF DNA METHYLATION IN RAPESEED (*BRASSICA NAPUS* VAR. *OLEIFERA* DEL.) UNDER SALT STRESS BASED ON METHYLATION-SENSITIVE AMPLIFIED POLYMORPHISM

PACE R.*, MARCONI G.**, RAGGI L.**, GUIDUCCI M.*, FALCINELLI M.**, BENINCASA P.*, ALBERTINI E.**

*) Department of Agricultural and Environmental Science, University of Perugia (Italy)
**) Department of Applied Biology, University of Perugia (Italy)

DNA-methylation, M-SAP, salinity, germination, growth

Salinity is an important limiting environmental factor for rapeseed production worldwide and can hamper initial developmental stages in Mediterranean climates where the crop is sown in late summer. The DNA methylation is known to play a crucial role in regulating plant development and tissue differentiation. In this study, we compared the extent and pattern of cytosine methylation in one tolerant and one sensitive rapeseed (*Brassica napus*) cultivar, germinated in distilled water and grown either in distilled water or in a 100 mM NaCl solution, by using the technique of methylation-sensitive amplified polymorphism (M-SAP). Analysis of amplification products generated by using eight primer combinations showed that the rapeseed genome is hypermethylated with several polymorphic fragments. In particular, under salt stress condition, the tolerant cultivar showed more DNA methylations than the stress sensitive one.

Forty-six methylation-related fragments were recovered from tolerant and sensitive cultivar, cloned, sequenced and subjected to BLAST search. Eight of them shared high homology with *Arabidopsis thaliana* genes somehow related to stress tolerance: trehalose phosphatase/synthase, LEUNIG, SH3 domain clathrin, radical SAM domain, fringe-related protein, glutamine fructose 6 phosphate, CYP86A8 and DNA methyltransferase.

Validation of results through the analysis of tissue-specific gene expression using real-time PCR is reported and discusssed.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.14

DISCOVERY AND DIFFERENTIAL EXPRESSION OF ARTICHOKE microRNAs IN RESPONSE TO SALT STRESS

DE PAOLA D.*, CATALANO D.*, FELICE N.**,***, CATTONARO F.***, PIGNONE D.*, SONNANTE G.*

*) Istituto di Genetica Vegetale (IGV), CNR, Via Amendola 165/A, 70126 Bari
**) Università degli Studi di Udine, Dipartimento di Scienze Agrarie e Ambientali, Via delle Scienze 208, 33100 Udine
***) Istituto di Genomica Applicata, c/o Parco Scientifico e Tecnologico L. Danieli, 33100 Udine

Artichoke, deep sequencing, microRNA, salt stress

MicroRNAs (miRNAs) are small noncoding endogenous RNAs playing a regulatory role by negatively affecting gene expression at the post-transcriptional level. miRNAs vary in length between 16 and 35 nucleotides with a mode of 22-nt, and are produced from longer hairpin-like precursor transcripts. Functional studies have demonstrated that miRNAs are involved in various developmental and physiological processes, and recent reports indicate their association with biotic and abiotic stress responses in plants.

High-throughput sequencing technology has allowed the identification and profiling of several conserved and non-conserved miRNAs. Additionally, deep sequencing provides quantitative expression information, since frequency of an individual miRNA generally reflects its relative abundance in the sample. This strategy has been successfully applied to both model and non-model plants.

In the present study, small RNA (sRNA) libraries were generated separately from roots and leaves of globe artichoke, obtained from plants subjected or not to saline treatment. Libraries were sequenced using Illumina technology, and results were analysed by bioinformatics tools.

The majority of sRNAs were 18-28 nt long with 21 nt and 24 nt sRNA as major peaks in the length distribution graph. To identify conserved miRNAs in globe artichoke, all sRNA sequences were Blastn searched against the currently known miRNAs contained in miRbase (http://www.mirbase.org/). Only perfectly matched sequences were considered as putative conserved miRNAs: this analysis highlighted the presence of at least 29 different miRNA families in artichoke.

Comparison to artichoke EST database was performed to find potential miRNA targets and precursors, and to predict hairpin structure. Subsequently, miRNAs were validated using a PCR approach.

Differential expression of miRNA between tissues (leaves and roots) and untreated and salt-treated samples was analysed after sample normalization (count per million).

In conclusion we have characterized the sRNA transcriptome in artichoke, in different tissues, in the presence or absence of salt stress, using deep sequencing strategy by Illumina platform.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.15

DRYRICE: DEVELOPMENT OF DROUGHT-TOLERANT RICE VARIETIES FOR A SUSTAINABLE RICE PRODUCTION IN ITALY

GRECO R.*, CASELLA L.*,**, BRUSCHI G.*,**, CAVIGIOLO S.**, LUPOTTO E.**, MANUELLO D.***, BORGO L.***, GIRONI R.***, SCHLUEPMANN H.****, OUWERKERK P.B.F.*****, PIFFANELLI P.*

*) Rice Genomics Group, Parco Tecnologico Padano, Lodi (Italy)
**) CRA - Unità di Ricerca per la Risicoltura, Vercelli (Italy)
***) Ente Nazionale Risi, Centro Ricerche sul Riso, Castello d'Agogna (PV, Italy)
****) Utrecht University, Department of Biology, Utrecht (The Netherlands)
*****) Institute Biology Leiden, Leiden University, Leiden (The Netherlands)

Rice, drought, genomics, stress response, marker-assisted breeding

Rice in Italy developed during the last two centuries essentially as a water demanding crop, completing the growth cycle under submersion. Reduced water availability due to climate changes, especially if occurring during critical phases of the plant growing cycle, dramatically affects crop production and quality. The DRYRICE project aims at understanding the mechanisms leading to drought tolerance in Italian rice, intended as capability to withstand scarce water input and/or lack of water for longer periods while maintaining yield stability. To date, this information is missing and the collection and characterization of germplasm carrying the drought tolerant traits is of utmost importance.

The DRYRICE project will focus on the improvement of Italian rice varieties of local (Lombardia) and national agronomic interest taking advantage of the most advanced genomics tools coupled to evaluations in field conditions. A reference collection of 96 rice varieties, including traditional and modern accessions representing the genetic diversity of the Italian rice germplasm, will be field evaluated for growth and production performances under control and water-limited conditions. Allelic variation in a set of candidate drought-resistance genes will be investigated using state-of-the-art genomics tools in the same set of varieties. Genetic data will then be integrated with changes in gene expression levels and field performances in water-limited conditions, with the final aim to identify new alleles and molecular markers with added value for the improvement of drought-resistance in rice.

In the frame of the DRYRICE project, we aim at developing relevant know how that will be of immediate use to devise efficient marker-assisted breeding strategies to improve Italian rice varieties for: 1) a more sustainable rice culture in the Lombardy region (which represents 50% of the area cultivated for rice in Italy); 2) their adaptation to mutated environmental conditions (water shortage in critical growth cycle periods) to ensure high and stable yields.

The DRYRICE project is funded by the CARIPLO Foundation.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.16

IDENTIFICATION AND CHARACTERIZATION OF SNPs MUTATIONS IN GENES INVOLVED IN DROUGHT AND SALT TOLERANCE OF DURUM WHEAT BY HRM TECHNOLOGY

MONDINI L.*, NACHIT M.M.**, PORCEDDU E.*, PAGNOTTA M.A.*

*) Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.C. de Lellis, 01100 Viterbo (Italy)
**) ICARDA p.o. box 5466 Aleppo, Syria

High Resolution Melting, SNPs, INDEL, stress tolerance, durum wheat

Salinity and drought stresses represent the most important factor inhibiting cereal yield throughout the world. Tolerance mechanisms to salinity and drought stresses can be considered to contain three main components: Na^+ exclusion, tolerance to Na^+ in the tissues and osmotic tolerance. Plants have developed a complex and elaborate signaling network that ensures their adaptation to those stresses. Recently, many transcription factors, able to considerably enhance tolerance for salt and drought stresses, have been identified. In the present work, conserved portions of DREB1, DREB2, AP2/EREB, WRKY and HKT1 transcription factors (TFs) have been utilized to design specific primers and to obtain amplification fragments not longer than 100bp. The selected portion were designed through multi-alignments in different species such as wheat, rice and *Arabidopsis*.. High Resolution Melting curve (HRM) technology represents one of the most recent and powerful tools for SNPs and INDEL mutations analyses. Here, HRM technology has been employed to detect the presence of SNPs and INDEL mutations into the TFs in durum wheat cultivars differently tolerant to salt and drought stresses. Seeds of Cham I (moderately salt tolerant), Jennah Khetifa (salt tolerant), Belikh 2 (moderately salt tolerant) and Trinakria (salt susceptible) varieties have been germinated in hydroponic solution containing $CaSO_4$ 10 mM. After germination, plants have been grown with a nutritive solution containing micro- and macro-elements and Fe^{++} for seven days. After which, NaCl at different concentrations, 0 M (as a negative control), 0.75 M and 1.5 M, has been added to the nutritive solutions. RNA have been extracted from root and leave materials, converted in cDNA, and used in HRM amplifications. Analysing the profiles observed in the resulting melting curves, some different samples corresponding to different treatment conditions have been chosen, sequenced and aligned with the homologue sequences present in genes databases in order to identify and characterize potential SNPs and INDEL mutations. The PCR amplicons, containing single and double SNPs, produced distinctive HRM profiles. By sequencing the PCR products, several SNPs have been identified and validated. All the revealed SNPs are located on salt and drought tolerant varieties confirming their value in breeding activities. All the characterized mutations are able to generate changing in amino acid sequence of the corresponding proteins. In addition, SNPs identified in present work, being absent in SNPs databases, will be registered.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.17

GENE EXPRESSION AND METABOLIC PROFILING IN *BETA VULGARIS* CULTIVATED AND WILD SPECIES

VASTARELLI P., PACIFICO D., MANDOLINO G.

CRA - Industrial Crop Research Centre, Via di Corticella 133, 40128 Bologna (Italy)

Beta spp., drought, real time PCR, sugars, HPLC

Beta genus comprises cultivated and wild/weedy species, these latter including accessions endowed with mechanisms of osmotic adjustment more effective than the cultivated *B. vulgaris* ssp. *vulgaris*.

The response of two subspecies of *Beta* (ssp. *vulgaris* and ssp. *maritima*) to a progressive water shortage applied by a computer-controlled system under rain shelters was compared. Water limitation was applied starting three months after emergence. Throughout the experiment, water supply and soil potential was monitored daily, and samplings of leaf tissue from control and stressed plants of the two accessions were carried out; osmotic potential and relative water content were measured, total RNA was prepared, and soluble sugars were measured by HPLC-ELSD. At the end of the growth season (in August, five months after emergence), root samples were also taken from some plants.

Drying of the soil due to controlled gradual decrease of the water supply during the growth season resulted in a declining soil water potential that did not affect growth and RWC of the plants, though a difference in leaf osmotic potential of 0.5-0.8 MPa was measured between plants grown in control and water-limiting environments.

In HPLC-analysed samples from individual roots of both subspecies, sucrose concentration was found to be 40% of dry weight. However, the HPLC profiles appeared more rich and complex in the roots and in leaves of *B. vulgaris* ssp. *maritma* compared to *B. vulgaris* ssp. *vulgaris*.

In the leaves, the genes for choline monoxygenase, known to be up-regulated by drought, and sucrose synthase 1, the sucrose-cleaving enzyme, are expressed at constitutively higher levels in *B. maritima* than in *B. vulgaris*, while no differences between the subspecies were recorded for transcription levels of the isoform sucrose phosphate synthase 2.

In the *B. maritima* leaves, the sucrose synthase 1 appeared to be up-regulated simoultaneously with an increased glucose concentration detected at the latest stages of growth, in stressed conditions.

In the roots of *B. vulgaris*, the gene for choline monoxygenase 1 was found to be strongly up-regulated at the later stages of growth in stressed plants compared to control ones.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.18

EFFECT OF DROUGHT STRESS ON THE EXPRESSION OF METABOLIC PROTEINS IN DEVELOPING GRAINS OF DURUM WHEAT

RIVERA ORTIZ L.M.*, PALERMO C.**, CENTONZE C.**, LAFIANDRA D.*, FLAGELLA Z.**, MASCI S.*

*) DABAC, University of Tuscia, Viterbo (Italy)
**) Bioagromed, University of Foggia (Italy)

Durum wheat, drought stress, developing grains, wheat kernel proteins, proteomics

Durum wheat (*Triticum durum* Desf.) is one of most relevant crop in the Mediterranean basin and represents the basis of several traditional foods. Mediterranean climate, characterized by sudden increases of temperatures and water deficit in spring time, during grain filling, may limit the optimal development of this crop with repercussions on grain yield. Moreover, heat and drought stress occurring during the grain-filling, can alter seed protein accumulation influencing both crop yield and yield quality.

Albumins, globulins and prolamins represent the principal protein groups in wheat endosperm. The non-prolamin fraction includes proteins with metabolic activity often implicated in allergies or intolerances in sensitive consumers. Prolamins (gladins and glutenins) constitute the gluten and their pattern influences rheological properties of dough.

This work aims to investigate the effect of drought stress on metabolic protein expression in durum wheat. Two commonly cultivated Italian cultivars (Svevo and Ciccio) were subjected to two irrigation regimes (well watered and water stressed) in growth chamber. Grain samples were collected at three grain-filling stages (Milk, Dough and Ripening stages) and seed metabolic protein expression was analyzed through 2D electrophoresis; spots were revealed with Coomassie Brilliant Blue and then processed with Progenesis SameSpots software.

The analyses performed show that most of the differential polypeptides after drought stress are in common with heat stress, as expected.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.19

DROUGHT RESPONSE IN TOMATO: MOLECULAR AND PHYSIOLOGICAL ANALYSIS

GRECO B.*, DE STEFANO R.*, DI MATTEO A.*, LOMBARDI N.*, GUIDA G.**, BARONE A.*

*) Dipartimento di Scienze del suolo, della pianta, dell'ambiente e delle produzioni animali, Università di Napoli "Federico II", Via Università 100, 80055 Portici (Na);
**) Istituto per i Sistemi Agricoli e Forestali del Mediterraneo - CNR, Via Patacca 85, 80056 Ercolano (Na)

SNP, tomato, drought, stress tolerance, re-sequencing

Drought stress in plants is one of the resulting effects of climatic change in the world and its consequences cause major yield losses. Most crop plants, including tomato (*Solanum lycopersicum*), are sensitive to drought stress. Substantial genetic variation for Drought Tolerance (DT) exists within the cultivated tomato, as well as in other related wild species. However, the genetic variability in the response to drought stress in tomato species is not well understood to warrant its use for developing drought-tolerant cultivars.

The aim of this work is to identify polymorphisms within genes involved in DT across tomato cultivars and wild species by re-sequencing. The tomato genotypes were tested, belong to different *Solanum* species and to a collection of cultivated varieties and ecotypes. Phenotypic characterization of genotypes was performed at the physiological level by determination of relative water content (RWC) and water loss rate (WLR) after many hours of dehydration. In addition, the effect of the water deficit was also assessed on the photosynthetic performance in leaves of 3 genotypes of tomato grown under a plastic tunnel. Photosynthetic performance of PSII, stomatal conductance, RWC and leaf water potentials in tomato leaf tissues were monitored during application of stress and after recovery watering.

In order to identify Single Nucleotide Polymorphisms (SNPs), specific primers were designed for sequences of 6 putative drought stress-related genes retrieved from GenBank. In particular, sequences annotated as MKP1 (MAP kinase phosphatase), Asr2 (ABA stress ripening), TSW12 (a lipid transfer protein gene), dehydrin TAS14, rd22 (dehydration responsive gene) and STO (putative zinc-finger protein) were analyzed. After amplification, SNPs discovery was achieved by re-sequencing PCR products on a ABIPRISM 3130 GENETIC ANALYZER. An average of 16 SNP and 2 IN/DEL were identified in these gene sequences. The wild species showed many mutations and this was predictable because the reference sequence reported in GenBank was from *S. lycopersicum*.

The identification of polymorphisms associated to the DT may lead to the development of useful molecular markers helping assisted selection programs.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.20

TRANSCRIPTOMIC ANALYSIS OF HIGH QUALITY FRUIT FROM AN INTROGRESSION LINE TOLERANT TO WATER DEFICIT

DI MATTEO A.*, VASCO M.*, DE STEFANO R.*, TROTTA N.*, LOTTI C.**, RICCIARDI L.***, BARONE A.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
**) Department of Agro-Environmental, Chemistry and Crop Protection, University of Foggia, Via Napoli 25, 71100 Foggia (Italy)
***) Department of Biology and Chemistry Agro-Forestal and Environmental, Genetics and Plant Breeding Unit, University of Bari, Via Amendola 165/A, 70125 Bari (Italy)

Solanum pennellii introgression lines (ILs), fruit quality, antioxidants, water deficit tolerance

In crop species such as tomato, drought stress can dramatically reduce fruit production and quality. Therefore, understanding a complex trait such as tolerance to drought is crucial to breed tomato for adaptation to this environmental constraint. Additionally, knowledge on the interaction between response to drought stress and fruit quality traits in tomato is still elusive. Elucidating the dynamic of this interaction may help breeders to develop new tomato genotypes combining increased drought tolerance to high fruit quality, thus leading to a better management of environmental resources. Previous screening of genetic resources carried out at the University of Bari allowed the identification of a QTL for drought tolerance in the IL9-2-5, an introgression line of *Solanum pennellii* into *S. lycopersicum cv.* M82 background. In fact, IL9-2-5 showed a small decrease in yield when lower water supply was applied.

The aim of this research was to characterize the molecular network controlling fruit quality under drought conditions in susceptible and tolerant tomato genotypes. In order to investigate effects of drought on fruit quality traits, IL 9-2-5 and M82 plants were grown according to a split-plot design with three replicates in semi-controlled conditions, that is in pots filled with a proper mixture of soil and sand, positioned under a Plariver roofing. Three treatments were applied: water supply at the field capacity; water supply at half of the control water volumes; no water supply. Different treatments were applied when 50% of the plants showed fruit set on the first differentiated flower truss. Fruits were harvested at red ripe stage. As water restitution decreased, M82 showed a lower yield compared to IL9-2-5. Also, fruit firmness, dry matter and soluble solid concentration were almost unaffected by water treatment in IL9-2-5 whilst they increased drastically in the M82 fruit. A similar pattern was observed for total phenols concentration. Interestingly, as for ascorbate IL9-2-5 and M82 did not differ in their ascorbate concentration in the control treatment whilst IL9-2-5 performed higher ascorbate concentration at lower water restitution. To investigate the molecular network controlling fruit quality-related processes in response to water deficit, we performed a transcriptomic analysis on a 90k Combimatrix TomatArray 1.0 comparing red-ripe fruit from IL9-2-5 and M82 at different water treatments. The transcriptomic approach allowed identifying 160 transcripts differentially expressed between genotypes and 241 differentially expressed among treatments. Based on functional annotation, gene ontology classification and hierarchical clustering, subsets of differentially expressed transcripts were used to develop model

networks describing mechanisms controlling fruit quality traits in response to water deficit. Interactions between stress perception and transduction, ethylene biosynthesis and antioxidant metabolism have been hypothesized and key transcripts are being validated by qPCR approach. Functional characterization of candidate genes will prove their involvement in fruit quality control and provide additional genetic means to breeders for tomato quality enhancement in sustainable cropping systems.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.21

ANALYSIS OF MOLECULAR VARIATIONS INDUCED IN TOMATO GENOTYPES BY WATER DEFICIT

LOTTI C.*, GRILLO S.**, MARCOTRIGIANO A.R.***, MIACOLA C.***, SCHIAVULLI A.*, PAVAN S.***, ZONNO V.***, RICCIARDI L.***

*) Department of Agro-Environmental, Chemistry and Crop Protection, University of Foggia, Via Napoli 25, 71100 Foggia (Italy)
**) Institute of Plant Genetics (IGV) – CNR, Portici, Via Università 133, 80055 Portici (Italy)
***) Department of Biology and Chemistry Agro-Forestal and Environmental, Genetics and Plant Breeding Unit, University of Bari, Via Amendola 165/A, 70125 Bari (Italy)

Solanum lycopersicum L., water deficit, gene expression analysis, stress tolerance

Plant responses to drought stress can be divided into three mechanisms: drought escape, dehydration avoidance and tolerance. These act, for example, through modification of phenological traits such us earlier flowering and fruit ripening; structural modifications, such as the increase of cuticle thickness, deep roots, reduced leaf area, higher stomatal resistances, etc.; changes in biochemical composition of macromolecules synthetized.

Important results have been achieved in several crops concerning morphological, physiological, biochemical and molecular studies dealing with plant response to water stress and drought. However, especially in open field conditions, results concerning the obtainment of cultivars adapted to water deficit conditions are rather poor. Furthermore, studies performed on model species were useful to identify a network of genes involved in plant response to water deficit, but limited information is available in tomato (*Solanum lycopersicum* L.).

Tomato is widely cultivated in Italy, mainly in Southern Italy, where crop are subject to detrimental effects of high temperatures, soil salinity and limited water availability, particularly during the crucial developmental stages of the plant. These environmental factors, limiting productivity and fruit quality of tomato, are avoided performing tomato crops in greenhouse or field using high water levels. However, in order to enhance the sustainability of the crop it could be of interest both for economic and environmental reasons to establish new cultivars with high yield stability and adapted to growth with reduced level of water.

On these purposes, within GENOPOM project, researches have been carried out on 10 tomato lines selected for water deficit tolerance and susceptibility. The trials have been performed in controlled and semi-controlled environment and in open field using different water deficit treatments. Moreover, all lines have been analyzed for their bioagronomical traits and damage indexes. Evidences on gene expression variation among treatments were also obtained using primers drawn on 15 gene sequences identified in genomic databases.

The results obtained confirm the water deficit tolerance of a selected tomato line and its higher quality and yield stability in respect to the other lines. Furthermore, for several lines and genes different gene expression levels were detected comparing the water deficit treatments imposed.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.22

TEMPERATURE AND WATER LOSS DIFFERENTLY AFFECT GENE EXPRESSION IN GRAPE BERRY CV "ALEATICO" DURING POST-HARVEST DEHYDRATION

CIRILLI M.*, BELLINCONTRO A.**, NICOLETTI I.***, MENCARELLI F.**, COLAO M.C.*, MULEO R.*

*) Department of Crop Production, University of Tuscia, Viterbo
**) Department of Food Science and Technology, University of Tuscia, Viterbo
***) Chemical Methodology Institute, CNR, Montelibretti (Roma)

Gene expression, grape, phenylpropanoid, post-harvest, wine

The increase of polyphenols content in wine is a common objective of wine producers for quality proposal. Recently the interest for these compounds has become even greater for their nutraceutical aspect. Regular, moderate consumption of red wine is linked to a reduced risk of coronary heart disease and to lower overall mortality. The most important problem in traditional sweet wine production is the decline of polyphenols pools during post-harvest dehydration of grape berry and increase in total volatile acidity due to the anaerobic metabolism. Management of post-harvest grape dehydration is affected by three factors: temperature, RH, and air flow. Temperature plays the main role because it affects not only the rate of mass transfer (water evaporation) but also the main metabolisms. Unravelling how the factors interact by their self and regulate the target genes of berry secondary metabolism and aroma development may help to improve production technology, sensorial profile and healthy value of this wine. The effect of temperature (T) and water loss [10, 20 and 30% of weight loss (wl)] on gene expression, berry metabolism and metabolite accumulation were studied on bunches of "Aleatico" variety dehydrated in an artificial tunnel for drying at three different T: 10°, 20° and 30° C, at constant 40% RH and air flow conditions. The lowest temperature strongly up-regulated PAL and STS transcripts, and in relative minor extent CHI and DFR. The highest level of all genes transcripts was reached at 20% weight loss. Similar trend was observed at 20° C for all gene tested except DFR gene, despite in relative minor amplitude respect 10° C treatment. In the skin of berries exposed to 30° C very low transcript levels were detected. Specific polyphenols content enclosed stilbenes reached maximum concentration at 20° C and 10% wl while at 10°C the peak was reached later and at lower level. ADH1-2 showed a pattern of temporally expression dependent from the temperatures: the highest amount of transcripts was found in berry at 10° C conditions when the wl was around 10%, while at the other temperature conditions the highest amount of transcripts was found at 20% wl. At 10°C and 20°C, 10% wl, higher content of acetaldehyde than ethanol was detected while at 30°C the opposed trend. During post-harvest dehydration, the norisoprenoid metabolism was also affected by the factors analysed, as resulted from the expression of CCD1 gene. From technical point of view, 20° C resulted to be the most suitable conditions to improve sweet wine quality in term of polyphenols due to a better compromise between gene induction and biosynthesis efficiency. For aromatic aspect, 10°C results the best temperature because of the least formation of acetic acid and ethanol. Although the largest amount of gene transcripts is greater at the lowest temperature, however, the highest accumulation of secondary metabolites was found at the T of 20° C. This event could be due either to a different

turnover of secondary metabolites or different level of preservation of the same metabolites (glycosylation, acetylation, p-coumaroylation) that might occur at the different temperature conditions.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.23

MOLECULAR AND PHYSIOLOGICAL ANALYSIS OF THE DROUGHT-INDUCED FLOWERING RESPONSE

RIBONI M., CONTI L., GALBIATI M., TONELLI C.

Department of Biomolecular Sciences and Biotecnology, Università degli Studi di Milano, Via Celoria 26, 20133 Milano (Italy)

Flowering time, drought stress, Arabidopsis thaliana

The decision to flower is a key step for reproductive success. Plants detect internal and environmental cues through different mechanisms and integrate these signals, allowing the floral transition to occur in favourable environmental conditions.

In *Arabidopsis thaliana,* physiological analysis and molecular genetics allowed to define four distinct flowering pathways: the photoperiodic, the autonomous, the vernalization and the gibberellins. These are responsible for the perception of the major internal and environmental signals, e.g. the photoperiodic pathway perceives the day-length. However, evidence indicates the existence of additional flowering pathways. These allow plants to detect other environmental conditions such as warm temperature (accelerating flowering) or salt stress (delaying flowering).

We find that drought also affects flowering time; in fact we notice a significant reduction of vegetative leaves in drought-treated plants compared to controls. This raises the interesting question of how drought stress is perceived and integrated into the floral transition mechanism.

Data will be presented illustrating our experimental approach and the initial characterization of mutants affected in the drought-induced flowering response.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.24

EFFECT OF WATER DEFICIT ON EXPRESSION OF STRESS RELATED GENES IN CAMBIAL ZONE OF TWO CONTRASTING POPLAR CLONES

PALLARA G.*, GIOVANNELLI A.**, TRAVERSI M. L.**, RACCHI M.L.*

*) Dipartimento di Biotecnologie Agrarie, Sezione di Genetica, Università di Firenze, Via Maragliano 77, 50144 Firenze (Italia)
**) Laboratorio di Xilogenesi, Istituto Valorizzazione Legno e Specie Arboree - CNR, Via Madonna del Piano, Sesto Fiorentino, 50019 Firenze (Italia)

Poplar genotypes, drought tolerance, cambium, antioxidant genes, metallothioneins

Poplar (*Populus sp*.) is of primary economic importance for the production of wood in temperate regions of the world. Long-lasting periods of water deficit strongly limit radial growth of plants, decreasing productivity of poplar plantations and wood quality. Until now, no study has dealt with the effect of genotype on regulation of genes involved in drought tolerance in cambial region of poplar. In this study we investigated the effect of prolonged water-shortage in cambial zone of two clones contrasting for their response to water deficit: 'Dvina' (*Populus deltoides*) and 'I-214' (*Populus x canadensis)*. For this purpose we monitored growth parameters of 'Dvina' and 'I-214' plants under well-watered and water-stressed conditions and analyzed lipid peroxidation of cellular membranes, proline content and the expression level of genes coding for antioxidant enzymes (SOD, CAT, APX, GR) and metallothioneins (MT) by RT-qPCR. Under limited water availability 'Dvina' maintained stem growth longer than 'I-214' and had lower leaf abscission at the end of the drought period. Lipid peroxidation analysis suggests a lower sensitivity to oxidative stress of cellular membranes of 'Dvina' compared to'I-214'. The effect of genotype on the level of gene transcription was prominent. While, the induction of stress defence genes appears marginally implicated in the response to drought, confirming the results obtained in other poplar tissues and clones. Under drought condition proline content increased both in 'Dvina' and in 'I-214' even at a different level, suggesting the occurrence of an osmotic adjustment in drought response. On the whole the results, though focused on cambial region, suggest the occurrence of a different strategy to protect the plant from dehydration in the two clones, underlining the importance of genotypic constitution in the response of poplar to prolonged water deficit.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.25

EFFECT OF PUTRESCINE ACCUMULATION IN PLANT RESPONSE TO ABIOTIC STRESS

IANNACONE R.*, TADDONIO G.*, PETROZZA A.*, QUINTO R.*, PLANAS J.**, ZARZA X.**, BORTOLOTTI C.**, TIBURCIO A.F.**, ALTABELLA T.**, CELLINI F.*

*) Metapontum Agrobios. S.S. Jonica, 106 Km 448,2 75010 Metaponto (MT, Italy)
**) Laboratori Fisiologia Vegetal. Facultat de Farmàcia. UB. Av. Diagonal, 643. 08028-Barcelona, (Spain)

Abiotic stress, tomato, Arabidopsis, Polyamines

In Arabidopsis, a model genus missing a functional ornithine decarboxylase pathway, putrescine content is modulated by the expression of two gene isoforms encoding ADC (*ADC1* and *ADC2*), which show contrasted expression patterns depending on the nature of the stress. *ADC1* is a constitutive gene whose expression is increased under cold stress, while *ADC2* is a stress-inducible gene whose expression is modulated by drought, wounding/jasmonate treatment, salinity and potassium deficiency. The different expression patterns of *ADC1* and *ADC2* upon stress are consistent with each isoform acquiring specific roles to cope with different stresses (Alcázar et al. 2010a). By using Arabidopsis mutants defective in Put biosynthesis (*adc1, adc2*) (Cuevas et al. 2008) and transgenic Arabidopsis lines over-expressing the homologous *ADC2* or *ADC1* genes (Altabella et al. 2010), we have shown that the accumulation of Put is essential for proper cold-acclimation and survival at freezing temperatures. Over-accumulation of Put also induces drought tolerance in Arabidopsis, but this effect is only achieved by over-expression of *ADC2* (Alcázar et al. 2010b). These observations demonstrate that Put is a protective compound in Arabidopsis, and open a new pathway for inducing stress resistance in crops through the manipulation of Put levels. In this work we undertake this approach to analyze the effect of Put accumulation in tomato resistance to different abiotic stresses (salt, drought and cold). For this, tomato plants have been transformed with the Arabidopsis *ADC1* or *ADC2* genes under the control of the constitutive promoter 35sCaMV. Several transgenic lines have been obtained and molecularly characterized.

References:

Alcázar et al. (2010a) Planta. doi: 10.1007/s00425-010-1130-0
Alcázar et al. (2010b) Plant Physiol Biochem doi:10.1016/j.plaphy.2010.02.002
Altabella et al. (2010) Patent Publication WO2010/004070 A1
Cuevas, JC et al. (2008) Plant Physiol 148: 1094-1105.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.26

CLONING AND ANALYSIS OF ARTICHOKE NBS-LRR RESISTANCE GENE HOMOLOGUES

FINETTI-SIALER M.M., CURCI P.L., PIGNONE D., SONNANTE G.

Istituto di Genetica Vegetale (IGV) - CNR, Via Amendola 165/A, 70126 Bari

Artichoke, RGA genes, TIR, non-TIR, NBS and LRR domains

The most abundant class of plant resistance genes encodes intracellular leucine rich repeats (LRR), together with a central nucleotide binding site (NBS). Encoded fragments are also provided with a variable amino terminal domain with either an *N*-terminal putative leucine zipper motif (LZ), and other coiled coil (CC) sequence or a region with high similarity to the Toll Interleukin I receptor protein (TIR). The latter is a protein involved in innate immunity factors found in animals. Based on this conserved sequences, NBS and LRR, it was possible to identify several putative resistance encoding genes, belonging to a wide range of different plant genomes. Degenerate primers designed on NBS conserved signatures regions, from known plant resistance genes, were synthesised to isolate Resistance Gene Analogues (RGAs) from cultivated (*Cynara cardunculus* subsp. *scolymus* [L.] Hegi) and wild (*C. cardunculus* var. *sylvestris* Lam.) artichoke. Amplification products of the predicted size were gel purified, cloned and sequenced. An identity search in public databases was then carried out using BLASTX algorithms. Characterization of the amplicons showed that each band included many different sequences, with a variable (44-100%) identity degree. The corresponding sequences belonged to TIR or non-TIR NBS-LRR groups. Other genome analyses suggested that the structural differences observed between the groups of sequences account for different functional roles. This is the first report on RGA isolation in globe artichoke. Further details on the isolated genes are briefly discussed.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.27

SCREENING OF ITALIAN RICE CULTIVARS FOR THE EXPRESSION OF *MYB* AND *WRKY* GENES UNDER ABIOTIC AND BIOTIC STRESSES

BALDONI E.*, MATTANA M.*, LOCATELLI F.*, PICCHI V.*, CONSONNI R.**, GENGA A.*

*) Institute of Crop Biology and Biotechnology, IBBA - CNR, Via Bassini 15, 20133 Milan (Italy)
**) Institute for the Study of Macromolecules, ISMAAC - CNR, Milan (Italy)

Myb, WRKY, osmotic stress, BTH, Italian rice cultivars

Italy is the largest rice (*Oryza sativa* L.) producer and exporter in Europe, with more than 50% of the total paddy production. Even if rice growth on the most productive irrigated lands in the world has reached almost the maximum potential production, the achievement of the optimum yield is made difficult by environmental stresses, such as water deficiency, soil salinity and pathogen attack. The development of new rice varieties with a higher tolerance/resistance to both abiotic and biotic stresses is of great interest also in our country, for the adaptation of rice to suboptimal climate and soil conditions. The molecular response to abiotic and biotic stresses has been largely studied on rice model cultivars, such as Nipponbare, whereas little is known on Italian varieties.

The aim of our research is to provide a characterization of Italian rice cultivars for the expression of genes coding for transcription factors involved in the response to water stress and pathogen attack.

Osmotic stress

PEG-mediated osmotic stress was imposed on hydroponically grown plantlets of 12 Italian cultivars, selected on the basis of their tolerance/sensitivity to water stress. The stress response was evaluated through measurement of physiological parameters (RWC, ion leakage or chlorophyll fluorescence). qRT-PCR expression analysis was performed on leaves and roots collected from plants grown under normal and stress conditions. The expression of the *OsDREB2A* gene, known to be induced by drought, was evaluated in order to verify the occurrence of stress response in treated plants. The expression analysis of 10 *myb* and three *WRKY* genes, known to be involved in drought response, is in progress on the selected cultivars.

BTH-induced stress

Benzothiadiazole (BTH), a functional analog of Salicylic acid (SA), is one of the so-called plant activators that protect various plants from infectious diseases. BTH treatments have been used by several authors to study disease plant response.

Plants of 12 rice Italian cultivars were grown in greenhouse in soil for 20 days and BTH was sprayed onto leaves. The cultivars have been selected on the basis of their resistance/susceptibility to blast. Expression analysis through qRT-PCR was first performed on the *WRKY45* gene, known to be induced by BTH, in order to verify the occurrence of the BTH response in treated plants. Among seven genes analyzed for the expression level, one *WRKY* and two *myb* genes seemed to be differentially expressed in resistant and susceptible cultivars. The qRT-PCR data revealed that the expression level of these genes increased in most of the resistant cultivars and decreased or did not

change in the susceptible ones, thus suggesting a positive correlation between their expression level and the resistance phenotype of the rice cultivars.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.28

THE MAIZE CHROMATIN REMODELING GENE *nfc102* REGULATES TRANSPOSON TRANSCRIPTION

MICHELOTTI A., BATTAGLIA R., ROSSI V.

Unità di Ricerca per la Maiscoltura, Consiglio per la Ricerca e la Sperimentazione in Agricoltura (CRA), Via Stezzano 24, 24126 Bergamo (Italy)

Chromatin, transposon, transcription, environment, Zea mays

The *nfc102* gene encodes for a WD-repeat protein belonging to the Multicopy suppressor of IRA (MSI) family. Members of this family are conserved among different plant species and are part of distinct multi-protein complexes, which are involved in the regulation of the chromatin structure in response to environmental stimuli.

On the basis of the sequence homology with the best characterized Arabidopsis counterparts, *MSI*-like genes can be grouped into two distinct classes. The first class refers to the Arabidopsis *MSI1*, which is involved in nucleosome deposition and in reproductive development. The second class refers to the Arabidopsis *FVE*, which is a member of the autonomous pathway of flowering and plays an essential role in controlling the epigenome stability. The maize *nfc102* displays high sequence homology with *FVE* and it is considered the maize *FVE* putative hortolog.

To investigate the functional role of *nfc102* we have obtained maize antisense and RNAi mutants that exhibit down-regulation of *nfc102* expression. These mutants also show a reduction of the mRNA level corresponding to the *nfc102* paralog, named *nfc101*, but not of other members of the *MSI* family with lower sequence homology with respect to *nfc102*. Using these mutants we observed that *nfc102* and *nfc101* down-regulation provokes an up-regulation of the transcription of RNA corresponding to the LTR region of different retrotransposons and of the TIR region of Mu-like sequences. Level of both RNA sense and antisense strand is affected. Conversely, qRT-PCR performed using primer combinations spanning the gene body region of the same retrotransposons and of Mu did not show significant difference of the RNA level in *nfc102* mutant compared to wild-type plants. A role of *nfc102* in establishing a non permissive chromatin environment to maintain the transcriptionally silenced state of transposons is discussed and a possible connection with the small RNA pathway is proposed.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.29

DIFFERENT APPROACHES TOWARD THE IDENTIFICATION OF PUTATIVE TARGETS OF THE *ARABIDOPSIS* GUARD CELL-SPECIFIC TRANSCRIPTION FACTOR ATMYB60

ALBERTINI A., CARAFA I., COMINELLI E., GALBIATI M., TONELLI C.

Department of Biomolecular Sciences and Biotechnology, University of Milan (Italy)

Transcription factor, guard cell, water stress, Arabidopsis thaliana

Water scarcity is a serious problem that will be exacerbated by global climate change.

Massive quantities of water are used in agriculture, and abiotic stresses, especially drought and increased salinity, are primary causes of crop loss worldwide. As plants lose over 95% of their water via transpiration through stomata, the engineering of stomatal activity is a promising approach to reduce the water requirement of crops and to enhance productivity under stress conditions.

We previously reported that AtMYB60 is a R2-R3 MYB transcription factor of *Arabidopsis*, specifically expressed in the guard cells and directly involved in the regulation of stomatal movements. Microarray analysis of gene expression indicated that a limited number of genes are altered in the *atmyb60-1* null mutant. We are carrying on different experiments toward the functional characterisation of the AtMYB60 putative targets. We also report an innovative technique for the *Arabidopsis* guard cells purification. All these approaches will allow the characterisation of new partners of the complex stomatal stress response network.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.30

SIMILARITY PATTERNS AND STABILITY OF ENVIRONMENTAL RESPONSE IN A SET OF SUNFLOWER HYBRIDS

ALBA V.*, MONTEMURRO C.*, SABETTA W.*, TURI M.**, RAVAGLIA S.***, TROCCOLI A.****, COLECCHIA S.A.****, BISIGNANO V.******, POLIGNANO G.B.******, ALBA E.*****, BLANCO A.*

*) Di.B.C.A., Section of Genetics and Breeding, University of Bari, Bari (Italy)
**) University Farm "A. Servadei", University of Udine (Italy)
***)Società Italiana Sementi – S.I.S., Bologna (Italy)
****) Cereal Research Centre – CRA, Foggia (Italy)
*****) Dep. of Agro-Forestry, Biology, Defence and Biotechnology, University of Basilicata, Potenza (Italy)
******) Institute of Plant Genetics – CNR, Bari (Italy)

Helianthus annuus L., GE interaction, stability, classificatory and ordination procedures

Sunflower (*Helianthus annuus* L.) hybrids have been investigated over the last four years within an experimental project involving the on-farm introduction of sunflower in rain-fed agricultural areas in Southern Italy. In particular, the investigation was carried out in order to analyze the response of sunflower hybrids with respect to different autumn-winter sowing data and to evaluate the responses of hybrids under critical conditions in terms of low winter temperature and water shortage. The application of pattern analysis to examine the responses of sunflower hybrids to changing environmental conditions are beyond the scope of this study. The data used in our study are from a test conducted in a location-year combinations during two years: 2007-09 at the experimental fields of the Agricultural Faculty of Bari-Italy and at the Cereal Research Centre of Foggia, Italy. Eleven commercial hybrids were tested following a randomized complete block design with three replications at each location-year combination. Eight agronomic characters including seed oil content were recorded. Classificatory and ordination procedures were used to investigate the performance of hybrids in each location-year combination in relation to three different autumn-winter sowing dates. The combined analysis of variance showed that hybrids, location-year combination, sowing date and their interactions were highly significant for all characters, which indicated that differences existed among hybrids in their response to changes of location-year combination and sowing dates. Hybrids grown at Valenzano in 2008/09 had highest mean values for plant height , stem branching, head diameter, 1000 seeds weight, seed yield; days to emergence and oil content resulted higher at Foggia in 2007/08; while, plants grown at Valenzano in 2007/08 showed more days to flowering. As regards to sowing dates factor results showed that for days to emergence, days to flowering , stem branching, head diameter and 1000 seeds weight the highest mean values occurred during the first sowing data; while, the second and third sowing data showed highest mean values for plant height, seed yield and oil content. Results of cluster and principal component analyses are presented separately for each sowing data. Cluster analysis classified the hybrid performances into groups that were differentiable in terms of means and stability. The hierarchy was truncated at 11 group level according to the number of hybrids tested. The significant grouping numbers obtained were 5 in each sowing date accounting for about

75% of total variation. Principal component analysis had indicated a different association among original characters in relation to the three sowing date. The first three components account for 74, 82 and 87% of the total variation for the first, second and third sowing date respectively. Thus a plot of component one against component two as Euclidean axes should provide a reasonable representation of the spatial arrangements of hybrid performances in the original multi-dimensional space. The applied statistical method gives full information on hybrid performances similarity in relation to location-year environments and different sowing dates.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.31

ROLE OF VvMYB14, A NOVEL R2R3 MYB FACTOR, IN BIOTIC AND ABIOTIC STRESS RESPONSE AND IN THE REGULATION OF STILBENE BIOSYNTHESIS IN GRAPEVINE

VANNOZZI A.*, BOSS P.K.**, WALKER, A.R.**, LUCCHIN M.*, DRY I.B.**

*) Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Padova, Via dell'Università 16, 35020 Legnaro (PD, Italy) - alessandro.vannozzi@unipd.it
**) CSIRO Plant Industry, Glen Osmond, South Australia 5064 (Australia)

Stilbene synthases, R2R3 MYB, downy mildew, abiotic stresses

Stilbene synthases (STSs) are a class of enzymes belonging to the general CHS type III polyketide synthase family involved in the last step of the biosynthesis of stilbenes. These enzymes, and their main products resveratrol or pynosylvin are detectable in only a limited number of unrelated plant species, including grape, and accumulate in response to biotic and abiotic stresses. Despite numerous studies that have been performed on the accumulation, metabolism and biological properties of resveratrol, little is known about the transcriptional regulation of this pathway. Based on microarray data obtained from grape cell cultures treated with jasmonic acid we identified a candidate R2R3 MYB transcription factor that shows an expression pattern similar to that observed for STSs and which could be involved in the regulation of stilbene biosynthesis in grape. This R2R3 MYB factor was designated *VvMYB14*, based on homology with the *AtMYB14* R2R3 MYB factor. Neither gene has previously been functionally characterized in either plant species. Analysis of *VvMYB14* expression in grape leaf discs treated with biotic (downy mildew infection) and abiotic stresses (wounding and UV-C exposure) known to be involved in the transcriptional activation of *STS* genes, showed a close correlation between the pattern and timing of expression of selected *STS* genes and *VvMYB14*.

Using a Dual Luciferase Reporter Assay System in transiently transformed grapevine cells, VvMYB14 was demonstrated to increase stilbene synthase promoter activity. Confirmation of the role of *VvMYB14* in the transactivation of VvSTS genes *in planta* is currently being examined using a transgenic grapevine hairy root system for testing the effect of both silencing and overexpression of *VvMYB14* on the response of VvSTS expression. Preliminary results indicate that roots in which VvMYB14 has been silenced, show significantly reduced levels of *VvSTS* transcription following the application of an abiotic stress. Further experiments are now underway to clarify the role of VvMYB14 in the regulation of both the stilbene synthase pathway and genes belonging to the general phenylpropanoid pathway in grapevine

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.32

EXPLORING ENVIRONMENTAL STRESS-INDUCED EPIALLELE FORMATION AND INHERITANCE IN *ZEA MAYS*

FORESTAN C.*, BATTAGLIA R.**, ENGLEDON S.***, ROSSI V.**, GUTIERREZ-MARCOS J.***, WEIGEL D.****, VAROTTO S.*

*) Department of Environmental Agronomy and Crop Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD, Italy)
**) CRA, Unità di Ricerca per la Maiscoltura, Via Stezzano 24, 24126 Bergamo (Italy)
***) Warwick HRI, University of Warwick, Wellesbourne CV35 9EF (United Kingdom)
****) Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen (Germany)

DNA methylation, environmental stresses, epigenetic marks, Zea mays

Since the central dogma of molecular biology articulation's in the late 50's, changes in the DNA sequences by mutation and gene recombination have been considered the only mechanisms able to create genetic variation among individuals. However, in the last years it has became evident that epigenetic marks such as DNA methylation, hystone modifications and histone variants play a primary role in creating alternative states of gene expression. The formation of epialleles that can be propagated mitotically and, in some instances, transmitted to the progeny remaining stable for several generations has been well documented in plants. In particular, environmental cues are thought to activate specific epigenetic mechanisms, which add epigenetic marks and in consequence alter patterns of gene expression, destabilize the plant genome and cause phenotypic changes. In this respect, environmentally triggered formation of epialleles and their maintenance represent an important, yet unexplored, source of variation and adaptive power that can contribute to improvement of crop plants.

In the framework of the FP7 European project entitled AENEAS (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) which aims to "explore" environmentally-induced epigenetic changes as the "new frontier" of natural and artificial variability, we are investigating the detailed mechanisms of epiallele formation in response to environmental cues and their heritable maintenance in a crop species like maize.

Starting from the knowledge produced by AENEAS participants in the model organism *Arabidopsis thaliana,* where evidences of the interaction between specific epi-regulators and environment in triggering epigenome changes are now available, we will develop reproducible stress protocols for inducing epigenetic changes in maize. Currently, we are developing protocols for shift of temperature treatments, salinity and drought stresses. In parallel we are analyzing at genome-wide level the DNA methylation profiles of cold stressed B73 and wt plants coupling bisulfite conversion of unmethylated cytosines with Illumina sequencing (BIS-Seq) in collaboration with the Max Planck Institute of Tubingen. Epigenetic regulation of gene expression in response to cold stresses will be also analyzed by mRNA-, miRNA- (UNIWA) and CHIP-seq (CRA Bergamo) All together the results obtained by these different approaches will allow to identify a robust list of sequences target of epigenetic regulation (epitargets) belonging to three main epigenetic pathways

(autonomous, small RNA and CpG methylation). Trans-generational inheritance of these epitargets will be analyzed in stressed maize wt and mutants for the three pathways.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.33

TRANSGENIC TOBACCO PLANTS EXPRESSING POXA1B GENE ARE ABLE TO REDUCE OLIVE OIL MILL WASTEWATER PHENOLS CONTENT

TATINO F., PALOMBA F., CHIAIESE P., FILIPPONE E.

Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)

Laccase, Nicotiana tabacum, phytoremediation, poxA1b, olive mill wastewater

Olive oil mill wastewaters (OMW) are one of major environmental issue in the Mediterranean area due to their high content of phytotoxic and low biodegradability of aromatic compounds such as phenols. Lignin, phenol and other aromatic compounds are degraded by white rot fungi that release in the environment oxidative enzymes, such as laccases. Our aim is to evaluate the ability of transgenic plants to reduce OMW phenol content by releasing a heterologous laccase enzyme. *In silico* analysis of *poxA1b* gene, encoding for a multicopper oxidase protein isolated from *Pleurotus ostreatus*, shown that signal fungi peptide is recognised in tobacco plants and therefore laccase protein might be release by root system. Transgenic tobacco plants were obtained by co-cultivation of leaf explants with *Agrobacterium tumefaciens* bearing the plant expression vector pGreen 0029::35S poxA1b. Putative transgenic shoots were screened by PCR, western blot and zymogram. The quantification of laccase activity was performed by 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,6 dimethoxyphenol (DMP) coupled assays on young leaves and roots exudates collected from eight tobacco plants growing *in vivo* in hydroponic culture. In leaf explants from transgenic lines the enzymatic activity was detected at an appreciable level and such activity was 2 times higher in transgenic lines 5 and 8 than in control plants. Roots exudates from the control plants did not showed any activities whereas in four transgenic plants a significant enzymatic activity was found. The ability of transgenic plants to reduce OMW-phenol content was carried out in hydroponic condition. Plants were left to grow into an OMW solution having a phenol content of 4.2 g l^{-1} . Two transgenic lines were able to reduce the phenol content to 0.08 g FW^{-1}, after only 10 days of growth in OMW solution. This figure was 3.5 times lower than the one observed in treatment with control plants.

Morphological variations were detected in five out eight transgenic plants. These alterations were observed for size, thickness, colour of leafs and plants inflorescences abortion before flowering.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.34

THE ROLE OF PHYTOCHELATIN OVERPRODUCTION IN Cd TOLERANCE OF ARABIDOPSIS AND TOBACCO PLANTS

BRUNETTI P.*, DE PAOLIS A.**, ZANELLA L.***, PROIA A.****, FALASCA G.***, ALTAMURA M.M.***, SANITÀ DI TOPPI L.*****, COSTANTINO P.*, CARDARELLI M.****

*) Dipartimento di Genetica e Biologia Molecolare, Università La Sapienza, P.le Aldo Moro 5, 00185 Rome (Italy)
**) ISPA-CNR, Lecce (Italy)
***) Dipartimento di Biologia Vegetale, Università La Sapienza, P.le Aldo Moro 5, 00185 Rome (Italy)
****) IBPM, CNR, Università La Sapienza, P.le Aldo Moro 5, 00185 Rome (Italy)
*****) Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parma (Italy)

Phytochelatins, Cd tolerance, vacuolar transporter, Arabidopsis thaliana, Nicotiana tabacum

Phytochelatins (PCs) are metal binding peptides synthesized from gluthatione by the enzyme PC synthase (PCS1). PCs are able to form specific complexes with Cd and other heavy metals in the cytosol, which are sequestered into the vacuoles by specific membrane transport proteins where metals are not harmful for the cell. We previously demonstrated that in tobacco seedlings PCS1 overexpression leads to PC overproduction and increases Cd tolerance in the presence of exogenous GSH (1). We analysed Cd tolerance of Arabidopsis plants overexpressing AtPCS1 (AtPCSox lines) and found profound differences between Arabidopsis and tobacco. Based on comparative analysis of seedling fresh weight, primary root length and alterations in root anatomy, we show that at relatively low Cd concentrations, Cd tolerance of AtPCSox seedlings is lower than wt, in contrast with what observed in tobacco, whereas at higher Cd concentrations AtPCSox seedlings are more tolerant to Cd as compared to wt Arabodopsis. Measurements of PC content in untransformed Arabidopsis and tobacco seedlings revealed that at 30 µM Cd level is 3 times higher in the former than in the latter, and differences were found also in the PC polymerization classes (Brunetti et al. submitted). The role of PCs in Cd tolerance will be discussed in view of recent data.

(1) Pomponi M., Censi V., Di Girolamo V., De Paolis A., di Toppi L.S., Aromolo R., Costantino P. and Cardarelli M.(2006) PLANTA 223(2):180-90

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.35

IMPROVING GENETIC TRANSFORMATION PROTOCOLS OF *LEMNA MINOR*, INVOLVED IN PHYTOREMEDIATION APPLICATIONS

IANNELLI M.A., FRUGIS G., IAFRATE S.

Institute of Agricultural Biology and Biotechnology - CNR, Via Salaria Km 29,300, 00015 Monterotondo Scalo (Rome, Italy)

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.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.36

THE DEFENSIN-LIKE GENE FAMILY OF GRAPEVINE

GIACOMELLI L.*, NANNI V.**, SILVERSTEIN K.***, TOWN C.****, ZANETTI M.*****, LENZI L.*, BARALDI E.**, DALLA SERRA M.*****, MOSER C.*

*) Centro Ricerca e Innovazione, Fondazione Edmund Mach, Via Mach 1, San Michele all'Adige (Trento, Italy)
**) DIPROVAL-Agraria, Università degli Studi di Bologna, Viale Fanin 46, 40127 Bologna (Italy)
***) Dept. Biostatistics and Bioinformatics, Masonic Cancer Center, University of Minnesota, 425 Delaware Street, SE MMC 806, Minneapolis, MN 55455 (USA)
****) The J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850 (USA)
*****) Istituto di Biofisica (CNR)-Fondazione Bruno Kessler, Via Sommarive 18, 38123 Povo (Trento, Italy)

Grapevine, defensin, antimicrobial peptides

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 since they might confer broad spectrum resi stance to pathogens in crops.

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 gene structure similarly to other defensins, 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 some of the identified DEFLs playing a role in the defense against pathogens in grapevine.

This work has been funded by the project “Le difensine quali mediatori dell'immunità innata: meccanismo d'azione e applicazioni nella difesa dalle malattie delle piante e dell’uomo” granted by the Fondazione Cassa di Risparmio Trento e Rovereto.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.37

COMBINATED TRANSCRIPTOMIC AND PROTEOMIC APPROACH TO IDENTIFY GENES AND PROTEINS INVOLVED IN FORL DISEASE RESPONSE IN TOMATO

FERRIELLO F.*, CARLI P.*, MAZZEO M.F.**, CACACE G.**, SICILIANO R.A.**, PUOPOLO G.***, ZOINA A.***, ERCOLANO M.R.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
**) Proteomic and Biomolecular Mass Spectrometry Center-Institute of Food Science - CNR, Via Roma 64, 83100 Avellino (Italy)
***) Department of Fruit Three Crop, Botany and Plant Pathology, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)

Tomato, microarray, proteomics, FORL resistance, isogenic lines

Fusarium oxisporum f. sp. Radicis lycopersicis (FORL) is one of the most destructive pathogen of tomato plants. This pathogen is responsible of the crow and roots rot until to determinate the plant's death. Early symptoms caused by FORL in tomato plants include stunting, yellowing, and premature loss of cotyledons and lower leaves. A pronounced brown lesion that girdles the hypocotyl (root/shoot junction), root rot, wilting, and death are advanced symptoms. The presence of the pathogen induces drastic changes in plant gene expression therefore several molecular approaches have been exploited to pinpoint changes in plant gene expression as a result of FORL infections. Large-scale microarray analysis and SSH library construction have proved to be useful tools for discovering new genes and genetic pathways and for studying gene expression in numerous systems, including the interactions between plants and pathogens. Furthermore proteomic studies were able to provide new insights on proteins involved in the resistance mechanism. In this study, we compare expression changes occurred in tomato root after infection by FORL of resistant and supscetible isogenic tomato lines. In particular, infected and non-infected root samples of resistance (Momor) and susceptible (Monalbo) genotypes were collected and used as starting material for the genomic and proteomic studies. We used microarrays and SSH analysis for assessing differential gene expression. A total of 226 different expressed genes were revealed. A semi quantitative polymerase chain reaction (RT-PCR), was used to validate changes 11 genes transcription variations.

Furthermore to highlight proteins differentially expressed after infection in susceptible and resistant cultivars of tomato, a comprehensive proteomic analysis was performed. Proteomes extracted from roots of the two tomato genotypes were separated by 2-DE and 51 spots, showing significant change in mean intensity in the 2-DE maps, were submitted to mass spectrometric analyses. Protein identification was achieved using mass spectrometric data for searches against the NCBInr database by means of the Mascot algorithm (http://www.matrixscience.com/). Proteins were also classified on the basis of their biological functions, using the KEGG tool (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/), to define the metabolic pathways mainly involved in the resistance mechanism. An integrated elaboration of transcriptomic and proteomic data will lead to a better understanding of the host response to FORL infection.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.38

INVESTIGATING THE ROLE OF THE PLANT IN THE INTERACTION WITH THE FACULTATIVE SYMBIONT *TRICHODERMA* SPP.

DE MASI L.*, RUOCCO M.**, DE PALMA M.*, LORITO M.***, TUCCI M.*

*) Institute of Plant Genetics, National Research Council, Via Università 133, 80055 Portici (Italy)
**) Institute of Plant Protection, National Research Council, Via Università 133, 80055 Portici (Italy)
***) Dept. ARBOPAVE, University of Naples Federico II, Via Università 100, 80055 Portici (Italy) - lorito@unina.it

Plant-microbe interactions, induced systemic resistance, plant defence, genetic variability, defence genes

Synthetic chemicals are widely used in agriculture for plant pest control but have serious negative effects both on human health and the environment. Besides, their production is energy-consuming, thus adding to their environmental cost. In this context, the use of Biological Control Agents (BCAs) as an alternative to conventional practices for plant pathogens control can significantly reduce the environmental and health impact of agriculture. Among the most popular BCA used in agriculture are rhizosphere-competent fungi of the genus *Trichoderma*, which exert beneficial effects on growth and disease resistance of interacting plants. Although they are widely used as bio-fertilisers and bio-pesticides in commercial formulates, knowledge on the molecular mechanisms underlying the plant response to the interaction is still lacking. Besides a direct plant pathogen control, *Trichoderma* spp. can also activate Induced Systemic Resistance (ISR), and sensitize plants to respond faster and/or more intensely to pathogen attack. The cross-talk that occurs between the fungal BCA and the plant is important both for the reciprocal identification of each player in the interaction and for obtaining beneficial effects. To contribute to the understanding of the plant role in the interaction, we investigated genetic variability among wild and cultivated tomato lines in their ability to interact with *T. atroviride* and *T. harzianum* and demonstrated that indeed the plant response to either *Trichoderma* species changes with the plant genotype both in terms of stem and root growth and in terms of induced defence mechanisms against the pathogen *Botrytis cinerea*. These findings indicate that the plant response to the interaction is under genetic control in tomato and demonstrate the feasibility of plant breeding aimed at obtaining tomato genotypes with improved ability to benefit from rhizosphere colonisation by *Trichoderma* strains. Besides, we demonstrate for the first time that *Trichoderma* is able to induce long-lasting over-expression of defence genes of the salicylic acid pathway in the absence of a pathogen, while its ability to stimulate plant resistance is accompanied by increased transcription of jasmonate-responsive genes upon pathogen challenge.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.39

NEMATODE TOLERANCE IN LETTUCE AND ITS RELATIONSHIP WITH APHIDS RESISTANCE

BERETTA M.*, SALA T.*, SABATINI E.**

*) CRA-ORL Research Unit for Vegetagle Crops, Montanaso Lombardo (LO, Italy)
**) CRA-ORA Research Unit for Vegetagle Crops, Monsampolo del Tronto (AP, Italy)

Lettuce, biotic stress, aphids, nematode, resistance

Meloidogyne spp., causal organisms responsible for the root-knot disease, cause serious damage in greenhouse crops, especially on lettuce, oriental melon, watermelon, pepper, tomato, and cucumber.

Macrosiphum euphorbiae (potato aphid) can distort leaves and stems, stunt, and cause necrotic spots on tomato leaves (Jayma L, 2007). Resistance against the aphid *Macrosiphum euphorbiae* was observed in tomato and attributed to Meu-1 gene, tightly linked to the nematode resistance gene, Mi. Some years later, cloning of Mi has allowed to determine the fact that Meu-1 and Mi are the same gene (Rossi, 1998).

Mi resistance gene effect on the feeding behavior of the potato aphid, *Macrosiphum euphorbiae* (Thomas). The isolation of the nematode-resistance gene *Gpa2* in potato is described, and it is demonstrated that highly homologous resistance genes of a single resistance-gene cluster can confer resistance to distinct pathogen species (van der Vossen, 2000).

The achieve of this study is to establish if a linkage between nematode and aphid resistance genes exists, observing the accordance in collected data and characterizing, with molecular technique, conserved sequences of tomato Mi gene in lettuce.

Ninety-six commercial cultivars of lettuce (*Lactuca sativa L.*) were evaluated under greenhouse conditions for resistance to nematode (*Meloidogyne spp)* and aphid (*Nasonovia ribisnigri*).

Plants were inoculated with eggs collected from roots of *Solanum lycopersicum* grown in a naturally infected soil. The degree of galling and number of egg masses were evaluated 8 weeks after inoculation. Beside, fifteen-days-old lettuce plantlets of the same variety were inoculated with aphids (*Nasonovia ribisnigri*) collected from susceptible plants. Screening were carried out after 3 weeks from inoculation.

The hypothesis of a relationship between nematode tolerance and aphids resistance has been investigated, based on phenotypic data as well as on the sequences of putative genes involved.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.40

OLIVE FRUITS ATTACKED BY *BACTROCERA OLEAE* REVEALED DIFFERENT PROFILES OF PHYTOHORMONES, VOLATILES AND DEFENCE RELATED TRANSCRIPTS

ALAGNA F.*, KALLENBACH M.**, PANDOLFI S.*, SACCHETTI P.***, BELCARI A.***, RAO R.****, BALDWIN I.T.**, BONAVENTURE G.**, BALDONI L.*

*) Institute of Plant Genetics – CNR, Research Division Perugia, Perugia (Italy)
**) Max Plank Institute for Chemical Ecology, Jena (Germany)
***) Department of Agricultural Biotechnology, University of Florence, Florence (Italy)
****) Department of Soil, Plant, Environment and Animal Production Sciences, University of Naples "Federico II", Portici (Italy)

Olea europaea, olive fly, plant defence, ethylene

Olive is the sixth most important oil crop in the world and its cultivation is mainly concentrated in the Mediterranean area, where the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera, Tephritidae), a monophagous insect, feeding only on cultivated olives and wild relatives, represents the most dangerous pest. Females lay their eggs on the fruits and larvae feed and grow in the drupe mesocarp. Infested drupes may drop before maturation or may undergo serious damages, rendering them unsuitable for direct consumption (table olives) or oil production, due to their very poor quality.

Clarifying the plant defence mechanisms is considered a primary target to develop new control strategies in order to drastically reduce the amount of chemicals applied to the crop. Up to now, this trait has never been investigated in olive and data about resistance and susceptibility to *B. oleae* are few and, sometimes, controversial. Resistant olive cultivars have never been described, but some of them show lower susceptibility, even though under strong infestations they may suffer severe attacks.

In order to investigate the plant defence mechanisms against this pest, we characterized olive fruits with *B. oleae* larval galleries by chemical and molecular analyses. Herbivore-induced plant volatiles (HIPVs) are known to control the interaction of plants with insects and their natural enemies. An unbiased analysis of HIPVs emitted from attacked olive fruits was performed by GCxGC-ToF and the results showed that 23 and 17 volatiles were differentially induced and repressed, respectively. Phytohormone analyses showed that attacked fruits strongly increased ethylene production compared to unattacked fruits, whereas jasmonates were not induced.

The expression of key genes of the ethylene biosynthesis and signalling pathways (ACS, ACO, ERFs) was quantified by Real time PCR and, consistent with the increased levels of ethylene, attacked olives presented increased levels of these transcripts compared to unattacked fruits. Moreover, the mRNA levels corresponding to a putative protease inhibitor (a protein related to plant defence response in many plant species) were significant induced in attacked olives compared to controls. Further experiments, aimed at assessing the involvement of ethylene in olive defence priming mechanisms and at characterizing the role of protease inhibitors in olive defence responses, are in progress.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.41

STUDY OF MOLECULAR BASES OF PLANT-APHID INTERACTION

COPPOLA V.*, CORRADO G.*, BUONOMO T.**, DIGILIO M.C.***, RENZONE G.****, D'AMBROSIO C.****, SCALONI A.****, ROCCO M.*****, MARTINELLI R.**, RAO R.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
**) CEINGE - Biotecnologie Avanzate s.c. a r.l., Via Comunale Margherita 482, 80145 Naples (Italy)
***) Department of Entomology and Zoology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
****) ISPAAM, National Research Council, Via Argine 1085, 80147 Napoli (Italy)
*****) Department of Biological and Environmental Sciences, University of Sannio, Via Port Arsa 11, 82100 Benevento (Italy)

Plant–insect interaction, *phloem-feeding insects, microarray, gene expression*

The development of an appropriate defence requires that plants recognize pests as early as possible. Consequently, plant responses are correlated to the mode of insect feeding and the degree of tissue damage at the feeding site. Although phloem-feeding pests, including aphids, cause extensive crop damage, only few data are available about how plants perceive these arthropods and activate endogenous defences.

Aphids are highly specialized in their mode of feeding and impose a complex stress on plants. Differently from herbivorous pests, the prolonged interaction of aphid stylets and plant tissue causes minimal mechanical damage and consequently, plants respond to aphids activating various defense mechanisms.

The potato aphid (*Macrosiphum euphorbiae* T.) is an important pest of tomato and other Solanaceae. Besides vectoring pathogenic viruses, this species causes physical damage to green tissues and removal of photoassimilates, which result in high yield loss when populations are high. Tomato (*Solanum lycopersicum*) transcriptional changes in response to aphids and molecular mechanisms associated with the development of symptoms are currently largely unexplored. To investigate tomato responses activated during a compatible interaction, we used a microarray analysis to monitor changes in host gene expression during *M. euphorbiae* attack.

We present a time series-based investigation of the tomato cv Microtom after *M. euphorbiae* infestation. Transcriptomic changes were studied 24h, 48h and 96h after infestation to monitor the progress of early induced responses. Transcriptional reconfiguration covered a broad range of biological processes, which include both primary and secondary metabolism. We also carried out a proteomic study 48h following aphid attacks to identify differentially expressed protein compared to uninfested control plants. About 87 differential expressed spot were identified and 49 out of them represented a single protein. These sequences were classified by Blast2go database and most of them were involved in functions highlighted by the transcriptomic analysis, e.g. primary and secondary metabolism of the plant, photosynthesis, oxidative and defence stress response.

This study showed how plant aphid response are complex and multifactorial. Because the modulation of endogenous defense may be a practicable strategy to improve plant resistance against

aphids, integrating transcriptomics and proteomics can greatly contribute to a systems-level understanding of host response.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.42

STUDY OF MOLECULAR BASIS OF PROSYSTEMIN INVOLVEMENT IN TOMATO RESPONSES TO APHIDS AND FUNGI

COPPOLA M.*, CORRADO G.*, BUONOMO T.**, RUOCCO M.***, DIGILIO M.C.****, MARTINELLI R.** , RAO R.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
**) Ceinge - Biotecnologie Avanzate s.c. a r.l., Via Comunale Margherita 482, 80145 Naples (Italy)
***) Department of Arboriculture, Botany and Plant Pathology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)
****) Department of Entomology and Zoology, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)

Plant–insect and plant-fungi interactions, prosystemin, microarray

In *Solanaceae*, a family of related peptide hormones called systemins are involved in the activation of defense genes in response to wounding and herbivory. A primary signal is an 18-aa oligopeptide, systemin (Sys), which is located at the C-terminus of a 200 aa pre-protein, prosystemin (ProSys). Sys is released from its precursor following wounding with an unknown mechanism. Sys locally promotes the biosynthesis of Jasmonic Acid (JA), the molecule responsible of the systemic wound signaling in tomato. ProSys involvement in tomato response to chewing insects has been described, but little is known about its role in modulating defense responses to phloem-feeding insects. Furthermore, as plant response to stress is regulated by coordinated and interconnected pathways, it is expected that the wounding specific defense responses modulate metabolic changes able to counteract further attack by pathogens. For these reasons we studied the biological and molecular involvement of Sys and its modified precursor, in the defense of tomato plants against aphids and fungi. We analysed two transgenic plant populations that constitutively express either *ProSys* or Δ*ProSys* (lacking the Sys coding region). Both sequences enhance the endogenous resistance against *Botrytis cinerea,* as their expression considerably reduce the necrosis areas on transgenic leaves inoculated with the fungus. In addition, a reduced aphid longevity was observed in transgenic plants with high expression level of the *ProSys* gene. The molecular basis of these observation were investigated through microarray analysis. The same approach was used to shed some light on the possible role of the ProSys N-terminal region in tomato defence. Overall our data show that the constitutive activation of *Sys* related defenses pre-adapt tomato plants to counteract aphid and fungi attacks.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.43

MULTIPLE RESISTANCE TO POWDERY MILDEW, LEAF RUST AND STEM RUST IN WHEAT CONFERRED BY GENES ON CHROMOSOME 6V INTROGRESSED FROM *DASYPYRUM VILLOSUM*

BIZZARRI M.*, PASQUINI M.**, NOCENTE F.**, SERENI L.**, MATERE A.**, VITTORI D.*, VIDA G.***, DE PACE C.*

*) Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo (Italy)
**) Agricultural Research Council - QCE, Rome (Italy)
***) Agricultural Research Institute - Hungarian Academy of Sciences, Martonvàsàr (Hungary)

Biotic stress, resistance breeding, genotype-by-environment interaction, GP-2, wild species

Stem rust caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) was effectively controlled worldwide, for the past half century, by systematically introducing stem rust resistance (*Sr*) genes in wheat cultivars. However, a new stem rust race, known as Ug99 or TTKSK is becoming a new potential threat to wheat production because it has broad virulence to currently deployed *Sr* genes. It has been shown that introgression of 6V chromosome into wheat genome bestow slow rusting type resistance to TTKSK isolate. Leaf rust and powdery mildew due to *Puccinia triticina* (*Pt*) and *Blumeria graminis* f.sp. *tritici* (*Bgt*) fungal infections, are also important diseases affecting wheat worldwide. The incorporation of effective resistance genes to these diseases into wheat is a breeding strategy for can be achieved using gene introgression from wild related species.

We report that chromosome 6V#4 from a *Dv* ecotype collected in Latium, when introgressed in *T. aestivum* cv. Chinese Spring (CS), determines multiple and simple inherited resistance to virulent races of *Bgt*, *Pt* and *Pgt*. Two wheat introgression inbred lines (IBL*s*), the 6V#4 disomic addition line CS-DA6V#4 and the monosomic 6V#4 substitution line CS-MS6V#4(6B) were studied. The former line was completely resistant to *Bgt* at seedling and adult plant stage, to *Pt* at adult plant stage (APR), and to *Pgt* at seedling stage. The latter line showed the same pattern of resistance as CS-DA6V#4, although its selfed progenies segregated due to the monosomic condition of the 6V#4.

The CS-DA6V#4 line was crossed to the susceptible CS-DA6V#1 line, obtained by Sears by utilizing a different *Dv* ecotype. An F_2 mapping population, segregating for response to *Bgt* infection, was obtained. The segregation fitted a 3:1 monogenic inheritance. These data suggest the presence of one dominant gene for resistance to powdery mildew (indicated *"PmVt*" and probably allelic to *Pm21* known gene). Molecular analysis using both the marker $OPH17_{1900}$ and $NAU/Xibao15_{902}$ (reported as linked to *Pm21*) were carried out to confirm the location of *"PmVt*"on the 6VS. In our 6V materials, $OPH17_{1900}$ was not linked to *PmVt*. $NAU/Xibao15_{902}$ was detected by PCR in both parental lines, amplicons displayed the same molecular weight but with significant difference in band intensity between the two parental lines, the CS-DA6V#1 expressing the fainter band. $NAU/Xibao15_{902}$ primers targeted a DNA sequence encoding for a serine-threonine kinase enzyme that might be involved in the resistance response. The amplified $NAU/Xibao15_{902}$ DNA fragments showed differences in the nucleotide sequences at the exons 3 and 4 which include the regions complementary to the primers. A nucleotide mutation in one of the primer pairing site in CS-DA6V#1 might explain the fainter band. Further molecular analyses are in progress. The F_3

progeny, from the F_2 mapping population, was naturally infected by *Pt* in the field. The APR response implied that resistance was not due to genes already present in CS, which was susceptible, but it was encoded at a locus on the 6V#4. Resistance to *Pgt* was observed in CS-DA6V#4 line by controlled infection at the seedling stage at CRA-QCE, and at the adult stage of CS-MS6V#4(6B) at ARI-HAS, using a total of four different isolates. Therefore 6V#4 is extremely important to deploy genes for multiple resistance to new virulent races of fungal pathogens in wheat germplasm.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.44

GERMIN-LIKE PROTEIN GENES FROM HOMOEOLOGOUS GROUP 5 CHROMOSOMES IN WHEAT

KALUNKE R.*, JANNI M.*, PALOMBIERI S.* GIORGI D.**, GROSSO V.**, LUCRETTI S.**, D'OVIDIO R.*

*) Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via San Camillo de Lellis, s.n.c., Viterbo (Italy)
**) ENEA Centro Ricerche Casaccia, Unità Tecnica AGRI, Laboratorio GEN, Via Anguillarese 301, 00123 Roma (Italia)

Defence gene, plant disease, Triticum spp., germin protein

The germin protein family comprehends two main groups of proteins: the oxalate oxidases (OXOs) and the germin-like proteins (GLPs). Both OXOs and GLPs are apoplastic proteins involved in several physiological plant processes including germination, development, and response to abiotic and biotic stresses. Wheat OXO and GLP gene expression is induced in response to powdery mildew infection and transient over-expression or silencing of a germin-like protein enhanced or reduced resistance against this pathogen, respectively. The ectopic expression of the wheat OXO gf2.8 gene in dicot species enhanced resistance against *Sclerotinia* pathogens. Germin probes co-localize to QTLs for broad spectrum disease resistance in rice, barley, maize and wheat, including resistance to *Pyrenophora tritici-repentis*, the causal agent of tan spot in wheat.

Since these observations suggest that OXO and GLP genes play a role in broad-spectrum defence against pathogens, they represent possible candidates for wheat improvement. With the aim to identify GLP members involved in plant defence, we are characterizing the sequence and functional features of GLP members from homeologous group 5 chromosomes. We have identified GLP genes from chromosomes 5A and 5D and their homologs in wild wheat relatives. Their coding region is strongly conserved and variation is present in the length of the single intron present at the beginning of the coding region. They are expressed in leaves and their transcript accumulation is regulated during seedling development and following infection with the fungal pathogen *Bipolaris sorokiniana*. One of the isolated GLP gene has been used to produced transgenic wheat plants to aid the understanding of its physiological role.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.45

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF PECTIN METHYLESTERASE INHIBITOR (*Pmei*) GENES IN WHEAT

ROCCHI R.*, JANNI M.*, KALUNKE R.*, CASTIGLIONI C.**, FAVARON F.**, BELLINCAMPI D.***, GIARDINA T.****, D'OVIDIO R.*

*) Department of Agrobiology and Agrochemistry, University of Tuscia, Via San Camillo de Lellis, s.n.c., Viterbo (Italy)
**) Te.S.A.F Department University of Padova, Padova (Italy)
***) Chemistry Department, University of Roma "La Sapienza", 00185 Rome (Italy)
****) ISM2/BiosCiences UMR CNRS 6263, case 342, Université Paul Cézanne (Aix-Marseille 3), 13397 Marseille cedex 20 (France)

PMEI, wheat, pectin, qRT-PCR

Plant cell wall is a highly complex structure composed of diverse components. Pectin is a major component of the middle lamella and primary plant cell wall in dicotyledonous species and its remodelling occurs during normal plant growth or following stress responses. Pectin degradation represents also an important step during the infection process of most pathogens. Cereal cell wall contains a lower amount of pectin compared to that present in dicot species, nonetheless pectinase activity has a primary role during pathogens infection of cereal tissues. 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 is secreted into the cell wall in a highly methylesteried form and here demethylesterified by pectin methylesterase (PME). 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. Based on sequence similarity we identified three *Tdpmei* genes (*Tdpmei1, Tdpmei2* and *Tdpmei3*) and demonstrated that *Tdpmei3* encode an active inhibitor of PME activity. By qRT-PCR analysis we showed that these genes are regulated during leaf development and *Tdpmei1* and *Tdpmei2* accumulated strongly in the ovary and stamen, whereas *Tdpmei3* accumulated mainly in the stem. Transcript analysis showed also that *Tdpmei1* and *Tdpmei3* are not induced following wheat leaf infection with the fungal pathogen *Bipolaris sorokiniana*, whereas *Tdpmei2* accumulated slightly.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.46

THE WHEAT POLYGALACTURONASE-INHIBITING PROTEIN GENES *Tapgip1* AND *Tapgip2* ARE UP-REGULATED BY FUNGAL INFECTION AND STRONGLY INDUCED IN RESPONSE TO MECHANICAL WOUNDING

JANNI M., BOZZINI T., LUPI R., MASCI S., D'OVIDIO R.

Department of Agrobiology and Agrochemistry, University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo (Italy)

Pgip, wheat, pathogen infection, wounding

Polygalacturonase-inhibiting proteins (PGIPs) are cell wall leucine-rich repeat (LRR) proteins involved in plant defence. Based on sequence similarity, three *Pgip* genes, one for each genome, have been identified in the hexaploid wheat genome. *Tapgip1* (B genome) and *Tapgip2* (D genome) are expressed in all tissues, whereas *Tapgip3* (A genome) is inactive because of an LTR, *copia* retrotransposon insertion within the coding region. To verify whether *Tapgip1* and *Tapgip2* are involved in wheat defence and encode active PGIPs, we have analyzed their expression following stress conditions and over-expressed them in transgenic wheats.

Both *Tapgip1* and *Tapgip2* are up-regulated by fungal infection and following treatment with oligogalacturonides (OGs) active as elicitor of defence responses, 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 restricted to the damaged tissues, and was not observed in the adjacent tissues.

Gain of function experiments by the ectopic expression of *Tapgip1* in a tetraploid wheat genotype carrying both endogenous *Pgip1* and *Pgip3* silent 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 over-expressing *Tapgip2* in a wheat genotype having only an active *Pgip1*.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.47

PROTEOMIC ANALYSIS OF TRANSGENIC BREAD WHEAT KERNELS FOLLOWING *FUSARIUM GRAMINEARUM* INFECTION

LAINO P.*,**,***, JANNI M.*, DI CARLI M.S.**, BENVENUTO E.**, LILLEY K.***, LAFIANDRA D.*, D'OVIDIO R.*, MASCI S.*

*) DABAC, University of Tuscia, Viterbo (Italy)
**) ENEA, Dipartimento BIOTEC, Sezione Genetica e Genomica Vegetale, C.R. Casaccia, Via Anguillarese 301, Rome, 00123 (Italy)
***) Department of Biochemistry, University of Cambridge, Cambridge (United Kingdom)

Wheat, FHB, wheat kernel proteins, transgenic wheat, proteomics

The effect of Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, was evaluated on the mature seed proteome of bread wheat. The analysis was performed on control and transgenic wheat plants expressing a defence related gene, a Poly-Galacturonase Inhibiting Protein (PGIP), conferring reduced FHB symptoms.

Control and transgenic lines showed comparable values in spikelet number and seed yield, indicating that constitutive expression of the PGIP transgene did not affect negatively these parameters. In contrast, comparison between *F. graminearum*-inoculated transgenic and control plants showed a significantly higher seed yield in transgenic plants, indicating a beneficial role of the PGIP transgene expression on grain productivity after *F. graminearum* infection.

The seeds obtained from transgenic and control plants, both without and after inoculation with *F. gramineaurum*, were used for proteomic analysis.

Metabolic proteins accumulation was analyzed by DIGE. Similarly, gluten proteins were analyzed by SDS-PAGE and A-PAGE.

Both metabolic and gluten proteins of mature kernels did not change significantly between infected and not infected control plants, as well as between control and transgenic plants infected with *F. graminearum.*

These results indicate that FHB does not modify significantly the mature wheat kernel proteome, neither in presence or absence of a transgenic PGIP. These results highlighted also that the expression of a PGIP gene in a transgenic wheat plants does not modify the mature seed proteome.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.48

BIORES** PROJECT: USE OF MAIZE RIP b-32 AS BIOACTIVE PROTEIN IN PLANT PROTECTION AGAINST PATHOGENS

LANZANOVA C., HARTINGS H., BERARDO N., MOTTO M., BALCONI C.

CRA-MAC Unità di Ricerca per la Maiscoltura, Via Stezzano 24, 24126 Bergamo (Italy)

Ribosome Inactivating Protein, b-32, plant protection, Fusarium verticillioides

One of the main topics of maize breeding is the improvement of plant protection against pathogens. Plants respond to attack by pathogenic fungi with a complex network of active responses such as the production and accumulation of proteins that are toxic or inhibitory to pathogens such as RIP (Ribosome Inactivating Protein). The role of RIP in the pathogens defense has been documented (Balconi et al., 2010).

In maize endosperm, a cytosolic albumin termed b-32 is synthesized in temporal and quantitative coordination with the deposition of storage proteins. In the past years b-32 was shown i) to enzymatically inactive ribosomes modifying rRNA inhibiting protein synthesis in vitro (Maddaloni et al., 1991) ii) to inhibit the growth of *Rhizoctonia solani* mycelia in an in vitro bioassay and plant assays (Maddaloni et al., 1997). In this context, we have recently shown and that maize b-32 was effective in wheat transgenic lines as an anti fungal protein by reducing *Fusarium culmorum* head blight (FHB) (Balconi et al., 2007) and in maize transgenic lines reducing *Fusarium verticillioides* attack symptoms in leaf tissues assays (Lanzanova et al., 2009).

Similarly to other RIPs, maize RIP is accumulated in the seed as an inactive precursor, which is converted into an active form by proteolytic processing which removes peptide segments from the N (residues 1-16 of pro-RIP) and C (residues 295-301) termini and also from the center of the polypeptide (residues 162-186 Hey et al., 1995).

Aims of the BIORES project are devoted to deepen our knowledge about relationships between structure and substrate specificity of b-32 protein, in order to clarify the role of the processed segments of *b-32* gene on the ability of maize RIP to inhibit fungal growth.

Thereby, a series of genetic constructions was made by selectively deleting the N-terminal, or C-terminal or internal linker domain. Genetic deletions of the b-32 gene, that is apparently responsible for suppressing enzymatic activity in the precursor, will be primarily expressed in *Escherichia coli* to produce sufficient quantities of modified proteins.To assess the role of bioactive b-32 modified protein protection against fungal pathogens (*F. verticillioides, Aspergillus flavus*), a series of *in vitro* bioassays are in progress to analyze their effect on the fungal growth and on mycotoxins accumulation in comparison with a commercial RIP (Saporin).

** Research developed in the "BIORES- Use of bioactive proteins in plant protection against pathogens - Utilizzo di proteine bioattive nella protezione contro patogeni in pianta." CRA-Consiglio per la Ricerca e Sperimentazione in Agricoltura-funded project.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.49

PROTEOMICS STUDY OF RESISTANCE OF BARLEY TO LEAF RUST MEDIATED BY Rph15 GENE

BERNARDO L.*,***, NEGRI A.S.**, PRINSI B.**, BISELLI C.*, URSO S.*, ESPEN L.**, VALÉ G.*, MOLITERNI V.M.C.*

*) CRA-GPG Genomics Research Centre, Via San Protaso 302, 29017 Fiorenzuola d'Arda (PC, Italy)
**) DiPROVE, University of Milan, Via Celoria 2, 20133 Milano (Italy)
***) CRA-RIS Rice Research Unit, Strada Statale per Torino Km 2,5, 13100 Vercelli (Italy)

Plant-pathogen interaction, proteomics, barley, defence responses, protein phosphorylation

Leaf rust represent one of the most important foliar diseases of barley (*Hordeum vulgare*) and is caused by the biotrophic fungal pathogen *Puccinia hordei* which penetrate barley leaves through stomata.

In this work, a proteomic approach was undertaken to study changes in total protein accumulation pattern in response to the leaf rust pathogen infection in two barley near isogenic lines (NILs), *Bowman* and *Bowman-Rph15*, which differ for the introgression of the leaf rust resistance gene *Rph15*. This gene represents a broad effective rust resistance gene conferring resistance to more than 300 different leaf rust strains and it is of outstanding interest for barley breeding activity. Changes in protein accumulation was monitored at 24 hours and four days after inoculation. No significant differences in protein accumulation were identified in the two lines at the early time point but analysis at 4 days after infection led to the identification of twenty-one protein spots significantly up or down regulated with a fold-change equal or higher than two. Most of down-regulated proteins were found in the *Rph15* near-isogenic resistant line while no significant differential proteins were basically identified in the susceptible line. Nineteen out of 21 protein spots were characterized by LC-MS/MS analysis and found to be involved in photosynthesis, sugar metabolism, energy balance and defence.

Protein phosphorylation, one of the most widespread regulatory mechanisms in nature, is a transient and reversible PTM involved in key intracellular processes as signaling, transcriptional and translational regulation, protein homeostasis, in cell proliferation and differentiation and also in plant-pathogen interaction responses. In order to highlight key components of the *Rph15*-mediated resistance to leaf rust, a study of phosphoprotein accumulation in response to pathogen infection was also carried out in the two NILs. Preliminary analyses allowed identification of 10 spots of differentially accumulated phosphoproteins at 4 days after pathogen infection.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.50

NEW MOLECULAR MARKERS FOR ASSISTED INTROGRESSION OF VIRAL AND FUNGAL DISEASE RESISTANCE IN TOMATO ITALIAN CULTIVARS

CIRIACI T., PULCINI L., FELICIONI N., FUSARI F., SABATINI E., ACCIARRI N.

C.R.A. - ORA, Agricultural Research Council, Research Unit for Vegetable Crops, Monsampolo del Tronto (AP)

Solanum lycopersicum, TYLCV, ToMV, Fusarium oxysporum

Fresh market tomato is traditionally wide spread cultivated in Italy; its domestication before and after its genetic improvement, started from the 17th century, resulted in a large diversity of plant and fruit shape, color, flavor and size. Local cultivars are more or less widespread and known by farmers and consumer in restricted areas, specially in the south Italy; only in some instances they are known and consumed away from traditional production sites. Many of them are highly appreciated by consumers and, in the local market, the price is often higher than that of ordinary commercial hybrids.

Since many years Ministry of Agriculture encourage and support the development of typical Italian products, funding research projects. Also some Italian private companies are interested in genetic improvement and commercial valorization of local tomato varieties with the aim of commercially playing in advance the multinational seed companies in this new offer.

The collaboration and integration of activities between public institutions and private companies started in the 1990s has first produced hybrids F1 improved in some agronomic characters but not for resistance to diseases: Cuorbenga F1 e Margot F1, belonging to the "Cuor di Bue di Albenga", Perbruzzo F1, belonging to "Pera d'Abruzzo" and Costiera F1 belonging to the "Rosa di Sorrento" were the first results.

The second step was to initiate a program of activities to obtain hybrids resistant to major diseases, better adaptability on greenhouse cultivation, new fruit color, better fruit firmness and improved content in antioxidants without change the main characters typical of local cultivars as shape and flavor.

To achieve these goals, a wide use of molecular marker assisted selection (MAS) has been made, not only by applying the knowledge already available in bibliography, but also improving protocols and identifying new molecular markers. After the initial progress allowed by new markers for fruit shape and resistance to *Verticillium,* research activity was devoted to the obtainment of new molecular markers linked to other disease resistance genes.

In the frame of this work, the results obtained in the search for new molecular markers associated to resistance to the most important parasitic virus and to *Fusarium oxysporum* f.sp. *lycopersici* are reported.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.51

EXPRESSION AND FUNCTIONAL ANALYSIS OF A FUNGAL CELL WALL DEGRADING ENZYME IN TOMATO-*PYRENOCHAETA LYCOPERSICI* PATHOSYSTEM

VALENTE M.T.*, INFANTINO A.*, ARAGONA M.*,**

*) CRA-PAV, Centro di Ricerca per la Patologia Vegetale Via C. G. Bertero 22, 00156 Roma
**) CRA-RIS, Unità di Ricerca per la Risicoltura, S.S. per Torino Km 2,5, 13100 Vercelli

Solanum lycopersicum, pathogen virulence, Real Time RT-PCR, β-1,4 endoglucanase

Corky root rot (CRR) is an important soil-borne disease of tomato (*Solanum lycopersicum*), caused by the hemibiotrophic fungus *Pyrenochaeta lycopersici*. Infected plants show a progressive deterioration of the roots, which develop dark necrotic lesions and large suberization. CRR infection usually does not kill plants but severely reduces crop yields.

The research is focused on the investigation of fungal genes likely candidate to infection success and to pathogen virulence. In a previous transcriptomic analysis of fungus-plant interaction several fungal ESTs were identified (Aragona and Infantino, 2008). Our attention focused on a EST, named PlGL1, having a high similarity with a fungal β-endoglucanase transcript. Similarly to many other plant cell wall degrading enzymes from different pathogens, this EST could be considered as a potential virulence factor. To assess the correlation of this transcript expression to the development of the disease, a relative quantitative Real Time RT-PCR was carried out on RNA purified from infected tomato roots at different time points, and from vegetative mycelium. Results showed an positive correlation between PlGL1 expression and disease development.

Full length cDNA sequence was identified by RACE analysis and gene sequence was obtained by PCR amplification on genomic DNA. Southern blot analysis demonstrated that PlGL1 gene is present in single copy. Bioinformatic analysis on the whole transcript revealed a translation product of 229 amino acid, containing a putative extracellular signal peptide. Protein BLAST analysis showed a very high homology with two endoglucanases from plant pathogenic fungi and detected a conserved domain of fungal glycoside hydrolase family 61. On the basis of previous data about regulation of cellulase production by carbon source in *P. lycopersici*, we evaluated PlGL1 expression and extracellular cellulolytic activity in different growth condition, with and without glucose. The results showed that cellulolytic activity takes place only in absence of glucose and it is enhanced by the presence of cellulose as the only carbon source. A relative quantitative Real Time RT-PCR of PlGL1 was carried out on RNA from mycelium growth with and without sugar: the results correlated the fungal cellulolytic activity with PlGL1 gene expression. In order to confirm the endoglucanase function of PlGL1, the heterologous expression of gene coding sequence in *Escherichia coli* was performed. Isolation and purification of the recombinant endoglucanase are currently in progress; the purified recombinant protein will be used for *in vitro* tests and on plant tissues (*in vivo*), in order to elucidate the role of PlGL1 in virulence and pathogenesis. Future investigations will be devoted to PlGL1 gene silencing and overexpression in *P. lycopersici*.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.52

IDENTIFICATION AND CHARACTERIZATION OF MARKERS LINKED TO *VERTICILLIUM DAHLIAE* (RACE 2) RESISTANCE GENES IN TOMATO

SALA T.**, BERETTA M.**, SABATINI E.*, CIRIACI T.*, ACCIARRI N.*, VALENTINO D.***, TAMIETTI G.***

*) C.R.A. - ORA, Agricultural Research Council, Research Unit for Vegetable Crops, Monsampolo del Tronto (AP)
**) C.R.A. - ORL, Agricultural Research Council, Research Unit for Vegetable Crops, Montanaso Lombardo (LO)
***) DI.VA.P.R.A, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco (TO)

Soil-borne disease, RAPDs, Solanum lycopersicum, Bulk Segregant Analysis, vascular wilts

Verticillium wilt is a common fungal disease that causes severe yield and quality losses in many crops, including *Solanaceae.* These kind of vascular wilts are particularly devastating since the pathogens, proliferating in the vascular system of host plants cannot be reached by many fungicides; vascular wilt fungi are able to survive in soil for many years thanks to persistent resting structures (microsclerotia) and the only effective control measure, soil fumigation, is expensive and may impact health and have environment effects. In a few cases, effective control of verticillium wilt has been reported in specific crops that exhibit race-specific resistance (Kawchuk et al., 2001; Fradin et al., 2009).

Plant resistance to viruses, bacteria, and fungi frequently involves specific host–pathogen interactions between the products of a plant resistance gene (R) and corresponding avirulence gene (Avr) in the pathogen (Kawchuk et al., 2001).

In tomato (*Solanum lycopersicum*), resistance to race 1 of *Verticillium dahliae* and other species is conferred by two inverted resistance genes from the tomato Ve locus; *Ve1* and *Ve2* genes, independently, also confer resistance to an aggressive race 1 of *V. alboatrum* in potato (Kawchuk et al., 2001). Both *Ve1* and *Ve2* were found to encode cell surface receptor proteins that belong to the extracellular Leu-rich repeat (eLRR) receptor-like protein (RLP) class of disease resistance proteins (Kawchuk et al., 2001; Wang et al., 2008). Sequence and expression analysis revealed that *Ve1* encodes a truncated protein in all susceptible genotypes and just *Ve1* expression (not *Ve2*) resulted in resistance against *V. dahliae* (Fradin et al., 2009).

New races of the pathogen have been identified that are capable of overcoming the resistance, so this locus is not sufficient to ensure verticillium wilt resistance: strains that are not contained by this locus are assigned to race 2 (Schaible et al., 1951; Diwan et al., 1999).

The aim of this work is the identification of markers associated to genes involved in *Verticillium dahliae* race 2 resistance.

Thanks to a BSA (Bulk Segregant Analysis) approach, performed on two population of 100 F_2 individuals obtained crossing resistant tomato cultivars (CampbellC28, H1350) with susceptible ones, we were able to identify two RAPD markers polymorphic between resistant and susceptible bulks. Sequence analysis on these markers allowed us to discovery a 100% similarity between one

of the identified RAPD markers and a *Solanum lycopersicum* gene encoding for a specific pathogenesis related protein (PR).

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.53

CHARACTERIZATION OF AN EST COLLECTION FROM POTATO GENOTYPES RESISTANT AND SUSCEPTIBLE TO *RALSTONIA SOLANACEARUM*

SACCO A.*, VITALE S.*, IORIZZO M.**, D'AGOSTINO N.*, DI MATTEO A.*, CHIUSANO M.L.*, BARONE A.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Via Università 100, 80055 Portici (Italy)
**) Department of Plant Pathology, University of Minnesota (MN, USA)

Potato, bacterial disease, cDNA AFLP-TP, microarray, gene annotation

Host resistance is the only control system available for the bacterial wilt of potato, caused by *Ralstonia solanacearum*. This resistance is horizontal, polygenic and its genetic basis is not understood yet. In order to identify genes involved into this plant-pathogen interaction, two different transcriptomic approaches have been used. In particular both a cDNA-AFLP-TP analysis and microarray experiments were performed on the resistant wild *S. commersonii* and the susceptible *S. tuberosum* cv. Blondy for monitoring gene expression during infection.

A total of 32 primer combinations were used to amplify cDNA-AFLP fragments and PCR products were sequenced resulting into 124 differentially expressed ESTs. Most of them (45.8%) were specific of the *S. commersonii* genotype after bacterial inoculation, whereas the remaining were obtained from both resistant and susceptible genotypes after inoculum (4.2%), or were over- or under-expressed in one of the two genotypes (36.7%). The EST electropherograms were processed to trim out the low quality bases from the ends of the reads. Vector contaminations were identified and removed and sequences that were < 25 nucleotides in length were discarded. This process reduced the original dataset to 36 sequences, which were fed into the assembling process resulting into 5 tentative consensus sequences and 25 singletons.

The microarray analysis was performed on a Combimatrix 4X2k chip on which around 650 oligos were synthesized based on an EST collection obtained from a PCR select experiment carried out on the resistant tomato cv. Hawaii 7996. After chip hybridization with RNA extracted from both resistant and susceptible genotypes, before and after inoculum, 59 differentially expressed sequences were selected.

Both differentially expressed sequences from the AFLP sequencing and the tomato EST collection used to design oligos for the synthesis of the Combimatrix chip were functionally annotated.

Automated annotation was performed by BLASTx searches against the UniProtKB/TrEMBL database and the *Arabidopsis thaliana* protein complement. Association to Gene Ontology terms (GO) was electronically inferred by exploiting TrEMBL entries.

Interestingly, among the 89 differentially expressed sequences the majority were related to metabolism, plant defence and signalling and transcription regulation.

Further investigations will focus on understanding the exact role of these sequences in protecting plants against pathogen attacks.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.54

HOUSEKEEPING GENE SELECTION USING AN EXTERNAL CONTROL FOR RT-PCR ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN EGGPLANT ROOTS DURING FUNGAL INOCULATIONS

BARBIERATO V., RINALDI P., CAPONETTO G., ROTINO G. L., TOPPINO L.

CRA-ORL, Unità di Ricerca per l'Orticoltura, Via Paullese 28, I-26836 Montanaso Lombardo (LO, Italy)

Fungal inoculation, quantitative RT-PCR, housekeeping gene, external reference

Fungal wilts caused by *Verticillium dahliae* and *Fusarium oxysporum f. sp. melongenae* are among the most serious diseases harming the eggplant production both in greenhouses and open field cultivation. In a previous work three cDNA libraries of differentially expressed genes involved in the plant-pathogen interaction have been isolated from roots of a *Fusarium* resistant introgression line after inoculation with *Fusarium, Verticillium* and both fungi together. Molecular studies enabled to identify a number of genes putatively involved in the plant pathogen interaction which need to be validated and subjected to characterization. We want to compare the expression profiles of these genes following the three different inoculations (*Fusarium, Verticillium* and *Fusarium+Verticillium*) and considering 0, 4, 8 and 24 hours after roots dipping in the fungal suspension.

Real-time PCR has greatly improved the ease and sensitivity of quantitative gene expression studies. Accurate measurement of gene expression with this method relies on the choice of a valid reference gene for data normalization which should be unaffected by experimental conditions. Usually, an internal control gene is used for this purpose. The most common housekeeping genes known in literature for plant-pathogen interaction studies are β tubulin, elongation factor 1-α, ubiquitin, 18s rRNA and glyceraldeyde-3-phosphate dehydrogenase (GAPDH). However, many recent studies showed that also internal standard genes could vary depending on different experimental conditions, and not often a reliable control has been reported. Therefore, in order to find the most suitable housekeeping gene for our study, a preliminary characterization was performed to confirm that the internal control gene chosen is expressed at a constant level in the three different fungal inoculations.

We first compared each other the expression of the above mentioned housekeeping genes and, in addition, we decided to use an exogenous reference gene to confirm the expression profiles of the internal control genes. A heterologous kanamycin transcript was co-transcribed with total RNA from eggplant roots to have an external reference in each sample. The expression of the external reference gene was invariable among all our experimental conditions. Therefore, we can use heterologous kanamycin transcript both as a reference gene and to check the expression profiles of putative housekeeping genes. Applying this approach to all our experimental treatments (i.e. type of fungal inoculation and timings), GAPDH showed an suitable stability in gene expression and it may be employed as internal reference for the evaluation of the expression of our collection of genes involved in eggplant/fungi interaction.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.55

HIGH-RESOLUTION GENETIC MAP OF THE *Rvi1* (*Vg*) APPLE SCAB RESISTANCE LOCUS

COVA V.*, LASSERRE P.**, VELASCO R.*, DUREL C.E.**, MALNOY M.*

*) IASMA Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, 38010 S. Michele all'Adige (TN, Italy)
**) UMR1259 Genetics and Horticulture (GenHort), Institut National de la Recherche Agronomique (INRA), BP 60057, 49071 Beaucouzé (France)

Resistance, Venturia inaequalis, apple, mapping

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, is one of the most prejudicial apple diseases for apple (*Malus* x *domestica*) commercial orchards, especially in temperate countries. Apple scab strongly decreases fruits quality and yield, whereas fresh market sales require high-quality fruits standards. In order to control this disease, traditional methods use 15 to 20 fungicide treatments per year, which causes health and ecological problems. Since genetic resistance is an alternative to the chemical control of this disease, it has been largely studied and many apple scab resistance genes have been identified in wild or domestic apple species (Gessler e*t al*, 2006, Soufflet-Freslon *et al,* 2008). One of them is the major gene *Vg* from the cultivar Golden Delicious, firstly identified by Bénaouf and Parisi (1997) and then confirmed and mapped on linkage group 12 by Durel et al (2000). This gene confers resistance towards *Venturia inaequalis* strains of race 7 and this race is virulent to varieties carrying the *Vf* major gene (confers resistance to races 1 to 5). Thus *Vg* and *Vf* genes are complementary to confer a large spectrum of resistance in breeding programs. The apple genome of the cultivar 'Golden Delicious' has been recently sequenced by the Research and Innovation Center of IASMA (Velasco *et al*, submitted). By this way, the fine mapping and cloning of the major gene *Vg* becomes strategic. This work will have practical issues supplying accurate molecular markers to screen the gene in Marker-Assisted Selection.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.56

IDENTIFICATION OF QTL FOR ALTERNARIA BLOTCH RESISTANCE IN A *MALUS* CROSS

ZINI E., PINDO M., VELASCO R., SALVI S., MALNOY M.

IASMA Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, 38010 S. Michele all'Adige (TN, Italy)

QTL, Alternaria blotch, resistance, apple

In the region Trentino - Alto Adige, which provide to almost 50% of the apple production in Italy, the apple pathotype of *Alternaria alternata* (Fr.) Keissler causing apple blotch has been identified in the late 90's in few trees, but now the disease is spreading around this area.

Symptoms on leaves appear as small, round, brown spots, 2–5 mm in diameter, with a purplish-black border. As the spots enlarge they merge to form larger blotch like areas of necrosis. Severe defoliation can occur. Fruit infection is uncommon, but inconspicuous, circular, black spots ,only 1–3 mm in diameter, may occur, centred on lenticels

In order to identify and estimate the effect of genes conditioning resistance to Alternaria blotch, a quantitative trait loci (QTL) mapping study was performed in F1 population derived from the cross between the cv. Rubinette and cv Royal Gala and genotyped with microsatellite and SNP markers. Macroscopic symptoms on leaves were evaluated, in the field, on the trees naturally infected. A QTL region for Alternaria resistance for both cultivars was found in linkage group 15, and confirmed in 2 years of evaluations (2008 and 2009). with a LOD score ranging from 7.5 to 10.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.57

ESTABLISHMENT OF AN *AGROBACTERIUM TUMEFACIENS*-BASED TRANSIENT TRANSFORMATION SYSTEM FOR FUNCTIONAL GENOMICS STUDIES IN GRAPE LEAVES THROUGH GENE SILENCING

URSO S.*, ZOTTINI M.**, RUBERTI C.**, LO SCHIAVO F.**, BISELLI C.*, VALE' G.*

*) CRA-Genomics Research Centre, Via San Protaso 302, Fiorenzuola d'Arda (Piacenza)
**) Department of Biology, University of Padova, Via Ugo Bassi 58/B, Padova

Transient transformation, gene silencing, Agrobacterium tumefaciens

Transient transformation of plants represents an useful tool to study genes function in different plant tissues and it is a time-saving procedure especially for species with long generation times avoiding stable transformation. In this work *Agrobacterium tumefaciens*, which can penetrate into leaf tissues, has been tested for grape leaves transient transformation assays. Two *A. tumefacinens* strains, AGL1 and GV3101, two grape genotypes (cv Superior and Mtp3294, carrying the *Run1* powdery mildew resistance gene) and two different growth conditions (*in vitro* and hydroponics) have been evaluated for transformation efficiency. The AGL1 strain demonstrates to be more efficient for transformation assay in the two genotypes and in the two growth conditions analysed. To establish the silencing procedure, a sequence coding for PDS (*Phytoene Desaturase,* gene coding for an enzyme involved in carotenoid biosynthesis) integrated in a Gateway® binary vector for gene silencing, has been used. The leaf lower surface has been agro-infiltrated by using a syringe without needle and the silencing symptoms were evaluated 6, 7 and 12 days after agro-infiltration. The presence of chlorotic spots due to photobleaching, considered to be a PDS silencing symptom, and GFP (green fluorescent protein) expression have been observed into agro-infiltrated leaf sites indicating an efficient gene silencing and transformation event. PDS transcripts down-regulation was analysed using qRT-PCR to verify gene silencing in transformed tissues.

In the near future this gene silencing system will be used as a functional genomic tool to study genes involved in powdery mildew resistance.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.58

UNRAVELLING THE MECHANISM(S) OF *ARTICHOKE ITALIAN LATENT VIRUS* ENTRY IN PLANT MERISTEMS

SANTOVITO E.*, MASCIA T.*,**, GALLITELLI D.*,**

*) Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari (Italy)
**) Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, c/o DPPMA, Via Amendola 165/A, 70126 Bari (Italy)

Artichoke viruses, PTGS, SRS, nepoviruses, plant virus tropism

Artichoke Italian latent virus (AILV) is a nepovirus that triggers detrimentally several globe artichoke varieties. A sanitation scheme based on meristem tip culture (MTC) proven useless in eliminating AILV while only a combined action of MTC and thermoptherapy eradicated the virus, so that stocks of virus-free plants were obtained. Recent evidences propose that RNA silencing could be involved in the degradation of viral RNAs before entering meristems, a mechanism that is probably enhanced by the exposure to high temperatures required by the thermotherapy so that the combined action produced AILV-free plants. Most plant viruses encode genes involved in RNA silencing suppression but no suppressors have been identified in nepoviruses. This information is not yet available for AILV, whose genome sequencing is still in progress. As a preliminary approach to understanding why AILV escaped MTC-induced sanitation, we studied the AILV infection pathway in tobacco. In the first stage of infection at 22 ± 2°C AILV induced a necrotic phenotype in inoculated leaves followed by appearance of chlorotic/necrotic ringspots on systemically infected leaves. From 21 days post-inoculation (dpi) onwards plants recovered from this condition and symptoms disappeared from new vegetation. This behaviour was not observed in infected plants grown at 15°C i. e. when PTGS is not operative, although overall symptoms were milder that those observed at higher growing temperatures. The recovery phenotype was attributed to the inability of nepoviruses to contrast silencing since it was active during AILV infection, as evidenced by the presence of AILV-derived small interfering RNAs (siRNAs) and by the recovery absence in plants grown at low temperature. Unlike other nepoviruses, disappearance of AILV-induced symptoms was accompanied by a proportionate reduction in viral RNA levels but not in its infectivity. While these results suggest that AILV does not posses a suppressor of RNA silencing, they do not explain how virus avoids silencing and invades meristems. The possibility that other mechanisms allow AILV to escape host defence responses is discussed.

Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress
Matera, Italy – 27/30 September, 2010
ISBN **978-88-904570-0-5**

Poster Communication Abstract – 4.59

DIFFERENT COMBINATIONS OF *CUCUMBER MOSAIC VIRUS* AND ITS SATELLITE RNAs SHOW COMMON AND DIFFERENTIAL EFFECTS ON TOMATO TRANSCRIPTOME AND HOST BIOLOGICAL PROCESSES

CILLO F.*, ZAMBONI A.**, MASCIA T.*,***, TULIPANO A.****, FERRARINI A.**, DELLEDONNE M.**, GISEL A.****, POLVERARI A.**, GALLITELLI D.*,***

*) Istituto di Virologia Vegetale - CNR, Via Amendola 165/A, 70126 Bari (Italy) - f.cillo@ba.ivv.cnr.it
**) Dipartimento di Biotecnologie, Università di Verona, Strada Le Grazie 15, 37134 Verona (Italy)
***) Dipartimento di Biologia e Patologia Vegetale, Università di Bari, Via Amendola 165/A, 70126 Bari (Italy)
****) Istituto di Tecnologie Biomediche - CNR, Via Amendola 122/D, 70126 Bari (Italy)

Solanum lycopersicum, virus disease, transcriptomics, microarray, plant-virus interactions

Transcriptional changes in tomato plants induced by the infection of *Cucumber mosaic virus* (CMV) and satellite RNA (satRNA) variants were profiled by microarray analysis. The analysis was performed on a Combimatrix platform using a tomato chip carrying 20200 specific probes from assembly of Tentative Consensus of the Tomato Gene Index (release 11.0, 2006).

Solanum lycopersicum cv. UC82 plants were infected with CMV-Fny, alone or in combination with three different satRNAs co-inducing lethal necrosis, stunting and symptomless phenotypes. Gene expression was examined at 2 and 9 days post-inoculation (dpi). After normalization, genes with a coefficient of variation < 0.8 in the three biological replicates and showing in infected vs. mock-inoculated plants a log2 (fold change) > 0.75 or < -0.75 were considered as differentially expressed.

CMV-Fny provoked wide transcriptional changes, with a peak of 3542 genes modulated at 2 dpi, whereas CMV-Fny/satRNA combinations affected to a lesser extent the host gene expression, with a minimum of about 1000 genes modulated by the symptomless combination at 2 dpi.

The effects of the selected CMV inocula on whole biological processes were evaluated by gene ontology terms enrichment analysis within the lists of modulated genes. Defense response, transcription and translation processes were the common over-represented categories among up-regulated genes, while down-regulation affected particularly genes involved in photosynthesis, chlorophyll biosynthesis and reductive pentose-phosphate cycle. Significant differences were also put in evidence, which provided new knowledge on how diverse CMV/satRNA combinations alter differentially vital biological functions in tomato and might account for different disease phenotypes.