HOW DO PLANTS PERCEIVE INSECT HERBIVORES AND TRIGGER SPECIFIC DEFENCE RESPONSES?

BONAVENTURE G., GILARDONI P., VAN DOORN A., KALLENBACH M., BALDWIN I.T.

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans Knoell Str. 8, 07745 Jena (Germany)

resistance gene, plant defence, Nicotiana attenuata, biotic stress, ethylene

Numerous hormones have been involved in the complex regulation of plant defence responses against insect herbivores (e.g., oxylipins, salicylic acid, ethylene, cytokinins) together with signal transduction components such as transcription factors (e.g., WRKY, MYB) and protein kinases (e.g., MAPK, CDPK). Studies performed in the model species *Nicotiana attenuata* demonstrated that the addition of insect-derived components in the wound of mechanically damaged leaves has the capacity to recapitulate insect-induced responses. In particular, fatty acid-amino acid conjugates (FAC) have been found to be necessary and sufficient for secondary metabolite elicitation, enhanced JA and ET production, suppression of nicotine synthesis, and for most of the transcriptional responses triggered by insect herbivory. FACs are molecules present in the oral secretion of a large number of larvae (e.g., *Manduca sexta*).

How plants perceive insect specific elicitors such as FACs and trigger downstream signaling cascades to induce insect-specific responses is largely unknown. Disentangling the effect of mechanical tissue damage and FAC elicitation will provide critical information on how plants tune defence responses to more efficiently protect themselves against insect herbivores.

In an attempt to unravel early mechanisms elicited by FACs, we combined a SuperSAGE approach for in-depth gene expression profiling with viral induced gene silencing (VIGS) and biological/biochemical assays for gene function characterization. We have identified several potential regulators of FAC-mediated responses. One of them encodes for a putative membrane-localized protein kinase involved in the FAC-mediated activation of the ethylene burst.
PLANT-MICROBE INTERACTIONS: EFFECTS ON HEAVY METALS UPTAKE AND ACCUMULATION IN *ARABIDOPSIS HALLERI*

FARINATI S., DALCORSO G., FURINI A.
Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy

*Arabidopsis halleri*, heavy metals, rhizosphere microorganisms, 2D-PAGE

*Arabidopsis halleri* is a plant species tolerant to Cd, Zn and Pb and hyperaccumulator of Cd and Zn. Being phylogenetically related to *Arabidopsis thaliana*, *A. halleri* is considered an important model system in studies that concern phytoextraction, a process that can be largely influenced by microbial community of the rhizosphere: in fact, it is able to influence, directly or indirectly, the heavy metal mobility in soil, influencing their absorption by plant roots. In this research it was considered an ecotype of *A. halleri* adapted to grow in a soil contaminated by heavy metals (Pb, Cd and Zn). With the aim to get insight on the complex genetic network responsible for heavy metal accumulation and detoxification in *A. halleri* in presence of the rhizosphere microbial community, shoot proteome analysis (2D-PAGE) was carried out for the identification of differentially expressed proteins in plants i) grown in the solely nutritive solution, ii) with the addition of Cd and Zn or iii) maintained with these two heavy metals plus soil rhizosphere microorganisms. Moreover, plants were also cultivated with the same heavy metal solution and eight microbial strains previously isolated from the rhizosphere of *A. halleri* plants grown on a contaminated site and selected for their tolerance to high concentration of Cd and Zn. Analysis of metal content suggested that populating the root zone with unselected microorganisms belonging to the autochthonous rhizosphere of a metal contaminated soil is sufficient to enhance plant metal uptake and translocation to the shoot. On the contrary, the massive inoculum with selected eight microbial strains has caused a minor heavy metal accumulation (both Cd and Zn) in the aboveground tissues.

Conversely to what normally observed in non-accumulator plants, heavy metal treatment induced in *A. halleri* a consistent up-regulation of photosynthesis related proteins. Subunits of the complexes responsible for light harvesting and electron transport were up-regulated by metals plus microorganisms treatment: these might be required for an enhanced energy demand of the entire cellular metabolism. Furthermore, the addition of these two metals caused a general down-regulation of proteins potentially involved in defence against herbivorous insects and pathogen attack. In particular, the expression of a myrosinase enzyme, two endochitinases and enzymes involved in the jasmonate biosynthetic pathway was strongly inhibited. This supports the view of a trade-off between metal hyperaccumulation and organic defences in *A. halleri*. In conclusion, metal uptake, transport and accumulation are energy-demanding processes, that can induce a general up-regulation of photosynthesis related proteins. This increased energy requirement is counteract by thrift defence system, therefore if high metal concentration in shoots provides a kind of protection system, other defence mechanisms are temporarily saved, highlighting a cross-talk between heavy metal signalling and defence signalling.

Furthermore, the attention was focused on the influence of the eight selected microbial strains: in particular each of them was singularly inoculated with *A. halleri* plants (in presence of
heavy metals) with the aim to understand its contribution on plant metal uptake and translocation to the shoot. Finally, a proteomic analysis was performed on some of these microbial strains to identify differentially expressed membrane proteins putatively involved in heavy metal tolerance and transport and results will be discussed.
FUNCTIONAL ANALYSIS OF AT4G16830 ENCODING AN RNA-BINDING PROTEIN INVOLVED IN PLANT OSMOTIC STRESS RESPONSE

AMBROSONE A.*, COSTA A.*, LEONE A.**, GRILLO S.*

*) National Research Council, Institute of Plant Genetics (CNR-IGV) Portici, Via Università 133, Portici (NA), Italy – alfredo.ambrosone@unina.it
**) Department of Pharmaceutical Science, University of Salerno, via Ponte don Melillo, Fisciano (SA), Italy

water stress/adaptation, qRT-PCR, RNA binding protein, knockout mutant, protein localization

Exposure to osmotic stress induces in plants a wide range of molecular and cellular responses. Recently, to characterize changes in gene expression occurring during stress adaptation, we performed a transcriptome analysis of potato cells exposed to gradual acclimation to water deficit induced by PolyEthyleneGlycole (Ambrosone et al., 2008. Options mediterraneennes). Among the genes consistently induced during long-term water stress, the \( \text{rgga} \) gene, coding for a novel Glycine-rich RNA-binding protein, was identified and isolated. BLAST analysis revealed that \( \text{rgga} \) is conserved in several species and contains an RNA binding domain and two RGG box. RNA binding proteins have been involved in the responses to different exogenous signals, including abiotic stresses, but their functions in stress adaptive mechanisms remain largely unknown.

To get insight into the biological role of \( \text{rgga} \) in plant stress response, a functional study of the hortologous gene (\( \text{Atrgga} \)) in \( \text{Arabidopsis thaliana} \) was performed. Similarly to the potato gene, qRT-PCR and Northern analyses revealed that \( \text{Atrgga} \) expression is regulated in response to different exogenous treatments (ABA, NaCl, PEG) in both cell cultures and young plants. In addition a strong \( \text{rgga} \) expression in guard cells and in vascular tissues was evinced by \( \text{rgga} \) promoter-driven GUS assay. To investigate the subcellular localization of the RGGA, transgenic plants overexpressing YFP-RGGA fusion protein were obtained. RGGA-YFP was localized prevalently in the cytoplasm and in the perinuclear region with no evidence of protein accumulation in root meristematic cells. A “gain and loss” of function study in Arabidopsis using \( \text{rgga} \) knockout mutants and transgenic plants overexpressing \( \text{rgga} \) under the control of 35S promoter was also performed. Overexpressing plants exhibited osmotic stress tolerance with high plant survival rates under stress conditions. Inversely seed germination and plant growth of \( \text{rgga} \) knockout mutant were severely affected by osmotic stresses.

These data taken together provide compelling evidence that RGGA affects the growth and stress tolerance of Arabidopsis plants under high salt and drought stress conditions, suggesting an important role in the complex machinery of plant adaptation to osmotic stress.
DROUGHT TOLERANCE IN TOMATO PLANTS EXPRESSING THE
ARABIDOPSIS TRANSCRIPTION FACTOR ATHB7

IANNACONE R., PETROZZA A., ARMENTANO N., LA VECCHIA G., TADDONIO G.,
CELLINI F.

Metapontum Agrobios, Metaponto (MT), Italy

drought resistance, tomato, Arabidopsis, transcription factors, Image analysis

ATHB7 is a member of HD-zip proteins that is involved in drought response in Arabidopsis. The gene is transcriptionally induced by dehydration in post-germinative stages of the life cycle and it is supposed to act as a negative regulator of growth.

To assess the effect of this gene in an heterologous system tomato plants expressing ATHB7 mRNA, by using a CaMV35S::ATHB7 construct were obtained. The expression of the inserted trait, monitored by real time PCR, was stable across six generations.

Drought tolerance was evaluated in tomato plants grown in pots in greenhouse. Trials were conducted for 3 years and several parameters (water potential, osmotic potential, spad, soil and leaves conductivity) were evaluated. In the last year parameters were evaluated on small plantlets. Four replicates for each line were grown under normal agronomic trial and under drought stress conditions. The drought trial was conducted for 18 days and daily leaf and stem water potential, leaf dry weight, and the foliar pigments (chlorophylls and carotenoids) were determined. Plant phenotype under normal and drought stress conditions was evaluated by using high-throughput non-destructive method. Automated screening of complete plants was led using Scanalyzer 3-D system (LemnaTec) under near-infrared (NIR) and visible (RGB) light conditions. NIR imaging was used to get information on watering status of plant leaves and their reaction to limited water availability. RGB imaging was used for assessing morphological parameters.

Transgenic tomato plants expressing the ATHB7 factor, showed a higher leaf water content than isogenic line grown under dry conditions. Furthermore morphological differences, in terms of dimensions of plants, visible leaf area and colour leaf, were detected between transgenic and UC82 control plants.

Data obtained by measuring physiological indexes and image analyses indicate an increased drought tolerance and a high level of recover after re-watering of ATHB7 plants compared to control plants.
THE ROLE OF PHYTOCHELATIN OVERPRODUCTION AND OF THE PUTATIVE VACUOLAR TRANSPORTER MRP3 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, enzymatically synthesized from glutathione by PC synthase (PCS1). PCs are able to form specific complexes with Cd and other heavy metals in the cytosol, that 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, leading to PC overproduction, increases Cd tolerance (1). In Arabidopsis thaliana Cd tolerance of PCS1 overexpressing seedlings is strongly dependent on growth conditions, possibly because PC content is 5 fold higher in wt arabidopsis compared to wt tobacco seedlings (Brunetti et al. manuscript in preparation).

Many laboratories are working to identify proteins involved in the transport of PC-Cd complexes into the vacuoles. In Arabidopsis thaliana proteins of the MRP family, a group of ABC proteins are good candidates for transporters of heavy metals complexed with PCs. In particular AtMRP3 is upregulated by Cd treatment, has vacuolar localization, and is able to complement the loss of YCF1, an ABC protein that is involved in the transport of GSH-Cd complexes in S.cerevisiae. To determine if MRP3 protein is involved in Cd sequestration into the vacuoles, we analysed Cd tolerance and accumulation of arabidopsis mrp3 knock out seedlings. Compared to wt, mrp3 seedlings show a slight sensitivity to Cd and an increased cadmium accumulation in the cytosol as detected by means of the fluorochrome BTC-5N (2). We also prepared AtMRP3 overexpressing arabidopsis and tobacco lines and we are currently comparing their tolerance and accumulation of Cd. Preliminary results show that arabidopsis seedlings overexpressing AtMRP3 are more tolerant to Cd than wt seedlings. To correlate Cd tolerance of these lines to PC content in the cell, we will analyse arabidopsis lines overexpressing both PCS1 and MRP3 (currently in preparation in our laboratory).

CANDIDATES OF THE BARLEY LEAF STRIPE RESISTANCE GENE
*Rdg2a* ARE INCLUDED IN A CLUSTER OF NBS-LRR ENCODING GENES

BISSELI C.*, BULGARELLI D.**, CONSONNI G.***, STANCA A.M.*, VALE’ G.*

*) CRA - GPG-Genomic and Post-Genomic Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy
**) Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, 50829 Cologne, Germany
***) DiPROVE, University of Milan, Via Celoria 2, 20133 Milano, Italy

Barley, *Pyrenophora graminea, Rdg2a, resistance gene*

*Rdg2a* is a mono-mendelian barley resistance gene that confers resistance against several isolates of the seed-borne fungal pathogen *Pyrenophora graminea* (the causal agent of barley leaf stripe) and immunity against isolate *Dg2*, the most virulent isolate of a collection of monoconidial isolates.

In order to characterize the genetic basis of *Rdg2a*-mediated leaf stripe resistance, a map-based cloning approach was undertaken for this gene. For this purpose the *Rdg2a* genomic region was saturated with molecular markers developed from shot-gun sequencing of Morex BACs covering the region. Because the cv. Morex does not carry a functional allele of the resistance gene, a 5X cosmid library of barley cv. *Thibaut* (bearing a functional allele of *Rdg2a*) was constructed. Screening of the cosmid library with markers co-segregating and tightly associated to *Rdg2a* yielded the identification of a 72Kbp cosmid contig encompassing the genomic region of the gene. Low-pass shotgun sequencing of this contig led to the identification of three sequences coding for NBS-LRR (Nucleotide Binding Site-Leucine Rich Repeats) proteins. Transcription analyses revealed that the three predicted genes are expressed only in the *Rdg2a*-near isogenic line (NIL) resistant genotypes but not in the corresponding susceptible NIL. The cloning of the full length cDNAs of the candidates confirmed the computational prediction for two of them, while in the third one a predicted intron is retained in the mRNA, causing a frameshift in the transcript that, most likely, lead to the production of non functional protein. Southern-blot analyses conducted on *Rdg2a*-resistant and three different susceptible genotypes as well as sequencing of the two NBS-LRR susceptible alleles highlighted genomic rearrangements in the locus suggesting that two NBS-LRR genes out of the three identified could represent good candidates for *Rdg2a*. Interestingly, single-cell transient assay revealed that the two predicted candidate proteins exist in the cell either inside and outside of the nuclei. The TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling) analysis of pathogen-challenged embryos of a resistant NIL revealed the absence of DNA fragmentation indicating that *Rdg2a*-mediated leaf stripe resistance may not involve programmed cell death at the host pathogen interface.
LASER DISSECTED CELLS REVEAL A CELL SPECIFIC DISTRIBUTION OF NUTRIENT TRANSPORTERS IN MYCORRHIZAL ROOTS

BALESTRINI R., GUETHER M., GOMEZ-ARIZA J., GIOVANNETTI M., BONFANTE P.

Istituto per la Protezione delle Pianta del CNR – UOS Torino and Dipartimento di Biologia Vegetale - UniTo, Viale Mattioli 25, 10125 Torino, Italy

laser microdissection, gene expression, arbuscular mycorrhizae, cell specificity, nutrient exchanges

Arbuscular mycorrhizal fungi (AMF) are an essential feature of the biology and ecology of most terrestrial plants and, as biofertilizers, AM fungi are an emerging issue in many projects focused on more sustainable, low-input agriculture practices. The identification of the events that lead to the formation of an AM, including the mechanisms involved in nutrient transfer, will be a challenging objective for a better exploitation of AMs in agricultural programs. The success of AM fungi in time and space is mainly linked to the nutritional benefits they confer to their plant hosts: they take up inorganic phosphate (Pi) and other macronutrients as well as microelements and water from the soil, and deliver them to the plant. The fungus, in turn, receives photosynthetic carbohydrates. Recent global transcriptomic studies of mycorrhizal roots revealed extensive changes in gene expression profiles. However, root colonization by AMF occurs by sequential steps only involving several cell types, with a specific functional role. Over the last few years, laser microdissection (LMD) has been used to study cell-specificity in arbuscular mycorrhizae and particular attention has been paid to the cortical cells containing the main feature of the symbiosis: the arbuscules. The LMD approach led to novel insights into the distribution of phosphate transporter (PT) transcripts during the AM interaction between tomato and *Glomus mosseae*. Transcripts of five tomato PT genes (*LePTs*) were, in fact, simultaneously detected in arbuscule-containing cells, unlike the neighbouring non-colonized cells. The contemporaneous presence of five plant PT mRNAs in the arbuscule-containing cells strongly suggests that the symbiosis enhances plant Pi uptake capabilities by recruiting additional PTs in this cell population. On the fungal side, the presence of *GmosPT* transcripts in the arbusculated cells suggest that the efflux of phosphate probably occurs in competition with its uptake and the fungus might exert control over the amount of phosphate delivered to the plant.

Recently, the gene expression of *Lotus japonicus* arbuscule-containing cells isolated by LMD was used to confirm array experiments. As in the previous work, three types of homogeneous cell populations were microdissected: cortical cells from non-mycorrhizal roots (C); non-colonized cortical cells from mycorrhizal roots (MNM) and arbuscule-containing cells (ARB). Taken together, the results provide novel information on the location of the genes that are involved in nutrient transport. Among the last, a plant ammonium transporter (AMT) that is involved in N-uptake during mycorrhiza symbiosis has been identified and transcripts are mainly localized to arbusculated cells. The transporter is exclusive of mycorrhizal roots, since it is not expressed during nodule symbiosis, and may represent a novel functional trait of AMs.
ANALYSIS OF GLOBE ARTICHOKE LEAF PROTEOME AFTER UV-C EXPOSURE

ACQUADRO A.*, FALVO S.**, DI CARLI M.***, DESIDERIO A.***, BENVENUTO E.***, GIULIANO ALBO A.*, MOGLIA A.*, LANTERI S.*

*) DIVAPRA, Plant Genetics and Breeding, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
**) Bioindustry Park del Canavese, Colleretto Giacosa (TO)
***) Sezione Genetica e Genomica Vegetale, Dipartimento BIOTEC, ENEA, Casaccia, Roma, Italy

UV-C stress, proteomic analysis, DIGE, globe artichoke

Plant responds to abiotic stress by altering its metabolism so as to switch on particular defense programs or to optimize its performance by other means. Mounting evidences indicate that UV light, reactive oxygen species (ROS) and photo-oxidative stress have effects on the regulation of different gene families members expression. Alterations in protein level or enzyme activities mainly concern: i) chloroplast localized proteins; ii) carbon metabolism protein; iii) protein involved in intracellular signaling; iv) heat shock proteins and v) proteins involved in phenylpropanoid biosynthesis.

Globe artichoke leaves (Cynara cardunculus var. scolymus L.) are highly rich in phenylpropanoids (e.g.: caffeoylquinic acids = CQAs; dicaffeoylquinic acids = dCQAs), and in previous experiments we evidenced that UV-C treatments (254 nm) boosted the production of dCQAs in young leaves while increasing the transcription of specific structural genes involved in their biosynthetic pathway.

In order to dig out proteins involved in early and late response to UV-C stimulus, we analysed the globe artichoke leaf proteome in a time-course DIGE (Differential In-Gel Electrophoresis) experiment. Four time points (6, 12, 18 and 24 h) following UV-C exposure were chosen, the latter corresponding to the peak of dCQAs production on the basis of our previous findings.

A total of 151 protein spots were selected as differentially expressed according to univariate ANOVA analysis. Among them, 111 showed to be modulated in the early stage response (6 h), 96 were modified after 12 h, while 18 and 39 appeared to be involved in late response, i.e. after 18 and 24 h of UV-C exposure. Multivariate analyses (k-means and hierarchical clustering) were performed and the protein expression vectors were divided into 15 groups.

Differentially regulated spots were excised and analysed with tandem mass spectrometry (MS/MS and de novo sequencing). A total of 137 out of 151 MS/MS spectra (91%) were identified on the basis of an annotated Compositae database, including a set of 19055 globe artichoke unigenes.

The functional categorisation showed a marked increase in some GO Cellular Components: “chloroplast” and “plastid”, where photosynthesis and production of the main building blocks take place; the GO Biological Process categorisation proved a high increment in protein involved in “response to abiotic stimulus and to stress”, such as heat shock proteins, chromosome scaffolds and proteins involved in redox reactions. Finally, the GO Molecular Function confirmed a marked regulation of enzymatic activity up-stream to the phenylpropanoids: glicolysis, glyoxylate cycle and
pentose phosphate pathway. All this aspects are known to be involved in high light response in other plants. The present results will contribute to the understanding of the molecular mechanisms taking place in early and late phase of globe artichoke UV-C responses.
ARBUSCULAR MYCORRHIZAL FUNGAL BIODIVERSITY IN A LOW IMPACT AGROSYSTEM

BERRUTI A.*, LUMINI E.**, CHERSICH S.***, SCARIOT V.*, BIANCIOTTO V.**

*) Department of Agronomy, Forest and Land Management, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy
**) Institute of Plant Protection IPP – CNR, c/o Department of Plant Biology University of Turin, Viale Mattioli 25, 10125 Torino, Italy
***) Department of Earth Science, Faculty of MMFFNN, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy

Symbiotic associations between arbuscular mycorrhizal fungi (AMF) and plant roots are widespread in natural environments and provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests, diseases, and drought, as well as tolerance to heavy metals. In addition, the presence of a well developed AMF hyphal network improve the soil structure. As obligate mutualistic symbionts these fungi colonize the roots of many agricultural crops and it is often claimed that agricultural practices (use of fertilizers and biocides, tillage, dominance of monocultures and the growing of non-mycorrhizal crops) are detrimental to AMF. As a result, agrosystems impoverished in AMF may not get the fully expected range of benefits from these fungi. We selected two different areas, respectively representative of a low impact agrosystem (Azienda Agricola Manenti, Sostegno – Biella, mainly involved in horticultural production) and its surrounding grassland, for an arbuscular mycorrhizal fungi (AMF) community composition analysis. Roots of a crop plant (Allium fistulosum), roots of two spontaneous plants (Trifolium spp., Plantago spp.) and their rhizospheric soil were sampled. AMF in roots and soils were identified by cloning and sequencing a region of 550bp of the 18S rDNA (SSU) and 600bp of the 28S rDNA (LSU). A morphological investigation was carried out too. All plants were well colonized and showed all the typical infection structures (inter and intracellular hyphae, arbuscules, coils). More than 250 clones were screened for RFLP and a total number of 32 RFLP types was found. Sequencing and phylogenetic analysis on the rDNA SSU and LSU highlighted a more widely distributed AMF community for spontaneous plants than for the crop plant. In particular, analysis on the SSU characterized three RFLP types which clustered into Glomus group Aa and Ab in all selected areas, hinting at a lower specificity for the AM fungal species they belong. Seven RFLP types, according to the phylogenetic tree built on the LSU, did not cluster with any previously published AM sequence. Shannon indexes showed higher AMF biodiversity values for the natural ecosystem than for the low impact agrosystem. Both values appeared to be significantly higher than the average found in literature related to conventional high-impact agrosystems, putting forward low-impact agrosystems as semi-conservative of the indigenous AMF natural composition. The results show that low impact practice (no fertilizers, no pesticide, minimum tillage) can preserve and enhance the population of AM fungi suggesting how AM fungi could effectively act as natural fertilizers to obtain a good plant productivity. On this basis it appears important to evaluate commercial AMF inocula as alternative to fertilization in field or greenhouse where natural
populations of this group of fungi are not always present. For this reason we undertook in parallel a molecular characterization analysis of some commercially available AMF inocula in order to verify their effectiveness on plant growth in nursery and in field.
CHANGES OF CYTOCHEMISTRY AND GENE EXPRESSION IN APPLE PLANTS RECOVERED FROM APPLE PROLIFERATION DISEASE


*) Dip. di Agrobiologia e Agrochimica, Università della Tuscia, Via S. Camillo de Lellis snc, I-01100 Viterbo, Italy
**) Dip. di Biologia e Protezione delle Piante, Università di Udine, Via delle Scienze 208, I-33100 Udine, Italy
***) Dip. Scienze e Tecnologie Chimiche, Università di Udine, Via Cotonificio 108, I-33100 Udine, Italy

apple, Apple Proliferation, calcium, gene expression, SAR

In Europe Apple Proliferation (AP) is one of the most economically important plant diseases requiring quarantine safeguard. ‘Candidatus Phytoplasma mali’ (‘Ca. P. mali’) is the disease agent associated to AP; it is a phloem-restricted and wall-less prokaryote. Most cultivars of apple, the main host of ‘Ca. P. mali’, appear susceptible, as well as wild and ornamental plants of the genus Malus. The AP disease causes a number of symptoms, the most typical of them is witches’ broom, and production of low quality fruits, which significant economic losses. Recovery is the spontaneous remission, sometimes permanent, from disease symptoms. The persistence of symptom remission is affected by host genotype and environmental conditions. The recovery potential is related to the colonization behavior of phytoplasmas in AP-infected plants. During winter the pathogen is almost eliminated from the aerial parts of the tree, due to the degeneration of the sieve tubes in the previous year’s phloem. In spring, the upper parts of the plant can be recolonised starting from the roots, where the phytoplasma persists throughout the year. In case of recovery, phytoplasmas surviving within the roots are not able to recolonise the plant crown. The precise causes that induce recovery remain still unknown and its physiological bases are poorly understood.

In this research the modifications in the phloem tissue related to recovery-induced resistance have been investigated through ultrastructural, chemical and cytochemical analyses of leaf tissues from recovered, healthy and diseased plants. In particular our study focused on: 1) ultrastructural detection of abnormal callose accumulations and P-protein pluggings in leaf tissues; 2) cytochemical localisation of Ca$^{2+}$ ions by potassium pyroantimonate precipitation and quantification in the cytosolic cell fraction; 3) cloning and expression analyses of genes involved in callose and phloem (P)-protein synthesis. The expression patterns of nine cDNAs, five of them encoding callose synthases (MDCALS1/5) and four phloem proteins (MDPP2-1/3 and MDERG1), were analysed by qRT-PCR. The pyroantimonate precipitates, indicating Ca$^{2+}$ presence, were localized in the phloem in all tested plants, but their concentration in the cytosol of the recovered apples was remarkably increased compared to the healthy or infected plants. Moreover, TEM showed structural modifications in the phloem of recovered apples, such as P-proteins accumulation (shifting from the unpolymerized to the polymerized form) and callose deposition in the phloem bundles. Callose synthesis and P-protein plugging are both Ca$^{2+}$-dependent phenomena and among the early key events forming physical barriers that might prevent the in planta movement of phloem-restricted
phytoplasmas. Analysis by qRT-PCR detected the differential expression of some analysed genes between diseased, healthy and recovered plants, in most cases the lowest expression was detected in diseased plants. Interestingly, two of the five analysed genes coding for callose syntases (MDCALS3 and MDCALS4) were strongly up-regulated in recovered plants in comparison to those healthy and diseased, then they may be involved in the enhanced callose synthesis observed in recovered plants. Present results support the hypothesis that a mechanism similar to SAR and mediated by Ca$^{2+}$ signal activities would be at the basis of recovery from Apple Proliferation.
**ISOLATION OF A **Debaryomyces hansenii** **YEAST STRAIN FROM FERMENTED LEAVES OF CAMELLIA SINENSIS**


*) IGB “ABT” – CNR, via P. Castellino 111, 80131 Naples, Italy
**) Department of Environmental Sciences, Second University of Naples, Caserta, Italy
***) DiSteBa, University of Salento, via Monteroni, 73100 Lecce, Italy

*Debaryomyces hansenii*, *Camellia sinensis*, tea fermentation, phyllosphere yeasts, yeast ecology

The surface of plants accommodates a varied microflora. This environment is usually named the phyllosphere, a term used in microbiology to refer to leaf surfaces or total above-ground surfaces of a plant as habitat for microorganisms (Ruinen 1956). In this environment a numerous and diverse community of microorganisms resides including bacteria, fungi, and yeasts. Some are beneficial to the plant, while others function as plant pathogens and may damage the host plant or even kill it (Lindow & Brandl 2003). In addition, many phyllosphere microorganisms are of great commercial importance to agricultural industry because they are involved in production of many fermented foods and beverages. Yeasts play an important part in this microflora. Very recently the microbial colonization of young leaves of tea plant (*Camellia sinensis* Kuntze), which are the commercial components of tea crop, has been studied with particular reference to the bacterial component (Gunasekera & Paul 2007). In contrast, the yeast component of *C. sinensis* phyllosphere remains very poorly characterized. Most studies have focused almost exclusively on the presence of several toxin-producing fungal species in tea factory atmosphere, due to their negative impact on human health (Dutta et al 2004). In the present study, four-years-old *C. sinensis* (Assam tea company India) tea plant leaves were used in a laboratory-scale tea fermentation process, and a yeast strain, predominant at the end of the fermentation, was isolated, identified and characterized. A psychrotolerant, halotolerant and alkalophilic yeast was isolated from fermented leaves of *C. sinensis* Kuntze, the tea plant. The yeast strain, named Tea-Y1, was both phenotypically and genotypically identified as belonging to the species *Debaryomyces hansenii*. This assignment was confirmed by scanning and transmission electron microscopy. The analysis of growth curves demonstrated the ability this yeast strain to grow in a temperature range between 4°C and 28°C, with an optimum of 23°C. There is evidence that environmental microorganisms inhibiting the tea factories and presumably derived from tea plant phyllosphere may be involved in the tea leaves fermentation process (Dutta et al 2004). Our result indicates that *Debaryomyces hansenii* may be one of these microorganisms.

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IDENTIFICATION OF UP-REGULATED GENES IN OLIVE FRUITS UNDER BACTROCERA OLEAE ATTACK

IMPERATO A.*, ALAGNA F.***, VARRICCHIO P.*, PASTORE N.*, BALDONI L.**, CORRADO G.*, RAO R.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy
**) CNR - Institute of Plant Genetics, Via Madonna Alta 130, 06128 Perugia, Italy

Olea europea, suppression subtractive hybridization, expressed sequence tags

The olive fruit fly Bactrocera oleae (Rossi) is a serious pest of olive in most of the countries around the Mediterranean Basin, where it causes significant yield losses. Olive cultivars are characterized by different susceptibility level possibly related to the variability in plant defence responses to the insect pest attack.

To gain insight into molecular mechanisms involved in olive defence response to olive fly, we have constructed a SSH (Suppression Subtractive Hybridisation) cDNA library from infested fruits of ‘Moraiolo’ cultivar. The cDNA library was constructed by using RNA from olive with feeding tunnels as tester and undamaged olives as driver. Sequence analysis of 220 expressed sequence tags (ESTs) using tBLASTx algorithm indicated that 48% of the ESTs could be classified into putative known functions. Several biological classes of genes were identified, implying that the molecular response of olives to B. oleae is complex and involves several pathways.

ESTs with functions typically involved in stress response or more represented in the cDNA library were analyzed by Real Time PCR. Some clones such as a pathogenesis related protein (PR27) and a Chitinase class I confirmed to be largely over-expressed in the olive tissue infested by the fruit fly, suggesting their involvement in olive defence response.

The rapid amplification of cDNA ends (RACE) is in progress for a subset of interesting genes both to obtain full-length cDNA not yet available in public databases and to confirm their putative functions.

To our knowledge, our study reports the first transcriptomic data of genes up-regulated under olive fruit fly attack, an initial step towards the understanding of the molecular basis B. oleae-olive fruit interaction.
GENES INVOLVED IN CHESTNUT RESPONSE TO INFESTATION BY DRYOCOSMUS KURIPHILOUS (YASUMATSU, HYMENOPTERA: CYNIPIDAE)

SARTOR C.*, TORELLO MARINONI D.*, QUACCHIA A.**, BOTTA R.*

*) Department of Arboricolturle and Pomology, University of Turin, Via Leonardo da Vinci, 10095 Grugliasco, Italy
**) Di.Va.P.R.A. - Entomology and Zoology applied to the Environment "Carlo Vidano", University of Turin, Via Leonardo da Vinci 44, Grugliasco (TO) 10095, Italy

Cynipid, cDNA, resistance gene, gall formation, differential display

Dryocosmus kuriphilus (Yasumatsu), a gall wasp introduced in Piedmont from China in 2002, has become one of the major threat to chestnut orchard and forests. In fact, C. sativa is highly susceptible to this insect. D. kuriphilus is univoltine and thelytokous; it lays eggs in chestnut buds in early summer. Larvae remain dormant until buds expand the following spring when they induce the formation of galls that are greenish-red, 8-15 mm large, located on leaves and twigs. Gall development suppresses shoot elongation, reduces fruiting, and causes twig dieback. Severe infestation can result in mortality of young trees. Chemical control is not effective. Although the biological control, that started in Italy by the introduction of the specific parasitoid Torymus sinensis, Kamijo from Japan, appears to be the most promising and efficient method for reducing the pressure of the pest in chestnut forests, it is likely that this will be not enough effective to guarantee high yield and good nut quality in orchards. Several cultivars, prevalently belonging to the species Castanea crenata and its hybrids, are considered resistant; among them, Bouche de Bétizac (C. sativa x C. crenata) was reported (Sartor et al. 2007). The genetic bases regulating this resistance and susceptibility in C. sativa cultivars are being investigated. Accessions of the cultivar Marrone (susceptible) and Bouche de Bétizac (resistant) were subjected to an intensive attack of D. kuriphilus, with the purpose of getting infested buds to be examined at different stages of development of larvae. At budburst and just afterwards (March - April) buds were collected for the extraction and isolation of mRNA. cDNA was obtained by reverse transcription and analysed using the differential display technique. Differentially expressed bands were blasted with sequences deposited in NCBI and TIGR database, and putative genes were identified.

The results showed that the mechanism of resistance may be due to the activation of a hypersensitive response at budburst in infested buds of Bouche de Bétizac. In fact, larvae are found also in this cultivar but die just at shooting time and do not develop galls. This mechanism is probably hindered in susceptible cultivars by the synthesis of proteins that prevent cell death. Concerning larva survival in galls, putative sequences of genes involved in differentiation and nourishing, such as those for biotin carboxylase carrier proteins, late embryogenesis abundant proteins, and plastid-lipid-associated proteins, were isolated providing confirmation to previous theories.

Acknowledgements: research funded by Regione Piemonte Administration
GENES INVOLVED IN CHESTNUT RESPONSE TO INFESTATION BY DRYOCOSMUS KURIPHILUS (YASUMATSU, HYMENOPTERA: CYNIPIDAE)

SARTOR C.*, TORELLO MARINONI D.*, QUACCHIA A.**, BOTTA R.*

*) Department of Arboriculture and Pomology, University of Turin, Via Leonardo da Vinci, 10095 Grugliasco, Italy
**) Di.Va.P.R.A. - Entomology and Zoology applied to the Environment "Carlo Vidano", University of Turin, Via Leonardo da Vinci 44, Grugliasco (TO) 10095, Italy

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Acknowledgements: research funded by Regione Piemonte Administration
GENETIC MAPPING OF A GENE FOR RESISTANCE TO SBCMV IN THE DURUM WHEAT CULTIVAR NEODUR

RUSSO M.A.*, MARONE D.*, DE VITA P.*, VALLEGA V.**, RUBIES AUTONELL C.***, RATTI C.***

*) CRA-Centro di Ricerca per la Cerealicoltura, S.S. 16, km 675, 71100 Foggia, Italy
**) CRA-Unità di Ricerca per la Valorizzazione Qualitativa dei Cereali, Via Cassia 176, 00191 Rome, Italy
***) Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Viale Fanin 44, 40127 Bologna, Italy

resistance gene, SBCMV, durum wheat

Soil-borne cereal mosaic virus (SBCMV), a Furovirus transmitted by Polymyxa graminis Led., is the causal agent of an important disease of wheat, widespread in Europe, where it causes losses in grain yield up to 70% with a great detrimental effects also on grain quality. Growing resistant cultivars represents the only effective and sustainable means of control.

Although valuable sources of resistance have been identified, little information is available on the genetic location of resistance determinants. This work aims to determine the genetic basis of resistance to SBCMV in the durum wheat cultivar Neodur by means of a bulk segregant analysis (BSA) approach. A population of 200 F8 recombinant inbred lines (RILs) obtained from the cross between durum wheat cultivars Neodur (highly resistant) and Cirillo (highly susceptible) was evaluated for SBCMV infection severity in a field with natural inoculum sources of SBCMV, located near Bologna (Italy), during the 2007-08 season. The infection severity was evaluated by visual scoring of symptoms and DAS-ELISA assay. Ten susceptible and ten resistant lines were selected and utilised to produce susceptible and resistant DNA bulks which were analysed with more than 200 microsatellite markers polymorphic between the parents. Results of the polymorphism analysis on bulks indicate the presence of a major gene on the short arm of chromosome 2B controlling the resistant trait; detailed results and microsatellites markers suitable for transfer of the resistance will be presented.
DIFFERENCES AND SIMILARITIES IN TOMATO DEFENSE RESPONSE TO NECROGENIC AND NON-NECROGENIC STRAINS OF CUCUMBER MOSAIC VIRUS

CILLO F.*, MASCIA T.*, DE LEONARDIS S.**, PACIOLLA C.**

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

*Solanum lycopersicum, Cucumber mosaic virus, ROS, plant defense response, programmed cell death

An early plant response to pathogen infection is an oxidative burst characterized by the production of reactive oxygen species (ROS) such as hydrogen peroxide. The increase of these ROS has been found in plant tissues in response to infection of various pathogens, including viruses, and enzymatic and not-enzymatic antioxidant systems are activated by the host in order to prevent potential cell damages. The analysis of some enzymatic defence components such as superoxide dismutases, catalases, total peroxidases and ascorbate peroxidases allowed to evaluate their contribution to the tomato defense response to infections of different Cucumber mosaic virus (CMV) strains.

In particular, these enzymes were examined in tomato (Solanum lycopersicum) cv. UC82 plants inoculated with the aggressive strain CMV-Fny and with the same strain co-inoculated with a necrogenic variant of satellite RNA (CMV-Fny/77-satRNA), a combination inducing systemic necrosis and plant death. Analyses included both the transcriptional activation and the enzymatic activity of such antioxidant components. Additionally, gene expression analysis was performed on genes involved in ethylene biosynthesis and signaling, in general defense responses and in programmed cell death processes.

The results obtained demonstrated that some scavengers of ROS and other defense responses were commonly activated by the two viral strains despite the different disease phenotype induced. Peculiar responses to either specific CMV inoculum were also evidenced, and their possible roles in the determination of the disease phenotypes are discussed.
TRANSCRIPTOMIC ANALYSIS OT TOMATO GENES FOLLOWING INFECTION BY THE GEMINIVIRUS TYLCSV

SARDO L., MIOZZI L., NORIS E., ACCOTTO G.P.
Istituto di Virologia Vegetale – CNR, Strada delle Cacce 73, 10135 Torino, Italy

microarray, whitefly-transmitted geminivirus, gene expression, plant-virus interactions

Several viruses can affect tomato (Solanum lycopersicon), a model species for the Solanaceae. Among them, geminiviruses cause yellow leaf curling and severe crop losses worldwide, but the molecular mechanisms underlying infection and symptoms development are far to be understood. To elucidate the transcriptional response of plants to Tomato yellow leaf curl virus infection, microarray analyses were performed. Plants were grown under controlled conditions in a growth chamber; viruliferous whitefly vectors (Bemisia tabaci) were caged on a fully expanded leaf, allowed to feed for 24h, and then removed. A young leaf was sampled after 1, 7 and 14 days. Control plants underwent the same treatment, with non-viruliferous whiteflies. In this work, using microarray technology and a set of approximately 12,000 tomato genes represented in the TOM2 oligonucleotide chip (Cornell University, USA), we analysed the genes differentially regulated during TYLCSV infection. Experiments were conducted with three biological replicates for each condition studied, and results were validated by qRT-PCR on selected genes.

At the onset of virus infection (1 day), the plant appeared to respond promptly, with more than 2000 genes regulated (FDR<0.05), including categories such as defense and stress response, primary metabolism, photosynthesis, DNA-protein complex assembly and organization and biogenesis of chromosomes.

This extended perturbation of the transcriptome decreased substantially after 7 and 14 days, when only 123 and 18 genes were regulated. The limited transcriptomic response observed 14 days after inoculation, when the systemic infection is well established, can be the result of a very fine strategy employed by the virus to escape the plant defence system. This strategy includes its strict phloem confinement and its capacity to produce silencing suppressors. In fact, symptoms caused by TYLCSV include leaf curling and yellowing, flower abortion, plant dwarfing, but no signs of hypersensitive reaction, necrosis or cell death, and no plant death.
COMPARISON OF EIGHT REFERENCE GENES FOR NORMALIZATION OF QUANTITATIVE RT-PCR GENE EXPRESSION ANALYSIS IN VIRUS-INFECTED TOMATO PLANTS

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

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

gene expression, quantitative RT-PCR, reference genes, Solanum lycopersicum, virus infection

Plant transcriptome analysis has been approached under a variety of experimental conditions. In the area of plant-virus interactions, transcript profiling is providing new perceptions into the mechanisms underlying pathogenesis, disease symptoms development and basal defense. Quantitative RT-PCR (qRT-PCR) is being largely used in gene expression analysis also as a mean to validate results obtained by wider analytical systems like microarray. The reproducibility of the results obtained by qRT-PCR strongly depends on accurate transcript normalization using stably expressed genes, known as housekeeping or reference genes.

We have evaluated the robustness of six usually employed housekeeping transcripts (β-tubulin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), elongation factor 1α (EF1α), ubiquitin 3 (Ubi3), actin (ACT) and 18S ribosomal RNA (18S), in addition to two novel genes that were shown to be stably expressed under viral infections in earlier microarray studies: namely urydilate kinase (UK) and cyclophilin (CYP), to be used as reference genes in tomato under viral infection pressure.

For this purpose, tomato plants were inoculated with different RNA and DNA viruses and a viroid, inducing different symptoms, RNA was extracted from leaf and root tissues and the stability of the above mentioned genes evaluated. Parallel analyses by three commonly used dedicated algorithms, GeNorm, NormFinder and BestKeeper, showed that, although both different viral infections and different tissue of origin influenced to some extent the expression levels of these genes, a set of genes resulted more stable than others. In particular, all algorithms showed good levels of stability of GAPDH, ACT and Ubi3 in both tissue types, while two of the widely employed reference genes, 18S and EF1α, demonstrated highly variable expression levels that should discourage their use to the purpose.

Thus, the results highlight the need for normalizing gene expression using carefully selected reference genes that may vary depending on specific experimental conditions.
DETECTION AND CHARACTERIZATION OF A MUTAGENIZED PEA LINE RESISTANT TO POWDERY MILDEW

PAVAN S.*, LOTTI C.**, FILIPPETTI A., MARZANO C. F.*, RICCIARDI L.*

*) Department of Biology and Chemistry Agro-Forestral and Environmental, Genetics and Plant Breeding Unit, University of Bari, Via Amendola 165/A, 70125 Bari, Italy
**) Department of Agro-Environmental, Chemistry and Crop Protection, University of Foggia, Via Napoli 25, 71100 Foggia, Italy

resistance gene, powdery mildew, pisum sativum

Powdery mildew caused by *Erysiphe pisi* is one of the most widespread fungal disease in pea. Following mutagenesis induced with diethyl sulphate, we identified a M2 line showing complete resistance to *E. pisi*. Occurrence of resistance was confirmed in the generations M3 and M4. We are currently developing a segregating F2 population in order to study the inheritance of the resistance and to develop a linkage map useful for marker assisted selection and positional cloning of the gene(s) responsible for resistance. Furthermore, we are studying the histological events associated with the failure in inducing disease of *E. pisi*. Powdery mildew resistance in pea cultivars is currently achieved through the exploitation of the recessive genes *er-1* and *er-2*. Future analysis will reveal whether the resistance source identified in this study is allelic to the two genes above mentioned.
A COMPARATIVE MAPPING STRATEGY PROVIDES EVIDENCE ABOUT THE DERIVATION OF THE BARLEY LEAF STRIPE RESISTANCE GENE Rdg1a


*) CRA-GPG - Genomic Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy
**) Germplasm Program, ICARDA, P.O. Box 5466, Aleppo, Syria

barley, leaf stripe, QTLs, Rdg1a, resistance gene

Leaf stripe of barley, caused by Pyrenophora graminea, is an important seed-borne disease in organically grown as well as in conventionally grown Nordic and Mediterranean barley districts. Two barley segregating populations represented by 103 recombinant inbred lines (RILs) of the cross ‘L94’ (susceptible) x ‘Vada’ (resistant) and 194 RILs of the cross ‘Arta’ (susceptible) x Hordeum spontaneum 41-1 (resistant), were analysed with two highly virulent leaf stripe isolates, Dg2 and Dg5, to identify QTLs for P. graminea resistance. A major QTL with its positive allele derived from ‘Vada’ and from H. spontaneum 41-1 was detected in both populations and for both the pathogen isolates on chromosome 2HL explaining 41.8% and 94.1% R^2 respectively for Dg2 and Dg5 in L94 x Vada and 97.8% and 96.1% R^2 respectively for Dg2 and Dg5 in Arta x H. spontaneum 41-1. Common markers mapped in the QTL region of the two populations allowed map comparison and highlighted an overlapping for the position of the resistance QTLs. Since the map position of the resistance QTLs identified in this report is at the same location as the leaf stripe resistance gene Rdg1a, mapped earlier in ‘Alf’ and derived from the ‘botanical’ barley line H. laevigatum, we propose that leaf stripe resistance in ‘Vada’ and H. spontaneum 41-1 is governed by the same gene, namely by Rdg1a, and that Rdg1a resistance could be traced back to H. spontaneum, the progenitor of cultivated barley. In the course of the mapping experiments, PCR-based molecular markers that can be used for marker-assisted selection (MAS) of Rdg1a were identified. An Rdg1a syntenic interval with the rice chromosome arm 4L was identified on the basis of rice orthologs of EST-based barley markers. Analysis of the rice genes annotated into the syntenic interval did not reveal sequences strictly belonging to the major class (nucleotide binding site plus leucine-rich repeat) of the resistance genes; nonetheless, four genes coding for domains which are present in the major disease resistance genes, namely receptor-like protein kinase and ATP/GTP binding proteins, were identified together with an homolog of the barley powdery mildew resistance gene mlo. Possible homologs of these genes in barley could represent candidates for Rdg1a or useful markers for fine mapping of the gene.
MORPHOLOGICAL AND MOLECULAR ANALYSIS OF THE INTERACTION BETWEEN DURUM WHEAT AND *Puccinia triticina*

LOSA A.*, GILARDETTI R.*, PASQUINI M.**, SARI GORLA M.*, GIANFRANCESCHI L.*

*) Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy
**) Unità di Ricerca per la Valorizzazione Qualitativa dei Cereali, CRA-QCE, Via Cassia 176, 00191 Roma, Italy

disease resistance, *Triticum turgidum*, brown leaf rust, host-pathogen interaction, laser microdissection

The main objective of the present study is the investigation of the molecular mechanisms involved in plant-pathogen interaction. In particular we are interested in unravelling the interaction of the durum wheat plant (*Triticum turgidum*) with the brown leaf blast pathogen (*Puccinia triticina*). Brown leaf rust is the most common disease of durum wheat. Unfortunately not much is known about the molecular processes going on during the interaction between the plant and the fungus.

We started our study with a microscopic analysis to morphologically describe the progression of fungus proliferation within plant tissues. We compared 3 different stages of the infection in resistant and susceptible varieties. Infections were performed in green house conditions on the first fully expanded leaf. Samples were collected at 18, 41 and 120 hours post infection (hpi), fixed and subsequently microscopically analysed. After an initial period dedicated to set up the procedures, the microscopic observation of the 18 and 41hpi samples allowed the identification of the pathogen within the leaves of the infected plants. The fungus resulted to be present in the tissues of both susceptible and resistant varieties. At the later stage (120hpi) some differences start to be evident, the most striking of which is the inability of the pathogen to sporulate within the tissues of the resistant variety. To investigate the molecular mechanisms of resistance we used a combined approach based on the exploitation of two new technologies: laser microdissection and microarrays expression profiling. Laser microdissection is a very powerful technique which allows to isolate and analyse selected plant tissues. We decided to isolate the mesophyl of infected and non infected plants by means of laser microdissection only at 41hpi, when the fungus is microscopically detectable but susceptible and resistant genotypes are still undifferentiated. mRNA was isolated, and amplified from laser captured cells and from the 18 and 120hpi samples. Real-time RT-PCR experiments were conducted to assess the quality and quantity of the mRNA. Here we present the results concerning the morphological analysis and the expression profile of few genes related to pathogenicity. Currently, mRNA has been extracted from each infection stage of both genotypes and microarray experiment are going to be performed to identify genes showing a modulated expression after the pathogen attack, either within genotype, or between resistant and susceptible genotypes. Such analysis should allow recognizing the host metabolic pathways related to resistance and possibly to identify the most relevant genes responsible for the host resistance.
**DASYPYRUM VILLOSUM 6V CHROMOSOME AS SOURCE OF ADULT PLANT RESISTANCE TO PUCCINIA TRITICINA IN WHEAT**


*) Department of Agrobiology and Agrochemistry of University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

**) C.R.A. Research Unit for Cereal Quality Improvement, Via Cassia 176, 00191 Rome, Italy

***) Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary

**leaf rust, adult plant resistance, durable resistance, Dasypyrum villosum, introgression line**

*Puccinia triticina*, the fungal pathogen causing leaf rust (LR) of wheat, occurs throughout the wheat growing regions of the world and the resistance to this disease represents a strategic aspect of many wheat breeding programmes. Adult plant resistance (APR) to leaf rust has been recognized as a major component of durable rust resistance.

The disomic addition lines CS+6V (DA6V#1; 2n=44) and CSxV63 (DA6V#4, 2n=44) and the disomic substitution line CSxV32 (DS6V#4, 2n=42), expressing different introgression events of chromosome 6V of *Dasypyrum villosum* (*Dv*) in the genome of the bread wheat cultivar “Chinese Spring” (CS), showed susceptibility to several selected pathotypes of LR when inoculated at the seedling plant stage. When controlled inoculations on the flag-leaf lamina of these lines were performed with a mixture of leaf rust pathotypes, the DA6V#4 and DS6V#4 lines evidenced strong APR (0 and 0-10 pustules respectively), while CS resulted highly susceptible (40-80 pustules per flag-leaf lamina). The APR of DA6V#4 and the susceptibility of CS with respect to natural LR infections were confirmed in multilocation epidemiological trials carried out in Italy during 2007 and 2008 (National Phytopathological Surveys).

Two cycles of selection within the CSxV32 disomic substitution line allowed the development of two sister lines. After genomic *in-situ* hybridization, it was evidenced that one of the sister line was a monosomic substitution line for chromosome 6V#4 (MS6V#4; 2n=41), most likely substituting chromosome 6D; this line (CSxV32-R) displayed APR to LR in the field in Italy (Rome and Viterbo) and Hungary (Martonvásár) when exposed to natural pathogen infections. In the same fields, the second sister line, lacking the 6V#4 chromosome, resulted susceptible to LR (CSxV32-S). These observations confirm the hypothesis that 6V#4 chromosome is carrying gene/s controlling APR to LR.

The genetic basis of APR to LR conferred by the 6V#4 chromosome, was studied in the F$_2$$_3$ progenies grown in Viterbo, derived from the cross between the DA6V#4 and DA6V#1 lines. Symptoms caused by air-born LR infections in the field were scored by counting the number of uredinia on the flag leaf and the leaf below it for each plant. DA6V#4 showed an average of 4.4 small uredinia (min.=0; max.=20; StEr=6.43) and was considered resistant (R); DA6V#1 evidenced an average of 80 uredinia (min. 30; St Er.=30.9) and was considered susceptible (S). About 55% of the F$_2$$_3$ progenies showed no more that 20 uredinia on the upper leaves and about 25% of the F$_2$$_3$ progenies showed a significant higher number (>50) of pustules. In total 236 F$_3$ plants were scored, 150 of which showed less than 20 pustules per plant (R) and 86 expressed over 20 and up to 350
pustules per plant (S); CS showed an average of 94.7 pustules. The null hypothesis of 10R:6S ratio for the bulk of the F2:3 plants was not rejected when chi-square test was adopted; that ratio was compatible with a 3R:1S segregation ratio among the F2 mother-plants from which the F2:3 progenies were derived.

Further analysis are in progress in order to confirm the hypothesis that the adult plant resistance to *P. triticina* surveyed in the 6V#4-introgression lines could be controlled by a single resistance gene, different from those already present in Chinese Spring (*Lr12, Lr34*).
RESISTANCE IN DURUM AND COMMON WHEATS TO STEM RUST DETECTED IN CENTRAL ITALY

PASQUINI M., NOCENTE F., SERENI L., MATERE A.

C.R.A. Research Unit for Cereal Quality Improvement, Via Cassia 176, 00191 Rome, Italy

wheat, stem rust, resistance gene, Ug99, gene pyramiding

Stem rust, caused by *Puccinia graminis f.sp. tritici*, is an age-old disease of wheat and it has been reported as one of the most destructive diseases worldwide. Its epidemics can be devastating when the most currently grown cultivars are susceptible. During the last fifteen years the presence of stem rust in Italy and in many other countries was detected at non-significant levels. This was probably due to the diffusion of more resistance genotypes, to the eradication of its alternate host (*Berberis vulgaris*) and to the cultivation of early heading wheat genotypes, able to escape disease infections. At the end of nineties a new virulent pathotype of *P. graminis*, known as Ug99 or TTKS, appeared in Uganda and, successively, it was detected in Kenya, Ethiopia, Sudan, Yemen and more recently in Iran. For the next years it is expected its dispersion also in South and Central Asia and in the future it could represent a potential threat to wheat production worldwide. During the last years very devastating stem rust epidemics have been detected in North Africa and around 85% of the wheat genotypes proved to be susceptible to this new stem rust strain, virulent against the traditional wheat resistance genes *Sr31* (located in the translocation 1BL.1RS from *Secale cereale*) and *Sr38* (derived from *Triticum ventricosum*). Moreover, during its migration, new variants of this pathotype, able to defeat the most effective resistance genes in wheat, have been identified. Wheat scientists from different countries were mobilitated for this rust emergency and different global initiatives were supported relating to stem rust surveillance and resistance screening and breeding.

A regular disease monitoring is currently carried out in the most important Italian wheat growing areas. Data on disease severity recorded in Italy during 2007-08 confirmed the absence of stem rust infections in almost all the locations tested except one, located in Central Italy (Montelibretti-RM), where two common wheat varieties Arsenal and Compair showed symptoms of this disease. Two pathotypes were identified within the pathogen population by testing in greenhouse a set of differential lines/varieties carrying known genes for resistance to *P. graminis*. Different resistance genes like *Sr24*, *Sr25* (both derived from *Thinopyrum ponticum*) and *Sr31*, showed their efficacy to the Italian pathotypes identified, while the lines carrying *Sr38* were susceptible.

Phytopathological analysis were carried out to test the seedling behaviour to stem rust of durum and common wheat cultivars grown in Italy. Many durum wheat cultivars resulted resistant to the pathotypes used for artificial inoculations, while several common wheat cultivars resulted susceptible. The different response of the two species could be due to the source (common wheat) of stem rust inoculum. Molecular PCR markers, closely linked to some *Sr* genes (*Sr24*, *Sr25*, *Sr31*, *Sr38*), were used to detect the presence/absence of the corresponding genes into the genetic background of the above mentioned materials. The presence of *Sr31* gene was evidenced in only two common wheat cultivars (Colledoro and Sollario), while several other genotypes resulted carrying *Sr38* gene. None of the durum wheat genotypes was positive to PCR analysis for the presence of *Sr31* and *Sr38*. A molecular marker assisted selection program was carried out to...
pyramid into elite common wheat cultivars different genes for resistance to rust diseases. Novel genotypes with the combined presence of resistance genes to *P. graminis*, *P. triticina* and *P. striiformis* were obtained.
MAPPING QTL FOR POWDERY MILDEW RESISTANCE IN DURUM WHEAT


*) Agroenvironmental Sciences and Technology Dept., University of Bologna, Bologna, Italy **) Società Produttori Sementi Bologna (PSB), Argelato (BO), Italy

Triticum durum Desf., disease resistance, powdery mildew, Quantitative Trait Loci (QTL)

Powdery mildew (Pm) is one of the main durum wheat fungal diseases in southern Europe. Susceptible hexaploid and tetraploid wheat cultivars have to be protected with fungicides (one to two treatments) to reduce the damage to the foliar apparatus. In durum wheat, a few resistance sources have been characterized and mapped. The durum wheat cv. Claudio shows a resistance response based on the results of field trials over several years (Italian variety testing network). To dissect the genetic basis of the Pm resistance carried by Claudio, a population of 181 RILs from the cross Claudio (resistant) x Meridiano (highly susceptible) has been developed and evaluated in two replicated field trials carried out under artificially inoculated conditions during 2007 and 2008 (Bologna, Italy). A parallel mapping effort has been conducted to produce a linkage map based on 158 SSR and 310 DArT markers. The distribution of the data collected four times during the disease developmental cycle over the two years showed a rather complex genetic control of powdery mildew response in this population. Two major QTLs controlling Pm resistance have been located on chr. 6BL (\textit{QPm.ubo-6B}) and 7BL (\textit{QPm.ubo-7B}) with the resistance alleles contributed by Claudio. In both years, the effect of \textit{QPm.ubo-7B} against Pm infection declined as the disease progressed, while \textit{QPm.ubo-6B} was undetectable in the early stage of the disease cycle and increased its effectiveness as the disease progressed. Additional minor QTLs with lower and less consistent effects across years were found on chrs. 2BS, 2BL, 6AS and 7AS, with both parents contributing resistance alleles. \textit{QPm.ubo-7B} is positioned in the distal end of chr. arm 7BL (\textit{Xbarc340, Xgwm146} and \textit{Xgwm344}) and possibly located in the homoeologous position of \textit{Pm1}. \textit{QPm.ubo-6B} is positioned near \textit{Xwmc539, Xbarc79, Xgwm1682} and \textit{Xgwm889}. Thus, Claudio is characterized by a polygenic resistance, based on two major genes/QTLs, showing effects consistent across years, and several minor genes.
In recent years, mainly due to climatic changes, Fusarium Head Blight (FHB) has become a severe problem in wheat cultivation in areas where it was not previously present, Italy included. The intensity of the attacks and their negative incidence on grain quality and yield, combined with the lack of resistant cultivars within adapted germplasm, have worldwide stimulated breeding efforts to develop FHB resistant wheats. In searching for effective resistance genes, exploitation of related species represents a valuable approach, especially for wheat, since gene synteny is well preserved among cereal species, and its polyploid nature represents a favourable condition to well tolerate the introgression of related alien DNA. We have used the decaploid wild wheat relative Thinopyrum ponticum as a source of FHB resistance to be introduced into wheat by chromosome engineering.

The donor accession carries the FHB resistance gene on the long arm of its 7el2 chromosome. On the other hand, the Lr19+Yp genes (for leaf rust resistance and yellow endosperm pigmentation, respectively) are located at similar position on the long arm of a homologous 7el1 chromosome of a different Th. ponticum accession, susceptible to FHB. In order to combine the potential benefit from both alien sources, durum and bread wheat recombinant lines, already possessing 7el1L segments including the Lr19+Yp genes, have been crossed with wheat translocation lines involving the 7el2L arm. Since 7el1 vs. 7el2 homology is full, their recombination is expected to allow pyramiding of all the desired traits in a single wheat genotype. Suitable, polymorphic DNA markers have been identified in the region of interest, which will facilitate the follow-up of the 7el1/7el2 alleles in the course of the multi-targeted transfer process.
INOCULATION TECHNIQUES FOR EVALUATING MAIZE RESISTANCE TO *FUSARIA VERTICILLIOIDES* EAR ROT*

FERRARI A., ABED N.J., BALCONI C.

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

*Fusarium verticillioides*, ear rot, artificial inoculation, genotype resistance, *Zea mays* (L.)

An efficient inoculation technique for Fusarium ear rot infection of maize, must be developed for a good and reliable differentiation between genotypes. Fusarium attack to maize ears can occur: i) by silks ii) by kernels. Some *Fusarium* strains produce mycotoxins which can be formed in infected plants before harvesting, or in grains during post-harvest storage. The occurrence of mycotoxins in cereal grains is a great concern worldwide, because their presence in feed and foods is often associated with chronic or acute mycotoxicoses in livestock and also in humans.

Our research is focused on the screening of maize genotypes for resistance to *Fusarium verticillioides*, fungal pathogen which attacks maize, causing root, stalk and ear rot diseases, producing mycotoxins (fumonisins).

During 2007, 33 maize genotypes (commercial hybrids) were tested in field experiments in various Northern Italy locations through artificial inoculation methods applied to each primary ear: i) the non-wounding Silk Channel Inoculation Assay (SCIA)-SPRAY technique, ii) the wounding SCIA-SYRINGE technique and iii) the Kernel Inoculation method. The test included: i) self pollinated non–inoculated ears, ii) self-pollinated inoculated ears, iii) open-pollinated non inoculated ears, iv) open-pollinated inoculated ears. At pollination, silk channel length was recorded. At maturity, ears were manually harvested; husk cover visual ratings ranging from 1 (good tight long husks extending beyond the tip of the ear) to 5 (poor:loose short husks with exposed ear tips) were recorded.

The severity of *F. verticillioides* attack was evaluated using ratings based on the percentage of kernels with visible symptoms of infection. After visual inspection ears were dried and shelled; the kernels were bulked within plots. To evaluate internal kernel infection, 50 kernels were randomly chosen from each sample, surface-disinfected, and plated on potato DRBC agar.

The non-wounding Silk Channel Inoculation Assay (SCIA)-SPRAY technique applied during 2007 was not efficient to evaluate maize commercial hybrids response to *F. verticillioides* attack, so during 2008, the 33 hybrids were tested in various locations by i) the wounding SCIA-SYRINGE technique and ii) the Kernel Inoculation method.

Variability in the response to Fusarium attack was evident after visual evaluation of the maize genotypes tested in field experiments through different artificial inoculation methods. The evaluation of symptomless infections result important for the final interpretation of data.

*Research developed in the Program MICOCER*
**Fusarium ear rot in maize: source of genetic resistance and analysis of the host response**

LANUBILE A., PASINI L., MAROCCO A.

Institute of Agronomy, Genetics and Crop Sciences, Università Cattolica delSacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy

*Fusarium verticillioides, breeding for resistance, microarray analysis, PR genes, oxidative stress*

Fusarium ear rot is caused by *Fusarium verticillioides*. Ear infection causes yield loss and the accumulation of fumonisins, frequently found in symptomless kernels. The aim of this study was: I) the evaluation of inbred lines for resistance to kernels infection by *F. verticillioides* and to fumonisins production; II) the introduction of sources of resistance into elite inbred lines and the evaluation of Fusarium ear rot severity and fumonisins contaminations; III) the analyses of the specific genes expression, induced and suppressed by *F. verticillioides* infection, in different maize tissues and time sequences. The resistance of the inbreeds was evaluated by silk-inoculation at 15 days after pollination with a suspension of $2 \times 10^7$ conidia ml$^{-1}$ of two toxigenic *F. verticillioides* isolates. Lines from the “breeding group” “Stiff Stalk” and “non Stiff Stalk” differed significantly for Fusarium ear rot incidence and fumonisin content in the kernels. Two F$_1$ populations, derived from the crosses CO430 x 1203 and Mp420 x 1203, were created. 143 families from Mp420 x 1203 and 124 from CO430 x 1203 were chosen and grown ear-to-row. A single self-fertilized ear was harvested from each row, until F$_5$-derived inbred lines were developed. The results obtained after natural and artificial infections suggest that sources of resistance against *Fusarium* and *Aspergillus* species can be used to improve resistance to *F. verticillioides* in elite inbreeds.

For the molecular evaluation, two inbred lines have been identified as resistant and susceptible to *Fusarium* infection and used. Their ears were infected with the fumonisin-producing strain 1744 of *F. verticillioides*, using the “pin-bar” technique, 15 days after pollination. The kernels were harvested 48 and 96 hours after infection and also from uninfected ears. RNA was extracted, labelled cDNA was prepared using fluorescent nucleotides. These probes were used for arrays hybridisation. 280 differentially accumulating maize gene transcripts in the *F. verticillioides*-treated plants compared with untreated plants were detected. Functional annotation of the transcripts revealed a variety of infection-induced host genes encoding defense response proteins, oxidative burst-associated enzymes, enzymes involved in sugar metabolism and proteins involved in protein synthesis, folding and stabilization. The differentially expressed genes were validated in RealTime PCR. Also silks derived from the same resistant and susceptible lines of maize were infected with the strain 1744 of *F. verticillioides*, 7 days after silking. The silks were harvested 12, 24, 48 and 72 hours after infection and also from uninfected ears. After RNA extraction the main genes identified in the array experiment and involved in pathogen response were tested using semiquantitative PCR and RealTime PCR.
AN INTEGRATED TRANSCRIPTION PROFILING TO INVESTIGATE THE RESPONSE TO RALSTONIA SOLANACEARUM IN POTATO

VITALE S., IORIZZO M., SACCO A., BARONE A.

Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

resistance gene, Solanum commersonii, cDNA-AFLP, microarray

Ralstonia solanacearum is a widely spread pathogen that causes severe damage to many cultivated species, including potato. No chemicals is active against this bacterium, therefore the selection of resistant varieties is the most reliable tool for disease control. Therefore, in order to identify genes expressed during this plant-pathogen interaction, two different transcriptomic approaches were used.

A cDNA-AFLP-TP analysis was carried out on the resistant Solanum commersonii and the susceptible S. tuberosum cv. Blondy. Various primer combinations were tested on RNA extracted from these genotypes at different times after inoculation. Up till now, out of 335 differentially expressed ESTs, around 70 were sequenced and selected for further analyses. In particular, one EST specifically expressed in the resistant genotype after the infection corresponded to part of a gene controlling the synthesis of an ABC transporter protein. This result will be further investigated since this type of protein has been hypothesized to play a role in protecting plants against pathogen attacks. Furthermore, the study of other ESTs is being aided by their alignment with BACs already sequenced in the framework of the International Tomato Sequencing Initiative.

In addition, a Combimatrix 4x2k array was synthesized using a tomato EST collection available in GenBank and obtained from a PCR select experiment performed on the resistant cv. Hawai 7996. The array slide, synthesized at the University of Verona, includes 4 subchip each one harboring probes designed to match specifically 658 transcripts. Probes are replicated 3 times within each subchip. This array is being hybridized with RNA extracted from the same potato genotypes used for the cDNA-AFLP analysis. Selected sequences obtained from both transcriptomic profiles will be validated by the qPCR real time, in order to identify those ESTs potentially involved in the resistant response to R. solanacearum.
IDENTIFICATION OF A LIPASE INVOLVED IN JASMONIC ACID BIOSYNTHESIS IN *NICOTIANA ATTENUATA*

ALAGNA F.*,**, BALDEN I.T.*, BONAVENTURE G.*

*) Max Planck Institute for Chemical Ecology, Department of Molecular Ecology, Hans Knöll Str. 8, 07745 Jena, Germany
**) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

*plant defence, virus induced gene silencing, lipase activity, subcellular localization*

Jasmonic acid (JA) is a multifunctional growth regulator widely distributed in the plant kingdom that modulates anther dehiscence, fruit ripening, root growth, tendril coiling, and plant resistance to insects and pathogens. The first step of JA biosynthesis is catalyzed by a lipase that hydrolyzes plastidial membrane lipids releasing free fatty acids. The identification of two Arabidopsis plastidial phospholipases, DAD1 and DGL provided genetic evidence that this is a critical step in the activation of JA biosynthesis.

We identified three putative functional homologues of DAD1 and DGL in *N. attenuata*: PLA1, PLA2 and PLA3 that were functionally characterized by Virus Induced Gene Silencing (VIGS) to investigate their involvement in JA biosynthesis.

After elicitation by wound and fatty acids coniugates (FACs), leaves of PLA2 and PLA3 VIGS-silenced plants accumulated similar amounts of JA and JA-Isoleucine (JA-Ile) compared to control plants. In contrast, PLA1 silenced plants showed significant reductions of >80% in JA and JA-Ile levels.

The full-length *PLA1* cDNA was obtained by 3' and 5' rapid amplification of cDNA ends (RACE). The sequence analysis revealed the presence of conserved lipase-3 domain with a catalytic triad consisting of glutamate-histidine-serine and of a putative signal peptide for plastid targeting.

To assess whether PLA1 encodes for an active glycerolipid acyl-hydrolase, we expressed PLA1 as a recombinant protein in bacteria and analyzed its activity towards different glycerolipid substrates. The enzyme hydrolyzed acyl groups of phospholipid (PC), galactolipid (MGDG) and triacylglycerol (triolein) with similar rates that ranged from ~1.5 to ~2 µg h⁻¹ µg protein⁻¹.

The subcellular localization of PLA1 was assessed by fusing the gene to EGFP by its C-terminus and cloned under regulation of the CaMV35S promoter. As controls, EGFP and a fusion EGFP protein carrying the first 273 amino acids containing the predicted plastid transit peptide of Lipoxygenase3 (LOX3) were used. *N. attenuata* leaves were infiltrated with Agrobacteria carrying the corresponding constructs and EGFP expression in mesophyll cells was analyzed by laser confocal microscopy. Protoplasts transformed with PLA1-EGFP and with LOX3-EGFP showed co-localization of a GFP green fluorescence and chlorophyll red autofluorescence, consistent with the predicted plastidial localization of PLA1, in contrast with protoplasts transformed with EGFP that showed a diffused green fluorescence characteristic of cytosolic localization that did not overlap with chlorophyll red autofluorescence.
These results indicate that the PLA1 protein is a plastidial lipase that catalyzes the release of fatty acids from chloroplast membranes and supplies linolenic acid (18:3) for de novo JA production in *N. attenuata* leaves.
DOES MYCORRHIZATION INFLUENCE TOMATO FRUIT QUALITY? A TRANSCRIPYOMIC APPROACH

SALVIOLI A.*, ZOUARI I.*, LACOURT I.**, BONFANTE P.*

*) Dipartimento di Biologia Vegetale dell’Università and Istituto per la Protezione delle Piante – CNR, Viale Mattioli 25, 10125 Torino, Italy
**) Sotral SpA, Via Livorno 60, 10144 Torino, Italy

Mycorrhiza, tomato, gene expression, fruit quality, Real time RT-PCR

Soil fertilization is considered one of the most important practices influencing plant productivity as well as fruit quality.

In this framework, the role of arbuscular mycorrhizal fungi (AMF), i.e. the symbiotic microbes which live in association with plants roots, may be crucial. While the positive effect of AMF as bio-fertilizers on plant physiology and productivity has already been reported, little is known about their effect on fruit quality traits. In particular, the potential effect of mycorrhization on fruit gene expression has never been considered so far. The aim of the investigation was therefore to assess the potential long-distance effect of AM fungi on tomato fruit transcriptomics.

Using cDNA microarray, transcription profiles of tomato fruit harvested at the breaker-turning stage from plants inoculated with *Glomus mosseae* and from non inoculated plants were compared. Twenty genes revealed an up- or down-regulation under the mycorrhizal versus the control condition.

Real time RT-PCR analysis confirmed the expression profiles of 58% of the selected genes. Interestingly, some of the genes whose expression appears to be influenced by mycorrhization have been described as ethylene- responding during fruit ripening.

In conclusion, our experiments reveal for the first time that AM fungi may have a long distance effect on the tomato fruit transcriptomics and in particular, genes related to the ripening process, aroma formation, sugar and aminoacid metabolism seem to be modulated by mycorrhization.
IDENTIFICATION OF GENETIC DETERMINANTS INVOLVED IN THE INTERACTION BETWEEN TOMATO AND THE BENEFICIAL ORGANISM TRICHODERMA SPP.

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

*) Institute of Plant Genetics (IGV), CNR, Via Università 133, 80055 Portici (NA), Italy
**) Institute for Plant Protection (IPP), CNR, Via Università 133, 80055 Portici (NA), Italy
***) Dept. Ar.Bo.Pa.Ve. - Plant Pathology, University of Naples, Via Università 100, 80055 Portici (NA), Italy

plant genetics, suppression subtractive hybridisation, Solanum lycopersicum, biocontrol, fungal antagonists

In the past few years several formulations of biopesticides based on fungal and bacterial antagonists have been introduced in the world market, mainly designed for organic farming but also for alternative, low input methods of disease management. Among the best characterised fungal antagonists are those belonging to the genus *Trichoderma*, that have developed the ability to directly interact with both plants and plant pathogens. During *Trichoderma*-plant interaction the antagonist can trigger systemic and localised resistance to pathogens as well as promote plant growth and development.

Using tomato as a model system, we have compared several genotypes treated with either *T. atroviride* P1 or *T. harzianum* T22 in terms of several biometric parameters. The results clearly demonstrate that all the considered parameters, including seed germination, plant development, tolerance to pathogens and specific gene expression, respond differentially to the interaction with *Trichoderma* in a plant genotype-depending way.

To identify the main genetic plant determinants involved in the processes of biocontrol and plant growth promotion by *Trichoderma* strains a subtractive hybridization approach has been adopted. RNA was extracted from *in vitro*-grown non *Trichoderma*-treated tomato plantlets (driver, Control plants) and from plantlets grown in the presence of *T. harzianum* T22 (tester, T22-treated plants) and used to construct subtractive libraries following the suppression subtractive hybridisation (SSH) procedure.

As a result of the screening 265 clones were identified. Differential expression of the clones was studied by dot blot analysis, revealing that 27% of clones were differentially up regulated. Sequence analysis showed that most of the clones were homologous to well-characterised genes involved in stress and disease response. However, about 22% of the clones matched nucleotide sequences annotated as unknown proteins. The expression of some of the isolated tomato genes was also confirmed by Real Time PCR.
LOCAL AND SYSTEMIC EFFECTS OF THE AM SYMBIOSIS ON GENE EXPRESSION PROFILES IN SOLANUM LYCOPERSICUM

FIORILLI V.*, CATONI M.**, NOVERO M.*, ACCOTTO G.P.**, LANFRANCO L.*

*) Department of Plant Biology, University of Turin, Viale Mattioli 25, 10125 Turin, Italy
**) Institute of Plant Virology – CNR, Strada delle Cacce 73, 10135 Turin, Italy

arbuscular mycorrhizal symbiosis, tomato, microarray, laser microdissection, Glomus mosseae

In nature one of the most widespread mutualistic association is the arbuscular mycorrhizal (AM) symbiosis formed between soil fungi belonging to Glomeromycota and most land plants. The symbiosis develops in the plant roots where the colonization involves epidermal and cortical cells. In the root cortex the fungus develops intercellular hyphae and extensively branched intracellular hyphae called arbuscules which are considered crucial components of the interaction. The AM symbiosis has a multifunctional character: AM fungi improve plant nutrient acquisition and provide protection from biotic and abiotic stresses. To obtain an overview of transcriptional changes triggered in roots and shoots of tomato (Solanum lycopersicum L.) as a result of the colonization by the AM fungus Glomus mosseae the TOM2 microarray platform (Cornell University) was used. Expression profiles of 17 selected genes was confirmed for qRT-PCR. This analysis revealed 362 up-regulated and 293 down-regulated genes in mycorrhizal roots. Significant gene modulation was also observed in shoots: 85 genes showed increased transcript levels while 337 genes were down-regulated. Most responsive genes are ascribed to the following functional categories: primary metabolism, defence and response to stimuli, cell organization, protein modification and transcriptional regulation. In addition, to identify possible plant determinants of arbuscule formation, the cell-type expression profiles of a subset of genes induced in mycorrhizal roots were monitored taking advantage of the laser microdissection technology. Six genes specifically expressed in arbusculated cells have been identified: they are involved in auxin and abscissic acid metabolism, cell wall biogenesis and cytoskeletal dynamics.
IDENTIFICATION OF A NOVEL SOURCE OF RESISTANCE TO CRENATE BROOMRAPE IN CULTIVATED PEA GERMLASM


*) Department of Biology and Chemistry Agro-Forestal and Environmental, Genetics and Plant Breeding Unit, University of Bari, Via Amendola 165/A, 70125 Bari, Italy
**) Department of Agro-Environmental, Chemistry and Crop Protection, University of Foggia, Via Napoli 25, 71100 Foggia, Italy

resistance gene, plant disease, pisum sativum, Orobanche crenata

Crenate broomrape (Orobanche crenata) is a parasitic plant threatening the cultivation of legumes in the Mediterranean basin and in the Middle East. Good level of resistance to O. crenata have been identified and exploited in some legume species, like faba bean and vetch. In contrast, only moderate tolerance has been detected in pea germplasm. Pea cultivation is not advisable in soils infested with Orobanche seeds, as yield losses up to 100% have been reported. We identified in the province of Bari (southern Italy) a pea landrace showing high level of resistance to O. crenata. We carried out a field trial with artificial inoculation of Orobanche seeds, and selected nearly completely resistant lines. Results clearly indicate that the lines developed in this study can be conveniently used in breeding programmes aimed to introduce resistance in pea cultivars. We are currently carrying out experiments aimed at: 1) the study of the inheritance of the resistance; 2) the characterization of the histological mechanisms preventing O. crenata colonization; 3) the identification of molecular markers suitable for assisted selection.
INTERACTION OF PATHOGENIC AND SYMBIOTIC FUNGI WITH TRANSGENIC RICE EXPRESSING THE MAIZE RIP b-32

VALLINO M.***, LANZANOVA C.***, REALI A.***, TRIULZI T.***, FAIVRE-RAMPANT O.****, PEROTTO S.**, BALCONI C.***, LUPOTTO E.*

*) CRA – Rice Research Unit, S.S. Torino, km 2,5, 13100 Vercelli, Italy
**) Plant Department – University of Torino and IPP-CNR, Viale Mattioli 25, 10125 Torino, Italy
***) CRA – Maize Research Unit, Via Stezzano 24, 24126 Bergamo, Italy
****) CIRAD-UMR BGP1, 34398 Montpellier Cedex 5, France

RIP (Ribosome Inactivating Protein), transgenic rice, pathogenic fungi, arbuscular mycorrhizal (AM) fungi

The maize gene b-32, normally expressed in the maize (Zea mays L.) endosperm, encodes for a RIP (Ribosome Inactivating Protein) exhibiting antifungal activity. Transgenic rice plants, in which the b-32 gene driven by the Ubi-1 promoter was inserted in association with the bar gene as a selectable marker, were obtained via biolistic transformation. A set of b-32 expressing homozygous progenies and the non-transgenic parental cv. Selenio as a negative control, were raised to maturity into a containment greenhouse. All transgenic plants exhibited a normal phenotype and were fully fertile and set seeds. Three homozygous b-32 rice progenies (SE7.1, SE7.2 and SE7.15) were characterized for the level of expression of the b-32 protein in various plant tissues, and for their interaction with pathogenic and symbiotic fungi. The different level of b-32 expression exhibited by the three transgenic lines allowed to set up pathogenicity experiments, in order to evaluate the level of response to Fusarium attack in leaf tissue colonization bioassays. The results obtained showed that the three transgenic progenies tested were more resistant than the cv. Selenio to Fusarium verticillioides, when leaves were inoculated with $10^6$ spores/ml and evaluated 2-4 days after inoculation. The same progenies were tested with Magnaporthe oryzae, a major rice fungal pathogen in the European area. Seedling tests, conducted on seedlings at the three leaf stage, did not confirm difference in response between the transgenic and non-transgenic rice plants. Antifungal proteins expressed in genetically modified organisms (AMPs) have the potential to affect non-target organisms. From the perspective of environmental risk-assessment, the effect of each new integrated gene on soil-borne microbiota must be evaluated. The effects of these AMPs on mycorrhizal fungi are accepted as good indicators of their effect on soil microbiota in general. Therefore, the arbuscular fungal (AM) species Glomus intraradices was tested for its ability to form mycorrhizal associations in the three transgenic rice lines and the control wild-type cv. Selenio. The experiments of colonization were performed by means of the sandwich system. Roots of young seedlings were carefully spread on a cellulose nitrate disc. Clamps of mycelium with spores were placed in direct contact with the roots; roots were subsequently covered with a second membrane, forming a “sandwich”. The inoculated seedlings were planted in sand in plastic pots, grown at 25°C and 16-hr photoperiod and supplied with water or Long Ashton solution. Five weeks after inoculation, the roots were stained with Cotton Blue and the presence of AM fungal infection was detected under light microscope. Results obtained showed that the mean percentage of
colonization by *G. intraradices* in the b-32 expressing lines was comparable with those observed in the wild type non-transgenic variety, showing a non-significant effect of the transgene on the mycorrhizal association. The results of the study are discussed in view of the effect of transgenic plants expressing antifungal genes on the rhizosphere microbiota population.

The work was developed within the framework of the EU-funded project EURICE (Ue QLK5-CT1999-1484) and present studies within the national project VALORYZA (MIPAAF DM 301/7303/06).
MOLECULAR CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI COMMUNITY IN A TYPICAL PIEDMONT GRAPEVINE CULTIVAR

MAGURNO F., BUGHI-PERUGLIA G., LUMINI E., BALESTRINI R., BIANCIOTTO V.

Istituto Protezione Piane - CNR and Dipartimento di Biologia Vegetale, Torino, Italy

arbuscular mycorrhizal fungi, Glomeromycota, grapevine, Vitis spp, molecular markers

The arbuscular mycorrhizal fungi (AMF) represent some of the most abundant organisms on this planet, where they form symbiotic associations with most land plants.

These organisms are considered crucial players in the interactions plant-environment in eco-agrosystems because of their ability to establish mycelial networks between roots and soil particles, roots on the same plant and roots of different plants. Benefits for plant in AMF symbiosis are well showed as an improvement in shoot/root growth, mineral transport, water-stress tolerance and resistance to certain diseases. In relation to this biofertilizer role, AMF species may differ in their effects on plant growth. So that, particular consortia of AMF species could result more adapted than others establishing an efficient symbiosis with a plant species. Therefore, with a view of an agricultural application, may be useful to investigate the most functional associations ‘plant-AMF community’, in order to take better advantage of AMF benefits.

One of the most relevant agro-food sectors for the Piedmont Region (Italy) is certainly represented by viticulture and wine production. Grapevines (Vitis spp.) roots are often heavily colonized by AMF under field conditions and in some cases AMF appear to be necessary for their normal growth and survival. Even so, little information, mainly related to morphological characterization, are available about composition of AMF communities living in the vineyards soil and in associations with grapevine roots.

Vineyard of Nebbiolo, one of the most important Piedmont cultivar, was selected in order to study the AMF community using a molecular approach. Sampling was performed in an experimental vineyard, located in Lessona (Biella, Piedmont, Italy), well characterized in relation to the chemical-physical profile of soil.

Soil samples and roots were analyzed using AM fungal-specific primers to partially amplify the small subunit (SSU) of the ribosomal DNA genes.

More than 700 clones were sequenced. Phylogenetic analyses highlighted an high rate of species richness, compared with similar studies already published on others plant cultures.

Moreover a particular phylogenetic group was the most represented in both compartments (soil and roots), suggesting a correlation between intra and extra radical communities.

These data, compared to others from an analogous study on a Nebbiolo vineyard in a different location, will be challenging to develop well adapted AMF inocula as additional tools to achieve good quality standard in the grapevine/wine chain.
NITRIC OXIDE ACCUMULATION DURING THE EARLY STAGES OF ARBUSCULAR MYCORRHIZAL SYMBIOSIS

CALCAGNO C., LANFRANCO L., NOVERO M., GENRE A., BONFANTE P.

Department of Plant Biology, University of Turin, Viale Mattioli 25, 10125 Turin, Italy

Nitric oxide (NO) is an important signalling molecule in plant systems, and it is involved in developmental processes and in response to several abiotic and biotic stresses. Evidence for the involvement of NO during symbiotic interactions has been recently found in nodules. Since rhizobial symbioses and arbuscular mycorrhizas (AM), share common features in their signalling pathways the aim of this work is to verify the involvement of NO in early plant responses to arbuscular mycorrhizal fungi. The accumulation of NO in root tissues was visualized by confocal laser microscopy using the cell-permeable NO-specific probe 4,5-diaminofluorescein diacetate (DAF-2DA).

Experiments were performed on transformed roots of *Medicago truncatula* treated with fungal exudates of *Gigaspora margarita* germinated spores. Wild type root fragments showed an increase of fluorescence during the first ten minutes following the application of the fungal exudate. The non mycorrhizal mutants *dmi1* and *dmi2* did not respond while a weak increment was recorded for *dmi3*. *M. truncatula* roots treated with Nod factor and the non host plant *Arabidopsis thaliana* treated with fungal exudates showed no increase in fluorescence. These data suggest that NO accumulation occurs down-stream DMI1 and DMI2 functions but up-stream DMI3. In conclusion, genetic and cellular evidences suggest that NO accumulation is a novel component in the signalling pathway leading to AM symbiosis.
CHARACTERIZATION OF WRKY CO-REGULATORY NETWORKS IN RICE AND ARABIDOPSIS


*) Rice Genomics Unit, Parco Tecnologico Padano, Lodi, Italy  
**) School of Computing, University of Leeds, Leeds, United Kingdom  
***) Embrapa Recursos Genéticos e Biotecnologia, Brasília DF, Brazil  
****) Department of Biomolecular Sciences and Biotechnology, University of Milan, Milan, Italy  
*****) Department of Molecular Genetics, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan  
******) Department of Biology, University of Milan, Milan, Italy

transcriptome analysis, phylogeny, co-regulatory pathways, transcription factors, rice

The WRKY transcription factor gene family has a very ancient origin in the plant kingdom and several studies have pointed out their involvement in a number of biological processes. To investigate the existence of WRKY co-regulatory networks in plants, an in depth transcriptome analysis of the whole WRKYs gene family upon biotic and abiotic stress conditions was carried out in rice (Oryza sativa) by integrating data from a custom 60-mer oligo array and from the 22K NIAS array. We defined the existence of nine clusters of OsWRKY genes tightly co-expressed. These clusters were found to contain phylogenetically unrelated genes, suggesting that specific sets of WRKY genes might act in co-regulatory networks. This hypothesis was tested in Arabidopsis thaliana by Pearson correlation coefficient analysis (PCC) of the Arabidopsis WRKY gene family, taking advantage of an extensive repository of Affymetrix expression data. PCC analysis and threshold value were validated using expression data of the AtMADS-BOX gene family, which is experimentally well characterized at the molecular and genetic levels. PCC (untransformed and log transformed) results revealed that more than 70% of the Arabidopsis WRKY genes analysed are co-regulated with other WRKYs. By PCC analysis, we identified two main co-regulatory networks (COR-A, COR-B) and two smaller ones (COR-C and COR-D) all including genes belonging to distinct phylogenetic groups. The COR-A network contained several AtWRKY genes known to be involved mostly in response to pathogens, whose physical and/or genetic interaction was experimentally proven. We also identified putative orthologs of a set of co-expressed WRKY genes in rice that are functionally likely to cooperate in the same signal transduction pathways. We propose that, making use of data from co-regulatory networks, it is possible to highlight novel clusters of plant genes contributing to the same biological processes or signal trasduction pathways. However, this is not currently feasible for rice, due to the paucity of rice transcriptome resources suitable to carry out PCC analysis. In addition, often it is difficult to establish orthology between rice and Arabidopsis WRKY genes. Therefore we decided to carry out extensive transcriptome analyses upon biotic and abiotic stress conditions, using the Affymetrix technology. This systematic analysis will enable us to develop working hypotheses on co-regulatory signal transduction pathways of rice WRKY genes to be experimentally tested.
EURIGEN: CHARACTERIZATION OF EUROPEAN RICE GERMPLASM FOR STRESS RESPONSE TRAITS


*) Rice Genomics Group, Parco Tecnologico Padano, Lodi, Italy
**) CRA-Unità di Ricerca per la Risicoltura (CRA-RIS), Vercelli, Italy
****) National Institute of Agriculture Botany (NIAB), Cambridge, United Kingdom
******) National Agricultural Research Foundation (NAGREF), Thermi-Thessaloniki, Greece

rice, genetic resources, biodiversity, SNP, stress response

The general objective of the EU-funded EURIGEN project is the characterisation and exploitation of European rice genetic resources of the temperate rice growing area, to enhance competitiveness of Europe in rice production, and alleviation of biotic and abiotic constraints typical of the Mediterranean area. This goal is achievable by means of the acquisition, evaluation and conservation of existing rice accessions, and identification of new genetic materials targeted at sustainable agricultural systems, making use of the most updated genomic tools. The project has two major targets: i) identification and conservation of genetic resources and ii) identification of valuable sources of new genes and alleles for agronomic and quality traits relevant to breeding programs.

The main platform of the project is the classification, maintenance and regeneration of the temperate rice germplasm bank. A panel of 455 rice accessions relevant for the breeding programs in European growing areas were analysed at both phenotypic and genotypic level. A centralised seed bank of the Eurigen collection was established and a DNA biorepository organised in bar-coded 96-well plates was created and made available to the Eurigen partners. A core collection of 200 rice genotypes was selected based upon phylogenetic analyses and phenotyped in field and controlled conditions for blast resistance, adaptation to reduced water availability and salinity tolerance. To identify favourable alleles as well as molecular markers correlating with improved performance under stress conditions, the 200 rice accessions were molecularly characterized by high-throughput SNP genotyping using the ILLUMINA BeadExpress platform. A panel of 384 SNPs was selected in candidate genes involved in stress responses based on literature data and preliminary results from ongoing projects at international level.

The integration of phenotypic and genotypic data will enable us to carry out association analyses to exploit the existing natural variation to devise novel strategies of rice improvement in EU countries.

The EURIGEN actions pursue the general objectives in accordance with the assessments of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture, and of the Council Regulation (EC) N° 870/2004 establishing a Community Programme on the conservation, characterization, collection and utilization of genetic resources in agriculture.
We acknowledge the financial support of the European Commission – DG Agriculture and Rural Development – AGRI GENRES Program to the 049 EURIGEN project.
ISOLATION OF POLYPHENOL OXIDASE GENE AND ITS EXPRESSION ANALYSIS UPON WOUNDING STRESS

QUARTA A., DURANTE M., MITA M., CARETTO S., DE PAOLIS A.

Institute of Sciences of Food Production, C.N.R., Unit of Lecce, Italy

Artichoke heads are rich in phenolic compounds which have many healthful properties. Particularly these compounds are known to possess antibacterial, antioxidant, anti-HIV, bile expelling, hepatoprotective, urinative, and choleric activities as well as to inhibit cholesterol biosynthesis and LDL oxidation. On the other side, phenolic compounds are responsible for the phenomenon of browning that reduces product quality in post-harvested-handling. There are different causes of browning in stored and/or processed vegetables, but the enzymatic browning due to the polyphenol oxidase enzyme has been reported to be the most important factor. Since browning affects the nutritional quality and appearance of vegetables it causes a significant economical impact, both to primary food producers and food processing industry.

Polyphenol oxidase (PPO; EC 1.10.3.1 also known as phenolase, phenol oxidase, catechol oxidase and tyrosinase) is a copper enzyme, widely distributed in nature, which catalyzes the formation of quinones from phenols in the presence of molecular oxygen. Polyphenol oxidase shows two different enzymatic activities: 1) the hydroxylation of monophenols to o-diphenols (monophenolase activity) and 2) the oxidation of o-diphenols to reactive o-quinones (diphenolase activity) which then polymerise to form brown, red or black pigments.

In order to isolate and characterize the ppo gene from artichoke, degenerate primers designed on conserved regions of ppo sequences of other plant species, were used to amplify a core fragment from leaf genomic DNA. The fragment obtained showed (BlastX) high amino acid sequence similarity with other plant ppo enzymes but no significant nucleotide sequence similarity. A complete coding sequence was obtained by using the 3’ and 5’ RACE-PCR technology. Preliminary data of Real-time expression on cDNA extracted from wounded artichoke heads, indicated an induction 24h after wound stress.
CHARACTERIZATION OF AN ARABIDOPSIS ABC1-LIKE GENE

MANARA A., DAL CORSO G., FURINI A.

Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

ABC1-like gene

The Abc1 (activity of bc1 complex) protein family originates from the *Saccharomyces cerevisiae* ABC1 gene which is required for the correct function of the bc1 complex of the mitochondrial respiratory chain [Bousquet et al., 1991]. In yeast Abc1 acts as a chaperone-like protein that is essential for the proper conformation and efficient functioning of the cytochrome b complex III. The Abc1 family has also been identified as a family of protein kinases. By functional complementation of the yeast *abc1* mutant an homolog of the yeast ABC1 have been isolated in *Arabidopsis thaliana* and was predicted to be localized in the mitochondria [Cardazzo et al., 1998]. Also in humans an homolog of the Abc1 proteins (CABC1) has been identified [Iiizumi et al., 2002].

Our work is focusing on the characterization of the putative *A. thaliana* Abc1-like gene homologous of a gene of *Brassica juncea* that has been found, by cDNA-AFLP technique, to be modulated following Cd-treatment.

*AtAbc-1 like* gene has an open reading frame coding for 695 amino acids and analysis of protein sequence revealed the presence of the conserved Abc1 region indicating that this gene belongs to the Abc1 protein family. The aminoacid sequence contains also a putative kinase domain and two transmembrane regions.

In wild-type *A. thaliana* plants the *Abc1-like* gene has been found to be induced after cadmium, treatment; homozygous *AtAbc1-like* mutant plants displayed no alteration of shoot phenotype, whereas roots elongation was observed in the presence of hydrogen peroxide. To date knowledge about the cellular localization of the gene protein product is limited.

The Arabidopsis genome contains 17 putative *Abc1-like* genes. The closest homolog to *AtAbc1- like* (45% amino acid identity) is AtOSA1, an oxidative stress-related protein. AtOSA1 is involved in plant response to oxidative stress generated by Cd$^{2+}$, hydrogen peroxide and light [Jasinski et al., 2008]. Up to now Abc1-like proteins have been identified in the mitochondria of eukaryotes, AtOSA1 is the first member of this family to be localized in the chloroplasts.

To analyse the subcellular localization, the AtAbc1-like protein was fused with the FLAG peptide DYKDDDDK at the C-terminus. Homozigous mutant *AtAbc1-like* plants were transformed with this construct, and protein localization will be determined immunologically.

Furthermore, the potential functional redundancy between AtOSA1 and AtAbc1-like will be analysed in the double mutant *AtOSA1xAtAbc1-like*.

TRANSCRIPT PROFILES IN ROOTS AND LEAVES OF THE MYCORRHIZAL-COLONIZED CLONE OF POPLAR AL35 GROWN ON HEAVY METAL POLLUTED SOIL

PALLARA G., RACCHI M.L.

Dipartimento di Biotecnologie Agrarie, sezione Genetica, Università di Firenze, Via Maragliano 77, 50144 Firenze

arbuscular mycorrhiza, Glomus mosseae, gene expression, heavy metal tolerance, poplar

The transcription profiles of genes coding for proteins involved in antioxidant defence and in heavy metal chelation and tolerance were investigated in mycorrhizal-colonized or not poplar plants of a tolerant clone (P. alba AL35) grown under heavy metal stress.

The aim of the work was to investigate the effect of mycorrhization on transcription of genes coding for proteins potentially relevant for heavy metal tolerance in a poplar clone previously selected on the basis of the capability to growth and accumulate metals and thus suitable for phytoremediation.

Transcription levels of the principal antioxidant defence genes and genes implicated in heavy metal chelation and transport were analyzed in roots and leaves of the clone AL35 plants grown on polluted or unpolluted soil and colonized or not with the arbuscular mycorrhiza Glomus mosseae. Analysis were performed using cDNA macroarray and Real-Time qPCR for validation.

The cDNA macroarray analysis revealed that heavy metals and mycorrhization significantly affected gene transcription and different transcriptions profiles in roots and leaves of plants grown in the different conditions were observed. Real-Time qPCR experiments performed largely corroborated cDNA macroarray data.

In polluted soil, transcript abundance of genes and in particular those involved in metal chelation and transport, increased in roots of mycorrhizal plants when compared with non-mycorrhizal ones. Conversely, in leaves mycorrhization resulted in a general down-regulation of the genes considered.

On the whole, the data so far obtained on transcription profiles of mycorrhizal-colonized AL35 plants grown into polluted soil, suggest that the arbuscular fungus Glomus mosseae seem contribute to tolerance of the poplar clone enhancing the mechanisms involved in chelation and in transport of heavy metals towards the vacuole and alleviating oxidative stress. Further analysis are in progress to assess this hypothesis.
MOLECULAR RESPONSE TO DIFFERENT SOILS AND ROOTSTOCKS IN GRAPEVINES

MARÉ C.*, APRILE A.**, TOCCI E.***, CORINO L.****

*) CRA - GPG - Genomic Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy
**) Department of Environmental and Biological Technologies and Sciences, University of Salento, Prov.le Monteroni, 73100 Lecce, Italy
***) CRA - Centro di Ricerca per l’Enologia, Via Pietro Micca 35, 14100 Asti, Italy
****) CRA - Unità di Ricerca per l’uva da tavola e la Vitivinicoltura Via Casamassima 148, 70010 Turi (BA), Italy

Vitis vinifera, Affymetrix oligonucleotide array, soils, rootstocks

Grapevine is an ancient culture that constitutes one of the most economically important fruit species worldwide. Soil conditions and rootstocks are two of the main causes that can influence quality and wine production. In this work the transcriptome variation to different soils and two different rootstocks in *Vitis vinifera* was investigated through Affymetrix GeneChip® microarray technology. The grapes cv. Pinot noir were grown in greenhouse with three different soils: sand, turf and vineyard soil from Asti. The plants were grafted with two different rootstock: 101/14, a weak rootstock and 1103 Paulsen, a vigorous one that enhances the growing rate, than the leaves samples were collected during two subsequent years. The results from the large-scale analysis of mRNA expression profiles in vegetative tissues of grapevine show that a mRNA abundance changes in response to different grafting and soil conditions is active. A greater number of transcripts are mainly involved in physiological and molecular processes as primary metabolism, secondary including phenylpropanoid, flavonoid and lignan biosynthesis, energy, cellular transport and communication as signal transduction mechanisms and biogenesis of cellular components. The transcript profiling pointed out genes and metabolic pathways regulated by soils and rootstocks conditions in specific manner in two different years. The object of this work is to identify the genes and the molecular bases that explain the soils and rootstocks role in grapevine development.
Glutathione S-transferases (GSTs) represent a multifunctional family of enzymes grouped into four main classes (tau, phi, theta, and zeta) conjugating endobiotic and xenobiotic compounds to glutathione. In plants, this is considered to be a crucial step in the detoxification process as the S-glutathionylated metabolites are tagged for vacuolar sequestration. In this work, we have isolated two glutathione S-transferases belonging to the tau class GSTs from sweet orange leaves. The cDNA clones contained a complete open reading frame of 651 bp encoding two 216 amino acid proteins. Homology search and sequence alignment showed that the deduced amino acid sequences shared high identity with GSTs from other plant sources, including several strictly conservative motifs and distinctive amino acid residues specific of the tau class GSTs. The genomic clones of both isoforms were also isolated and the analysis of the gene organization confirmed the membership of both enzymes to the tau class GSTs. The encoded proteins differ only for three amino acids: the triplet R89, E117 and I172 found in the isoform named GSTU1 is replaced by the triplet P89, K117 and V172 in the GSTU2 isoform. The successful in vitro expression of the proteins led to the functional active form of both enzymes which showed different specific activity against CDNB as substrate, the GSTU1 showing values three fold lower than that observed for the GSTU2 enzyme. The analysis of the gene expressions suggested that the GST isoforms show either different distribution between leaf and flesh, the isoforms being decidedly expressed in the leaf, or cultivar related specificity, the U2 being highly expressed in the leaves of red orange whereas the U1 in the blond orange leaves. Furthermore, we also showed that the expression of U1 gene was remarkably induced in response to cadmium sulphate, CDNB and cyhalothrin treatments as well as to cold stress. On the contrary, the U2 isoform was constitutively expressed probably playing some sort of “default scavenging” activity in vivo. Taken together these results suggested that GSTU1 is a stress responsive gene and can be considered as potential target that is genetically modified so as create novel germoplasm with enhanced stress tolerance.
ECTOPIC EXPRESSION OF THE UVR8 GENE MIMICS STRESS-INDUCED CHANGES IN PLANT ARCHITECTURE IN A. THALIANA

FASANO R.*, GRILLO S.**, LEONE A.*

*) Department of Pharmaceutical Sciences, University of Salerno
**) Institute of Plant Genetics, IGV, National Research Council

plant stress, uvr8, A. thaliana

Plants undergo architectural and developmental changes as a common response to many distinct sub-lethal environmental stresses and a general acclimation strategy. Key elements in these stress-induced morphogenic responses appear to be endogenous molecules like flavonoids, auxin, ethylene and ROS that affect cell division, cell elongation and/or cell differentiation. Although this response has been widely reported, the underlying molecular mechanisms are poorly understood. Starting from the finding that the uvr8 gene was able to restore the growth of the yeast osmo-sensitive mpk1/ppz1- mutant, we found that uvr8 gene expression is also up-regulated by osmotic stress (PEG-mediated water deficit and NaCl). In absence of stress, ectopic uvr8 overexpression in Arabidopsis plants causes a reduced vegetative growth, early flowering, reduced primary root and leaf expansion, reduction of silique and seed number per plants, mimicking constitutively stress-induced changes in plant architecture. Interestingly, down-regulation of the protein triggered diametrically opposite morphological effects, suggesting that somehow the uvr8 gene coordinates growth reprogram and redistribution in stressed plants, to enable them to acclimate to sub-optimal external growth conditions. The opposite phenotypes of uvr8 sense and antisense plants were also associated to modification of expression of cell cycle genes and other genes controlling plant morphogenesis as well as to a different content of flavonoids, causing perturbation of auxin transport. Sense and antisense uvr8 plants respond also differentially to abrupt osmotic stress. Altogether these data support a novel role of the uvr8 gene in the plant stress-induced morphogenic response, beside that previously reported in the UV-B response.
STUDY OF MECHANISMS INVOLVED IN SALT TOLERANCE BY cDNA-AFLP METHOD IN DURUM WHEAT GERMPLASM

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

*) Università della Tuscia, Via S. C. de Lellis, 01100 Viterbo, Italy - pagnotta@unitus.it
**) ICARDA p.o. box 5466 Aleppo, Syria

salt tolerance, durum wheat, cDNA-AFLP

Salinity stress is one of main factors that are able to influence cereal yield throughout the world. Mechanisms involved in tolerance to salinity stress consist of three main components: Na+ exclusion, tolerance to Na+ in the tissues and osmotic tolerance. Plants, in the time, have developed a complex and elaborate signaling network that guarantees their adaptation to salinity; therefore, the comprehension of these mechanisms is of fundamental importance. cDNA-AFLP represents an easy and powerful method to identify sequences differently expressed under stress conditions. In present work it has been used durum wheat varieties with different degree of tolerance. The varieties have been grown under different salt stress conditions and the cDNA–AFLP technology has been employed to detect and analyse the differently expressed bands. Seeds of Cham I, Jennah Khetifa, Belikh 2 and Trinakria, have been germinated in hydroponic solution containing CaSO4 10 mM; after germination, plants have been grown for seven days in a nutritive solution containing micro- and macro-elements and Fe++. There after, NaCl at different concentrations, 0 M (as a negative control), 0.75 M and 1.5 M, has been added to the nutritive solutions. Some morphological traits such as root and leaf turgor, length and weight, have been recorded after one hour and after 5 days since the supply of salt solutions. In the same way, RNA has been extracted from roots and leaves material and converted in cDNA through reverse transcription. The resulting cDNA has been amplified with different AFLP primers combinations and loaded on a high resolution acrylamide gel. The bands present in the tolerant lines, but absent in the susceptible one have been isolated, cloned, and sequenced. The obtained sequences have been finally blasted. Some of them show homology with sequences involved in ABA, osmotin, selenium-binding protein-like, CTP synthase-like protein, etc. Present work confirms the existence of salt tolerance in durum wheat germplasm, highlighting the importance of these genetic resources to isolate the genes involved and their use in breeding activities aiming to face the climatic global changes which see an increasing of salty soils all around the world.
CHLOROPASTIC YCF2 GENE EXPRESSION IN STRESSED PLANTS

DURANTE M., PIERETTI M., BERNARDI R.

Department of Crop Biology, Genetics section, University of Pisa, Via Matteotti 1/B, 56124 Pisa, Italy

*chloroplast ycf2* gene, *plant disease*, *stress tolerance*

The chloroplast *ycf2* gene plays a vital unknown function in the higher plants: gene silencing or reduction in mRNA synthesis induce cell death (Drescher *et al*., 2000).

Using the SSH technique, Bernardi *et al*. (2008) isolated a cDNA sequence in cold tolerant plants of *Olea europaea* L. showing a very high expression of the *ycf2* gene induced by cold treatment.

The olive gene was isolated and sequenced. It presents a 6385 nucleotide length and the sequence is highly conserved in the plants, also in the rudimentary plastid genome of the non-photosynthetic parasitic *Epifagus virginiana* (Wolfe *et al*., 1992), thus confirming an essential role for the plant survival. The hypothetical protein sequence is constituted of 2278 amino acids presenting an ATPase domain and a DUF825 domain with unknown function.

We have focused our work on the fruit development of *O. europaea* in order to verify if *ycf2* may have a specialized function in non-photosynthetic tissues during seed maturation, as reported for tomato by Richards *et al*. (1994). Semi-quantitative RT-PCR using mRNA extracted at different maturation stages indicated that mRNA is present in fruits of all stages, although the higher concentration was found in leaves.

Analysis of *ycf2* transcripts in poplar plants treated with ozone and in plane infected with *Ceratocystis fimbriata*, agent of the colored canker, confirmed a differential expression of the gene after the stressing treatments.

All the results point to a defense activation of *ycf2* both in biotic and abiotic stresses.
MYBLEU OVEREXPRESSION IN CITRANGE CARRIZO IMPROVES TOLERANCE TO HYPOXIC STRESS


*) C.R.A.-ACM Centro di ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale, Italy
**) Istituto di Biologia e Biotecnologia Agraria, C.N.R., Via E. Bassini 15, 20133 Milano, Italy

Mybleu, plant transformation, Carrizo citrange, flooding tolerance

Classical breeding and genetic transformation have been used to improve deficit oxygen tolerance in different crops. However, by classical breeding successes have been achieved only in rice (*Oryza sativa*). The Italian citrus cultivation is, in some areas, performed on soils characterized by a content of clay and/or loam in excess compared to optimal levels, determining the inability to eliminate water excesses during years of heavy and prolonged rainfall. In this kind of soils, humidity retention, flooding and/or waterlogging, represent a serious environmental stress that can lead to anaerobic conditions affecting many plant processes. Anaerobiosis may cause premature senescence which results in leaf chlorosis, necrosis, defoliation, cessation of growth and in extreme cases even plant death.

A large number of Myb transcription factors are involved in plant responses to abiotic and biotic stresses. The Mybleu ectopic expression in *Arabidopsis thaliana* plants positively affects cellular metabolism and increases tolerance to oxygen deficit. This Myb transcription factor, isolated from rice (a monocot, herbaceous), is able to activate the anaerobic response pathways in *Arabidopsis* (a dicot, herbaceous). In order to clarify the mechanisms involved in the response to oxygen deficit and to improve tolerance to flooding in citrus species, we overexpressed Mybleu in Carrizo citrange, one of the most popular citrus rootstocks, by *Agrobacterium tumefaciens* mediated transformation.

Analysis by polymerase chain reaction (PCR) and sequencing showed that stable integration of the transgene in the plant genome was obtained in the 71.6% of the analysed plants. Real time PCR performed on a random selection of transgenic plants lines confirmed the expression of the inserted transgene and allowed a classification of the transgenic plants based on the different expression levels of the transgene. Wild type and transgenic lines (both low and high expressing lines) were tested for their tolerance to hypoxia/anoxia by physiological, biochemical and molecular analyses. The results indicated that high expressing plants are more tolerant to hypoxia than wild type and low expressing plants.

Our findings on Carrizo citrange confirm previous results of improved tolerance in *Arabidopsis* by Mybleu supporting the hypothesis of a conservation of the regulatory pathways involved in the anaerobic response among species. Therefore, our results indicate that the overexpression of Mybleu may represent a powerful tool to improve hypoxic stress tolerance in crops.
FLOWERING ECOPHYSIOLOGY OF HEMP (CANNABIS SATIVA L.) AS A FIRST STEP TOWARDS SPECIES BREEDING

FAUX A.-M., RAULIER P., BERTIN P.

Research Laboratory in Crop Physiology and Plant Breeding, Faculty of Bioengineering, Agronomy and Environment, Université catholique de Louvain, Place Croix du Sud, 2-11, 1348 Louvain-la-Neuve, Belgium

crop breeding, flowering model, hemp, monoecy, photoperiodic response

Hemp (Cannabis sativa L.) is a naturally dioecious species with flowering responding to daylength variations. The acquisition of monoecious varieties remains an eternal renewal because of their instability: firstly, the late apparition of sexual dimorphism prevents the total eradication of dioecious male plants during the selection process, and, secondly, the monoecious state presents a continuous distribution between the male and female extreme phenotypes. Hemp is also known to be a quantitative short-day plant. Maximum stem yield seems to occur shortly after flowering. Therefore, photoperiodic conditions have a key influence on the determination of yield potential of the crop. In the context of renewed interest for alternative fibre crops, understanding the flowering system of hemp could offer valuable information to support species breeding and cultivation. The present study aims to describe the floral development, growth and biomass production of hemp as well as the expression of the sexual phenotype in response to photoperiod and temperature conditions.

Trials were settled in the field and in controlled conditions. Five monoecious varieties of different precocities were cultivated in 2007 and 2008 during five culture periods in two sites with different edapho-climatic conditions in Belgium. The first culture period ran from Mid-April to end of September and the last one from end of June to Mid-November. The same varieties were cultivated from January to June 2008 in growth chambers under five controlled photoperiods from 10 to 18 hours.

In the field, mean thermal times to reach a particular development stage were congruent between both years. Growth rhythm and biomass production appeared to be correlated with floral development rhythm. Lower floral development and growth rates, and the consequent higher biomass production, were observed for the first culture periods and could be explained by the longer daylength conditions experimented by these treatments. Three phases of growth were determined: an accelerating phase before 400-500°Cd, a linear phase between 400-500 and 1200-1500°Cd and a saturating phase after 1200-1500°Cd (base 1°C). The effect of temperature on growth, pointed out in 2007 during the linear phase of growth, was less obvious in 2008. Temperature and length of cultivation cycle seemed to influence positively the production of biomass. The highest yields were observed when sowing and harvesting were achieved in Mid-April and end of September, respectively.

In controlled conditions, sex ratio and evolution of flowering stages were observed at each node separately. All varieties presented a typical floral response of short-day plants, with flowering occurring faster at daylengths shorter than 14 hours. The analysis of the distribution of the sexual
phenotypes allowed the identification of times intervals and nodes ranges which could be used for future description of the sexual phenotype of hemp plants.

Finally, the observations and results of this first ecophysiological approach should lead us to propose a flowering model for hemp and provide us precious information which may be introduced in a second step, the approach of sex determinism by the QTL method.
EVALUATION OF GENE EXPRESSION AND PHOTOSYNTHESIS ACTIVITY IN DIFFERENT FAGUS SYLVATICA GENOTYPES UNDER HIGH CO2 LEVEL


*) Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
**) Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Forest Genetics, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany
***) Department of Environmental and Forestry Technologies and Sciences, University of Florence, Via San Bonaventura 13, 50145 Florence, Italy
****) Freie Universitaet Berlin, Institut fuer Biologie, Altensteinstr. 6, D-14195 Berlin, Germany
******) Dipartimento di Biologia Evoluzionista “Leo Pardi”, University of Florence, Via Romana 17, 50125 Florence, Italy
*******) Institute of Plant Genetics – CNR, Research Division Florence, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy

CO2, microarray, European Beech, photosynthesis

The problems related to global changes, mainly caused by human activities, are the origin of much concern for the health of the environment. Oil and carbon combustion, the use of chlorofluorocarbons, and deforestation are some of the principal factors responsible for CO2 production and for air temperature increase. The scenario is factors leading to global changes affecting precipitation patterns, nitrogen concentration in the atmosphere, UV-B radiation increase and temperature increase. Forest trees constitute a relevant economic and ecological resource that is under severe treat by environmental changes.

The principal aim is to investigate the response to CO2 from three different genotypes of Fagus sylvatica by gene expression and ecophysiological analyses. Shoots of F. sylvatica (Montieri (GR), Italy) and F. sylvatica “purpurea tree” were grafted on F. sylvatica rootstocks. Plants were grown under controlled conditions in a climate chamber using the same temperature and light parameters, while CO2 concentrations were approx. 380-400 ppm (ambient) in the control room and 1000 ppm (high) in the experimental room.

A PAM fluorescence system (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) with a 6 mm diameter standard fibre optic was used for the measurements of the in vivo photosynthesis. Under ambient conditions photosynthesis (expressed as electron transport rate) was higher in the Italian compared to the German genotype. After 4 d under high CO2 treatment, electron transport rate showed increased values compared to the plants growing under ambient CO2. Photosynthesis of plants (Italy) adapted to high CO2 decreased immediately after been exposed for 2 h to ambient CO2. No down-regulation of photosynthesis could be observed in leaves at high CO2 level. Microarrays analyses have already been done and preliminary results will be discussed.
DIFFERENT RESPONSE TO THE UV-STRESS AMONG *POPULUS ALBA* L. CLONES


*) Department of Environmental and Forestry Technologies and Sciences, University of Florence, Via San Bonaventura 13, 50145 Florence, Italy
**) Institute of Plant Genetics – CNR, Research Division Florence, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

*UV-B stress, poplar, ecophysiology, gene expression*

To evaluate a possible differential behaviour of 5 white poplar genotypes towards UVB radiation, we studied the association between polymorphism data (SNPs) and expression level on genes (such as *chs*, *comt*, and *rbcL*) putatively related to UVB response. Molecular data were integrated with ecophysiological observation. From the ecophysiological data obtained we observed, in stressed plants, a decrease of the maximum efficiency of the PSII, in particular for 3 clones increasing the hours of UVB treatment. Furthermore an abrupt decrease of the values is already present from the second day of stress. The analysis using qRT PCR for the genes studied, for the leaf tissue of the 5 clones treated for 3, 6 and 12 hours with UVB, showed a different pattern of expression, with a dissimilar response among the clones analyzed towards the stress. Both the ecophysiological and the expression data showed a different response particularly for 3 clones that resulted more sensitive to the treatment than the others. The analysis of the polymorphism of the sequences has instead not allowed the construction of associations with the ecophysiological and expression data for absence of missense mutations.
MOLECULAR RESPONSE TO DIFFERENT SOILS AND ROOTSTOCKS IN GRAPEVINES

MARÉ C.*, APRILE A.**, TOCCI E.***, CORINO L.****

*) CRA - GPG - Genomic Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy
**) Department of Environmental and Biological Technologies and Sciences, University of Salento, Prov.le Monteroni, 73100 Lecce, Italy
***) CRA - Centro di Ricerca per l’Enologia, Via Pietro Micca 35, 14100 Asti, Italy
****) CRA - Unità di Ricerca per l’uva da tavola e la Vitivinicoltura Via Casamassima 148, 70010 Turi (BA), Italy

Vitis vinifera, Affymetrix oligonucleotide array, soils, rootstocks

Grapevine is an ancient culture that constitutes one of the most economically important fruit species worldwide. Soil conditions and rootstocks are two of the main causes that can influence quality and wine production. In this work the transcriptome variation to different soils and two different rootstocks in Vitis vinifera was investigated through Affymetrix GeneChip® microarray technology. The grapes cv. Pinot noir were grown in greenhouse with three different soils: sand, turf and vineyard soil from Asti. The plants were grafted with two different rootstock: 101/14, a weak rootstock and 1103 Paulsen, a vigorous one that enhances the growing rate, than the leaves samples were collected during two subsequent years. The results from the large-scale analysis of mRNA expression profiles in vegetative tissues of grapevine show that a mRNA abundance changes in response to different grafting and soil conditions is active. A greater number of transcripts are mainly involved in physiological and molecular processes as primary metabolism, secondary including phenylpropanoid, flavonoid and lignan biosynthesis, energy, cellular transport and communication as signal transduction mechanisms and biogenesis of cellular components. The transcript profiling pointed out genes and metabolic pathways regulated by soils and rootstocks conditions in specific manner in two different years. The object of this work is to identify the genes and the molecular bases that explain the soils and rootstocks role in grapevine development.
MOLECULAR CLONING AND EXPRESSION ANALYSIS IN RESPONSE TO ABIOTIC STRESSES OF TWO TAU-TYPE GSTs FROM ORANGE LEAVES

LO PIERO A. R., MERCURIO V., PUGLISI I., PETRONE G.

Department of Agronomic, Agrochemical and Animal Production Sciences, University of Catania, Via S. Sofia 98, 95123 Catania, Italy

Glutathione S-transferase, sweet orange, xenobiotics, heavy metal, in vitro expression

Glutathione S-transferases (GSTs) represent a multifunctional family of enzymes grouped into four main classes (tau, phi, theta, and zeta) conjugating endobiotic and xenobiotic compounds to glutathione. In plants, this is considered to be a crucial step in the detoxification process as the S-glutathionylated metabolites are tagged for vacuolar sequestration. In this work, we have isolated two glutathione S-transferases belonging to the tau class GSTs from sweet orange leaves. The cDNA clones contained a complete open reading frame of 651 bp encoding two 216 amino acid proteins. Homology search and sequence alignment showed that the deduced amino acid sequences shared high identity with GSTs from other plant sources, including several strictly conservative motifs and distinctive amino acid residues specific of the tau class GSTs. The genomic clones of both isoforms were also isolated and the analysis of the gene organization confirmed the membership of both enzymes to the tau class GSTs. The encoded proteins differ only for three amino acids: the triplet R89, E117 and I172 found in the isoform named GSTU1 is replaced by the triplet P89, K117 and V172 in the GSTU2 isoform. The successful in vitro expression of the proteins led to the functional active form of both enzymes which showed different specific activity against CDNB as substrate, the GSTU1 showing values three fold lower than that observed for the GSTU2 enzyme. The analysis of the gene expressions suggested that the GST isoforms show either different distribution between leaf and flesh, the isoforms being decidedly expressed in the leaf, or cultivar related specificity, the U2 being highly expressed in the leaves of red orange whereas the U1 in the blond orange leaves. Furthermore, we also showed that the expression of U1 gene was remarkably induced in response to cadmium sulphate, CDNB and cyhalothrin treatments as well as to cold stress. On the contrary, the U2 isoform was constitutively expressed probably playing some sort of “default scavenging” activity in vivo. Taken together these results suggested that GSTU1 is a stress responsive gene and can be considered as potential target that is genetically modified so as to create novel germoplasm with enhanced stress tolerance.
ECTOPIC EXPRESSION OF THE UVR8 GENE MIMICS STRESS-INDUCED CHANGES IN PLANT ARCHITECTURE IN A. THALIANA

FASANO R.*, GRILLO S.**, LEONE A.*

*) Department of Pharmaceutical Sciences, University of Salerno
**) Institute of Plant Genetics, IGV, National Research Council

plant stress, uvr8, A. thaliana

Plants undergo architectural and developmental changes as a common response to many distinct sub-lethal environmental stresses and a general acclimation strategy. Key elements in these stress-induced morphogenic responses appear to be endogenous molecules like flavonoids, auxin, ethylene and ROS that affect cell division, cell elongation and/or cell differentiation. Although this response has been widely reported, the underlying molecular mechanisms are poorly understood. Starting from the finding that the uvr8 gene was able to restore the growth of the yeast osmo-sensitive mpk1/ppz1- mutant, we found that uvr8 gene expression is also up-regulated by osmotic stress (PEG-mediated water deficit and NaCl). In absence of stress, ectopic uvr8 overexpression in Arabidopsis plants causes a reduced vegetative growth, early flowering, reduced primary root and leaf expansion, reduction of silique and seed number per plants, mimicking constitutively stress-induced changes in plant architecture. Interestingly, down-regulation of the protein triggered diametrically opposite morphological effects, suggesting that somehow the uvr8 gene coordinates growth reprogram and redistribution in stressed plants, to enable them to acclimate to sub-optimal external growth conditions. The opposite phenotypes of uvr8 sense and antisense plants were also associated to modification of expression of cell cycle genes and other genes controlling plant morphogenesis as well as to a different content of flavonoids, causing perturbation of auxin transport. Sense and antisense uvr8 plants respond also differentially to abrupt osmotic stress. Altogether these data support a novel role of the uvr8 gene in the plant stress-induced morphogenic response, beside that previously reported in the UV-B response.
STUDY OF MECHANISMS INVOLVED IN SALT TOLERANCE BY cDNA-AFLP METHOD IN DURUM WHEAT GERMPLASM

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

*) Università della Tuscia, Via S. C. de Lellis, 01100 Viterbo, Italy - pagnotta@unitus.it
**) ICARDA p.o. box 5466 Aleppo, Syria

salt tolerance, durum wheat, cDNA-AFLP

Salinity stress is one of main factors that are able to influence cereal yield throughout the world. Mechanisms involved in tolerance to salinity stress consist of three main components: Na+ exclusion, tolerance to Na+ in the tissues and osmotic tolerance. Plants, in the time, have developed a complex and elaborate signaling network that guarantees their adaptation to salinity; therefore, the comprehension of these mechanisms is of fundamental importance. cDNA-AFLP represents an easy and powerful method to identify sequences differently expressed under stress conditions. In present work it has been used durum wheat varieties with different degree of tolerance. The varieties have been grown under different salt stress conditions and the cDNA–AFLP technology has been employed to detect and analyse the differently expressed bands. Seeds of Cham I, Jennah Khetifa, Belikh 2 and Trinakria, have been germinated in hydroponic solution containing CaSO_4_10 mM; after germination, plants have been grown for seven days in a nutritive solution containing micro- and macro-elements and Fe++. There after, NaCl at different concentrations, 0 M (as a negative control), 0.75 M and 1.5 M, has been added to the nutritive solutions. Some morphological traits such as root and leaf turgor, length and weight, have been recorded after one hour and after 5 days since the supply of salt solutions. In the same way, RNA has been extracted from roots and leaves material and converted in cDNA through reverse transcription. The resulting cDNA has been amplified with different AFLP primers combinations and loaded on a high resolution acrylamide gel. The bands present in the tolerant lines, but absent in the susceptible one have been isolated, cloned, and sequenced. The obtained sequences have been finally blasted. Some of them show homology with sequences involved in ABA, osmotin, selenium-binding protein-like, CTP synthase-like protein, etc. Present work confirms the existence of salt tolerance in durum wheat germplasm, highlighting the importance of these genetic resources to isolate the genes involved and their use in breeding activities aiming to face the climatic global changes which see an increasing of salty soils all around the world.
CHLOROPASTIC YCF2 GENE EXPRESSION IN STRESSED PLANTS

DURANTE M., PIERETTI M., BERNARDI R.

Department of Crop Biology, Genetics section, University of Pisa, Via Matteotti 1/B, 56124 Pisa, Italy

chloroplast ycf2 gene, plant disease, stress tolerance

The chloroplast ycf2 gene plays a vital unknown function in the higher plants: gene silencing or reduction in mRNA synthesis induce cell death (Drescher et al., 2000).

Using the SSH technique, Bernardi et al. (2008) isolated a cDNA sequence in cold tolerant plants of Olea europaea L. showing a very high expression of the ycf2 gene induced by cold treatment.

The olive gene was isolated and sequenced. It presents a 6385 nucleotide length and the sequence is highly conserved in the plants, also in the rudimentary plastid genome of the non-photosynthetic parasitic Epifagus virginiana (Wolfe et al., 1992), thus confirming an essential role for the plant survival. The hypothetical protein sequence is constituted of 2278 amino acids presenting an ATPase domain and a DUF825 domain with unknown function.

We have focused our work on the fruit development of O. europaea in order to verify if ycf2 may have a specialized function in non-photosynthetic tissues during seed maturation, as reported for tomato by Richards et al. (1994). Semi-quantitative RT-PCR using mRNA extracted at different maturation stages indicated that mRNA is present in fruits of all stages, although the higher concentration was found in leaves.

Analysis of ycf2 transcripts in poplar plants treated with ozone and in plane infected with Ceratocystis fimbriata, agent of the colored canker, confirmed a differential expression of the gene after the stressing treatments.

All the results point to a defense activation of ycf2 both in biotic and abiotic stresses.
MYBLEU OVEREXPRESSION IN CITRANGE CARRIZO IMPROVES TOLERANCE TO HYPOXIC STRESS


*) C.R.A.-ACM Centro di ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale, Italy
**) Istituto di Biologia e Biotecnologia Agraria, C.N.R., Via E. Bassini 15, 20133 Milano, Italy

Mybleu, plant transformation, Carrizo citrange, flooding tolerance

Classical breeding and genetic transformation have been used to improve deficit oxygen tolerance in different crops. However, by classical breeding successes have been achieved only in rice (*Oryza sativa*). The Italian citrus cultivation is, in some areas, performed on soils characterized by a content of clay and/or loam in excess compared to optimal levels, determining the inability to eliminate water excesses during years of heavy and prolonged rainfall. In this kind of soils, humidity retention, flooding and/or waterlogging, represent a serious environmental stress that can lead to anaerobic conditions affecting many plant processes. Anaerobiosis may cause premature senescence which results in leaf chlorosis, necrosis, defoliation, cessation of growth and in extreme cases even plant death.

A large number of Myb transcription factors are involved in plant responses to abiotic and biotic stresses. The Mybleu ectopic expression in *Arabidopsis thaliana* plants positively affects cellular metabolism and increases tolerance to oxygen deficit. This Myb transcription factor, isolated from rice (a monocot, herbaceous), is able to activate the anaerobic response pathways in *Arabidopsis* (a dicot, herbaceous). In order to clarify the mechanisms involved in the response to oxygen deficit and to improve tolerance to flooding in citrus species, we overexpressed Mybleu in Carrizo citrange, one of the most popular citrus rootstocks, by *Agrobacterium tumefaciens* mediated transformation.

Analysis by polymerase chain reaction (PCR) and sequencing showed that stable integration of the transgene in the plant genome was obtained in the 71.6% of the analysed plants. Real time PCR performed on a random selection of transgenic plants lines confirmed the expression of the inserted transgene and allowed a classification of the transgenic plants based on the different expression levels of the transgene. Wild type and transgenic lines (both low and high expressing lines) were tested for their tolerance to hypoxia/anoxia by physiological, biochemical and molecular analyses. The results indicated that high expressing plants are more tolerant to hypoxia than wild type and low expressing plants.

Our findings on Carrizo citrange confirm previous results of improved tolerance in *Arabidopsis* by Mybleu supporting the hypothesis of a conservation of the regulatory pathways involved in the anaerobic response among species. Therefore, our results indicate that the overexpression of Mybleu may represent a powerful tool to improve hypoxic stress tolerance in crops.
FLOWERING ECOPHYSIOLOGY OF HEMP (CANNABIS SATIVA L.) AS A FIRST STEP TOWARDS SPECIES BREEDING

FAUX A.-M., RAULIER P., BERTIN P.

Research Laboratory in Crop Physiology and Plant Breeding, Faculty of Bioengineering, Agronomy and Environment, Université catholique de Louvain, Place Croix du Sud, 2-11, 1348 Louvain-la-Neuve, Belgium

crop breeding, flowering model, hemp, monoecy, photoperiodic response

Hemp (Cannabis sativa L.) is a naturally dioecious species with flowering responding to daylength variations. The acquisition of monoecious varieties remains an eternal renewal because of their instability: firstly, the late apparition of sexual dimorphism prevents the total eradication of dioecious male plants during the selection process, and, secondly, the monoecious state presents a continuous distribution between the male and female extreme phenotypes. Hemp is also known to be a quantitative short-day plant. Maximum stem yield seems to occur shortly after flowering. Therefore, photoperiodic conditions have a key influence on the determination of yield potential of the crop. In the context of renewed interest for alternative fibre crops, understanding the flowering system of hemp could offer valuable information to support species breeding and cultivation. The present study aims to describe the floral development, growth and biomass production of hemp as well as the expression of the sexual phenotype in response to photoperiod and temperature conditions.

Trials were settled in the field and in controlled conditions. Five monoecious varieties of different precocities were cultivated in 2007 and 2008 during five culture periods in two sites with different edapho-climatic conditions in Belgium. The first culture period ran from Mid-April to end of September and the last one from end of June to Mid-November. The same varieties were cultivated from January to June 2008 in growth chambers under five controlled photoperiods from 10 to 18 hours.

In the field, mean thermal times to reach a particular development stage were congruent between both years. Growth rhythm and biomass production appeared to be correlated with floral development rhythm. Lower floral development and growth rates, and the consequent higher biomass production, were observed for the first culture periods and could be explained by the longer daylength conditions experimented by these treatments. Three phases of growth were determined: an accelerating phase before 400-500°Cd, a linear phase between 400-500 and 1200-1500°Cd and a saturating phase after 1200-1500°Cd (base 1°C). The effect of temperature on growth, pointed out in 2007 during the linear phase of growth, was less obvious in 2008. Temperature and length of cultivation cycle seemed to influence positively the production of biomass. The highest yields were observed when sowing and harvesting were achieved in Mid-April and end of September, respectively.

In controlled conditions, sex ratio and evolution of flowering stages were observed at each node separately. All varieties presented a typical floral response of short-day plants, with flowering occurring faster at daylengths shorter than 14 hours. The analysis of the distribution of the sexual
phenotypes allowed the identification of times intervals and nodes ranges which could be used for future description of the sexual phenotype of hemp plants.

Finally, the observations and results of this first ecophysiological approach should lead us to propose a flowering model for hemp and provide us precious information which may be introduced in a second step, the approach of sex determinism by the QTL method.
EVALUATION OF GENE EXPRESSION AND PHOTOSYNTHESIS ACTIVITY IN DIFFERENT *FAGUS SYLVATICA* GENOTYPES UNDER HIGH CO₂ LEVEL


*) Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
**) Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Forest Genetics, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany
*****) Department of Environmental and Forestry Technologies and Sciences, University of Florence, Via San Bonaventura 13, 50145 Florence, Italy
******) Freie Universitaet Berlin, Institut fuer Biologie, Altensteinstr. 6, D-14195 Berlin, Germany
*****) Dipartimento di Biologia Evoluzionista “Leo Pardi”, University of Florence, Via Romana 17, 50125 Florence, Italy
******) Institute of Plant Genetics – CNR, Research Division Florence, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy

CO2, microarray, European Beech, photosynthesis

The problems related to global changes, mainly caused by human activities, are the origin of much concern for the health of the environment. Oil and carbon combustion, the use of chlorofluorocarbons, and deforestation are some of the principal factors responsible for CO₂ production and for air temperature increase. The scenario is factors leading to global changes affecting precipitation patterns, nitrogen concentration in the atmosphere, UV-B radiation increase and temperature increase. Forest trees constitute a relevant economic and ecological resource that is under severe treat by environmental changes.

The principal aim is to investigate the response to CO₂ from three different genotypes of *Fagus sylvatica* by gene expression and ecophysiological analyses. Shoots of *F. sylvatica* (Montieri (GR), Italy) and *F. sylvatica* “purpurea tree” were grafted on *F. sylvatica* rootstocks. Plants were grown under controlled conditions in a climate chamber using the same temperature and light parameters, while CO₂ concentrations were approx. 380-400 ppm (ambient) in the control room and 1000 ppm (high) in the experimental room.

A PAM fluorescence system (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) with a 6 mm diameter standard fibre optic was used for the measurements of the *in vivo* photosynthesis. Under ambient conditions photosynthesis (expressed as electron transport rate) was higher in the Italian compared to the German genotype. After 4 d under high CO₂ treatment, electron transport rate showed increased values compared to the plants growing under ambient CO₂. Photosynthesis of plants (Italy) adapted to high CO₂ decreased immediately after been exposed for 2 h to ambient CO₂. No down-regulation of photosynthesis could be observed in leaves at high CO₂ level. Microarrays analyses have already been done and preliminary results will be discussed.
DIFFERENT RESPONSE TO THE UV-STRESS AMONG POPULUS ALBA L. CLONES


*) Department of Environmental and Forestry Technologies and Sciences, University of Florence, Via San Bonaventura 13, 50145 Florence, Italy
**) Institute of Plant Genetics – CNR, Research Division Florence, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

UV-B stress, poplar, ecophysiology, gene expression

To evaluate a possible differential behaviour of 5 white poplar genotypes towards UVB radiation, we studied the association between polymorphism data (SNPs) and expression level on genes (such as chs, comt, and rbcL) putatively related to UVB response. Molecular data were integrated with ecophysiological observation. From the ecophysiological data obtained we observed, in stressed plants, a decrease of the maximum efficiency of the PSII, in particular for 3 clones increasing the hours of UVB treatment. Furthermore an abrupt decrease of the values is already present from the second day of stress. The analysis using qRT PCR for the genes studied, for the leaf tissue of the 5 clones treated for 3, 6 and 12 hours with UVB, showed a different pattern of expression, with a dissimilar response among the clones analyzed towards the stress. Both the ecophysiological and the expression data showed a different response particularly for 3 clones that resulted more sensitive to the treatment than the others. The analysis of the polymorphism of the sequences has instead not allowed the construction of associations with the ecophysiological and expression data for absence of missense mutations.
EFFECT OF SHADING ON THE FLAVONOID PATHWAY DURING GRAPE BERRY RIPENING IN *VITIS VINIFERA CV AGLIANICO*

FOGARTY F., FAILLA O., ROSSONI M., SCIENZA A.

Dipartimento di Produzioni Vegetali, Università degli Studi di Milano, Facoltà di Agraria, Via Celoria 2, 20133 Milano

*Anthocyanin, shading, Vitis vinifera, Aglianico, Real Time PCR*

Polyphenols are very important secondary metabolites in plants and they are responsible for the chemical, sensorial and nutraceutical properties of wine. Among them, anthocyanins are particularly important as they are the blue, red and purple pigments of fruits and flowers and they are responsible for the red colour of wine. Polyphenolic compounds are synthesised in the well-known flavonoid pathway.

Previous studies (Downey et al, 2004; Fujita et al. 2007; Rustioni et al, 2006) demonstrated that cluster shading significantly affects accumulation levels for anthocyanin and other main products of the flavonoid pathway, but more studies are needed to fully elucidate the effect of shading on the regulation of this pathway. For this reason we have chosen to study the response to shading in Aglianico, a red berry *Vitis vinifera* cultivar widely grown in Southern Italy.

The experimental vineyard was situated in Galluccio (CE) and the plants were maintained in good sanitary and agronomic condition. Ten days before veraison, a shading screen was applied to the grape bunches. Control bunches were fully exposed to sun light trough leaf removal. From veraison to full maturity, samples of both shaded and control grape berry skins were collected and frozen in liquid nitrogen. Whole berries samples where collected and stored at -20°C for technological and chemical assays. Samples were collected in triplicate.

The maturation pattern of the whole berries were evaluated measuring soluble solids, pH and titrable acidity. We also measured total polyphenols, total flavonoids, non anthocyanin flavonoids, tannins, and total anthocyanins by spectrophotometry. The anthocyanin profiling was carried out with HPLC.

Total RNA was extracted from berry skins and the transcription levels of the key genes of the flavonoid pathway were determinated by Real Time PCR.
BIO-AGRONOMICAL CHARACTERIZATION AND GENE EXPRESSION STUDY OF GENOTYPES GROWN UNDER DIFFERENT WATER CONDITIONS


*) 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

drought stress, tomato, bio-agronomical traits, gene expression

Abiotic stresses such as low or high temperature, high salinity and drought affect the growth and yield of crops.

Actually, drought, defined as the occurrence of a substantial water deficit in the soil or atmosphere is an increasingly important constrain to crop productivity and yield stability worldwide.

Most crops, including (*Solanum lycopersicum* L.), are sensitive to drought stress throughout the ontogeny of the plant, from seed germination to harvest.

An approach to minimizing agricultural losses incurred by drought stress is to develop cultivars that can escape or withstand periods of drought.

To survive under such unfavourable growth conditions, plants have developed a number of unique defence mechanism that enhance their tolerance to detrimental conditions. Tolerance to the environmental stresses has a complex genetic base and in the recent years research has allowed the identification and characterization of a great number of genes involved in the mechanism of plant response to abiotic stresses.

In the present work data both on bio-agronomical characterization of tomato genotypes grown under two different water conditions and on preliminary studies of genetic mechanism involved in drought stress response are reported.

Ten genotypes of tomato, including M82 cultivar, two Introgression Lines (IL) previously selected for their higher tolerance to drought stress conditions and seven Albanian and Apulian landraces, were grown using a split-plot experimental design with three replicates.

In order to evaluate the effect of drought stress on yield the main bio-agronomical data such as flowering and ripening time, number and weight of fruits per plant, solid soluble content etc., were recorded.

Furthermore, the expression of a set of ten genes involved in water stress response has been evaluated.
PHYSIOLOGICAL AND TRANSCRIPTIONAL ANALYSES OF FACTORS AFFECTING WATER USE EFFICIENCY IN A MODERN AND AN OLD DURUM WHEAT CULTIVAR


*) CRA-GPG Centro di Ricerca per la Genomica e la Postgenomica Animale e Vegetale, Via S.Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy
**) CRA-CER Centro di Ricerca per la Cerealicoltura, SS 16 Km 675 - 71100 Foggia, Italy
***) Potsdam Institute for Climate Impact Research (PIK) PF 60 12 03 -14412 Potsdam, Germany

drought, durum wheat, stomatal conductance, water use efficiency

The aim of this work was to analyse the response to water stress in two durum wheat cultivars (the modern cv “Ofanto” and the old “Cappelli”) employed as parents of a segregating population, being contrasting for their yield performance. In a growth chamber experiment plants were grown at early stage under dry and irrigated conditions. Both physiological evaluations and target gene expression analyses have been conducted throughout the experiment. Gas exchange and mass accumulation measures indicated that “Ofanto” consumed more water and used soil water resources more rapidly on incipient drought. Consistently “Ofanto” showed a higher stomatal conductance, resulting in a lower water use efficiency (WUE), compared to “Cappelli”, probably sustained by a better capacity of osmotic adjustment (OA). These results can suggest a constitutive difference in stomatal responses in “Ofanto” and “Cappelli”, with consequences for water economy and yield stability.

In order to unravel the molecular basis of these physiological responses, a number of genes known to be involved in the control of stomatal aperture as well as in osmotic adjustment have been selected for Real Time PCR. Some genes showed differential expression between the two cultivars with profiling related to the differential physiological behavior.
TRANSRIPTOMIC ANALYSIS OF DROUGHT AND HEAT RESPONSES IN DURUM WHEAT

APRILE A.*, PANNA R.*, PERROTTA C.*, BORRELLI G.**, DE BELLIS L.*

*) Department of Environmental and Biological Technologies and Sciences, University of Salento, Prov.le Monteroni, 73100 Lecce, Italy
**) CRA-CER S.S.16 km 675, 71100 Foggia, Italy

drought stress, heat stress, Triticum durum, stress tolerance, transcriptome analysis

The productivity of wheat, one of the most important crops worldwide, is often limited by an array of abiotic stresses that avoid a successful growth and a complete grain filling. The water shortage is the major limiting factor affecting crop production. Nevertheless drought stress never comes alone. Sometimes drought stress is associated to freezing temperatures that reduce water availability, sometimes to pathogens involved in vascular mobility, or to high soil salinity. But dramatic effects occur when high temperatures and drought stress happen simultaneously.

Recently several researchers endeavoured to dissect the complex responses to drought and heat stress in bread and durum wheat. Anyway the above works are far from field condition, where, in summer period, the two stresses occur at the same time.

In this experiment two different durum wheat cultivar were analyzed. Ofanto cv. was achieved from recent breeding programs and is characterized by high yield and good stability of production in stress conditions (drought and high temperature). On the contrary Cappelli cv. is one of the first italian cultivars and was developed in 1915. As consequence it is a low performance cultivar mainly in stressed conditions. An Ofanto x Cappelli molecular map and several QTLs for abiotic stress tolerances are also available.

These genetic materials were grown in drought stress condition, subjected to high temperature and to both stresses. A transcriptome analysis was carried out using the Affymetrix 61K wheat chip on three biological replicates of mRNA extracted from flag leaves during booting stage, the stage more susceptible to drought and heat stress.

The poster will present data on genes and pathways up- or down–regulated highlighting the common and the different molecular mechanisms actived by the two cultivars. Moreover a clustering organization of the genes will show specific drought and heat responses, with particular regard to specific genes activated only in the combined stress.

Final purpose of this work is to associate specific QTLs to the expression values of drought and heat tolerance candidate genes.
ENVIRONMENT SPECIFIC AND COMMON QTLS FOR ADAPTATION OF BARLEY TO A WIDE RANGE OF MEDITERRANEAN DROUGHTED ENVIRONMENTS


*) CRA – Genomic Research Centre, I-29017 Fiorenzuola d’Arda (PC), Italy
**) Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, I-42100 Reggio Emilia, Italy
***) CRA – Cereal Research Centre, I-71100 Foggia, Italy
****) MABDE consortium (Details in poster/presentation)

barley adaptation, drought, QTL, yield, NT doubled haploids

The Nure x Tremois (NT) doubled-haploid population (namely Italian Barley Mapping Population, IBMP) has been exploited in order to map QTLs for yield adaptation to drought environments. The winter parent - Nure - Italian two-rowed feed-barley cultivar, showed a wide range of adaptability, including South European environments. The spring parent - Tremois - French two-rowed malting variety was adapted to fresh, fertile environments. A multilocalational irrigated vs. non-irrigated field trial of the IBMP population has being carried out during two years (2003/04 and 2004/05) in Mediterranean Europe, North Africa and West Asia to map QTLs of yield and drought adaptation traits, under the frame of the EU-MABDE project. Candidate genes (CGs) involved in barley development and response to abiotic stresses have been placed on the NT map, as well, to build a DArT/SSR/AFLP medium density map of some hundred markers. After statistical and QTL analysis for yield as well as for other agronomic traits, recorded in 18 different trials, a clear picture of stable through environments QTLs, plus environment-specific QTLs was drawn. The impact of the two components on yield and adaptation are presented and discussed. Moreover, the co-location of yield, yield components and phenology QTLs is presented and discussed, with particular emphasis on few major developmental loci responsible of a large part of the response to different environmental conditions. Lastly, QTLs of yield adaptation found by association mapping with DArT markers on the DBG (Diverse Barley Germplasm) collection in the same Mediterranean fields are compared with the QTLs found in the biparental IBMP population.
Poster Abstract – 2.55

A SEQUENCE ANALYSIS OF GENES INVOLVED IN DROUGHT STRESS IN THE CULTIVATED SUNFLOWER AND IN THE HELIANTHUS GENUS


*) Department of Crop Plant Biology, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
**) Institute of Applied Genomics, Via Linussio 51, 33100 Udine, Italy
***) Department of Crop and Environmental Sciences, University of Udine, Via delle Scienze 208, 33100 Udine, Italy

A major goal of population and quantitative genetics is to identify the polymorphisms underlying phenotypic variation, particularly in traits that are important for ecological adaptations. While the accumulation of functional genomics data over the last decades has provided detailed information on the genetic basis of many traits in a number of model organisms, genetic variation in non-model species remains largely unknown.

Despite the importance of genes related to abiotic stress in environmental adaptation, studies on DNA sequence polymorphism of such genes in plant species are rare mainly because of their organization in gene families. This can lead to errors in comparison since, for example, non-orthologous loci can be incorrectly compared. A search for genes that are in a unique copy in the genome usually involves a Southern blot hybridization. Possible candidates are further amplified by PCR on genomic DNA from a completely homozygous plant (i.e. a highly inbred line) and their products are directly sequenced. In the absence of single nucleotide polymorphisms the gene can be considered as unique and compared to other allelic variants of other genotypes. In our study, we have analysed sequence variability of four unique genes involved in drought response in eight inbreds of sunflower of different origin, eleven Helianthus species, as well as in Viguiera multifolia and Tithonia rotundifolia that are two species related to the Helianthus genus, by isolation and analysis of allelic sequences. The four selected genes encode a dehydrin (DHN), a drought-responsive-element binding protein (DREB2), an early light-induced protein (ELIP), and a non-specific lipid transfer protein (LTP). Within cultivated sunflower, nucleotide diversity per synonymous and nonsynonymous sites was calculated for each gene. The $\pi_s/\pi_a$ ratio range was very low indicating strong evolutionary constraints, though with differences among genes. As far as noncoding regions, the intron showed a larger variability than the other regions only in the case of DHN gene. In the other genes tested, in which one or more introns occur, variability in the introns was similar or even lower than in the other regions. On the contrary, 3'-UTRs were usually more variable than the coding regions.

A comparison of orthologous loci in different Helianthus and Asteraceae species was performed using the NJ method. The dendrogram obtained confirms the subdivision of the genus between annual and perennial species and is highly significant (all nodes showed bootstrap values higher than 50%), indicating that these genes could be useful to clarify Helianthus phylogeny by applying to the 53 wild sunflower species.
EFFECTS OF IN VITRO BACTERIAL INOCULATION (BACILLUS SUBTILIS) ON THE RESPONSE TO PEG-INDUCED WATER STRESS IN MEDICAGO SATIVA L.

CALVIO C.*, BALESTRAZZI A.*, GALIZZI A.*, PIANO E.**, CARBONERA D.*, CONFALONIERI M.**

*) Department of Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy  
**) CRA - Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, Viale Piacenza 29, 26900 Lodi, Italy

Bacillus subtilis, Medicago sativa, PEG-induced water stress, bacterial inoculation

It is known that water stress has a strong impact on plant growth and development, causing lower yields and, possibly, crop failure. Plants can interact with several microorganisms able to enhance their growth ability under adverse environmental conditions. The plant growth-promoting bacteria (PGPBs) exert beneficial effects by different mechanisms, among which increased nutrient availability and reduced ethylene production in planta. PGPBs can also produce auxins, cytokinins and gibberellins, promoting plant growth through phytohormone-mediated signaling pathways.

The beneficial effects of the gram-positive bacterium Bacillus subtilis have been documented in field crops challenged with abiotic stresses. However, information concerning the mechanisms involved in the B. subtilis-plant interaction are still scanty.

In the present work, we tested a wild type B. subtilis strain (NCIB3610) for its ability to support the in vitro growth of alfalfa (Medicago sativa L.) seedlings in presence/absence of water stress induced by PEG6000. Different bacterial concentrations were tested during plant cotyledon inoculation and the occurrence of both leaf-associated epiphytic and endophytic B. subtilis populations was assessed at different times following inoculum. The effects of bacterial inoculation on alfalfa biomass production were also evaluated.
METHYLATION-SENSITIVE AFLP MARKERS TO INVESTIGATE DROUGHT-STRESS RESPONSE IN MONTEPULCIANO AND SANGIOVESE CULTIVARS

MARCONI G.*, RAGGI L.*, PALLIOTTI A.**, NAPOLITANO M.*, ALBERTINI E.*

*) Department of Applied Biology, University of Perugia, Italy  
**) Department of Agricultural Sciences, University of Perugia, Italy

Vitis vinifera, drought-stress, DNA methylation, methylation-sensitive AFLP markers

The interest in the identification of drought-resistant genotypes of Vitis vinifera that can optimise their water use is dramatically increasing especially in areas where it is difficult to extend the irrigation or which are undergoing a progressive shift towards sub-tropicalisation. Previous results indicate that in contrast to those of Montepulciano, Sangiovese vines in presence of severe, multiple summer stresses, show morpho-biochemical and physiological behaviours that result in the optimisation of the whole-vine carbon gain. Therefore, in comparison to Montepulciano, Sangiovese can be considered as being well adapted to drought conditions. The cultivar genetic background appears to have a crucial role in adaptation to multiple summer stresses, and in the ability of the grapevine for CO₂ uptake and for accumulating non-structural carbohydrates into reserve organs.

DNA in plants is highly methylated, containing methylated bases such as 5-methylcytosine (m⁵C) and N⁶-methyladenine (m⁶A). This phenomenon in plants is species-, tissue-, organelle- and age-specific. Changes in DNA methylation are present throughout the entire life cycle of plants, starting from seed germination up to the plant death either programmed or induced by various agents such as biotic and abiotic stresses. It is well known that changes in DNA methylation results in alteration of gene transcription, DNA replication and repair, gene transposition and cell differentiation.

In the present study Montepulciano and Sangiovese cultivars were compared with the aim of understanding if drought-stress tolerance involves DNA methylation. The analysis was performed using methylation-sensitive AFLP (M-SAP) markers on genotypes with contrasting response to drought-stress. Results of genome analysis and isolation of fragments related to methylation/drought-stress is reported and discussed.
HEAT INDUCTION VS CONSTITUTIVE OVEREXPRESSION OF HSP70 PROTEIN IN ALFALFA


*) Dipartimento di Biologia Applicata, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
**) Metapontum Agrobios, S.S. Jonica, 106 Km 448,2, 75010 Metaponto (MT), Italy

HSP70, heat shock, GSA-AT, gabaculine, Medicago sativa

Heat shock-proteins are involved in multiple functions in response to stress and constitutive stimuli such as controlled protein degradation, de novo folding and refolding in response to stress. Recent findings indicate HSP70 to be particularly important also as carrier molecule in anti-tumoral and anti-viral vaccines, enhancing immune response.

To assess the feasibility of HSP70 production in alfalfa (Medicago sativa L.), we used two different approaches. First, we evaluated the accumulation of HSP70 in 12 alfalfa varieties of diverse origin, upon heat shock for 5 min at 60°C.

In the second approach, transgenic lines over-expressing the HSP70 gene from Arabidopsis thaliana were assessed as bioreactors.

HSP70 accumulation in the Wt cultivars and in the transgenic lines was evaluated through Western blotting analyses using anti-HSP70 specific antibody.

The results obtained thus far showed that some cultivars the expression of HSP70 increased by heat shock whereas other varieties did not respond efficiently to the induction.

On the other hand, the transgenic lines bearing A. thaliana HSP70 gene under CaMV35S promoter control showed the expected protein overexpression.

Direct comparisons of the two “production systems”, is underway, to determine the economic feasibility of using alfalfa as a bioreactor for producing HSP70.
SILENCING OF PLASTIDIAL \textit{OMEGA-3 FATTY ACID DESATURASE 7 (FAD7)} IN TOMATO INCREASES PLANT HEAT TOLERANCE

SANAMPUDI V.R.R., MAZZUCATO A.

Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.C. de Lellis snc, 01100 Viterbo, Italy

Omega-3 Fatty acid desaturase, RNAi, histochemical GUS assay, \textit{Solanum lycopersicum}, heat tolerance

Exposure to high temperature causes reduced yields in tomato (\textit{Solanum lycopersicum} L.). Objective of this study is to obtain tomato plants resistant to high temperature by silencing the omega-3 fatty acid desaturase 7 (FAD7) gene. Fatty acids play an important role in tolerance to high temperature by altering their composition of bonding pattern in the membranes. Plastid omega-3 fatty acid desaturase catalyzes the conversion of dienoic fatty acids (16:2 and 18:2) to trienoic fatty acids (16:3 and 18:3) in glycerolipids which are the main constituents of chloroplast membranes. In tobacco, it has been shown that an increase of dienoic fatty acids in plastid membranes was correlated with the acquisition of termotolerance. We produced transgenic tomato plants that express the transcript of double-stranded RNA (dsRNA) of the tobacco plastid FAD7 gene to induce post transcriptional gene silencing and reduce the expression omega-3 fatty acid desaturase. The transcription of the transgene was confirmed by histochemical GUS assay. The steady state messenger-RNA level of the targeted gene was low in transformed plants when compared to the controls. Under the heat stress, the transformed plants silenced for FAD7 showed a higher number of viable pollen grains and higher fruit set and yield when compared to untransformed plants. These results indicates that post transcriptional gene silencing of omega-3 fatty acid desaturase is useful to increase tolerance to high temperature in plants by changing composition of membrane fatty acids.
Towards Physical Mapping and Sequencing the FR-H2 (Frost Resistance-H2) Region of Barley Chromosome 5H

PASQUARIELLO M.*, BARABASCHI D.**, TONDELLI A.**, SCHULTE D.***, STEIN N.***, STOCKINGER E.****, PECCHIONI N.*, FRANCIA E.*

*) Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42100 Reggio Emilia, Italy
**) CRA–Genomic Research Centre, Via San Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy
***) Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Departement of Genebank, AG Genome Diversity, Correnstrasstra_e 3, D-06466 Gatersleben, Germany
****) Department of Horticulture and Crop Science, the Ohio State University/OARDC, 1680 Madison Avenue, Wooster OH 44691

barley, 5H, Fr-H2, CBF, 454 sequencing

Frost resistance-H2 is one of two major quantitative trait loci, located on chromosome 5H, that affect freezing tolerance and winter hardiness of barley. Coincident with Fr-H2 is a cluster of more than 14 genes encoding CBF transcription factors, that are at present the best candidates in barley to explain the effects of frost tolerance given by the QTL. It is not known whether the effect of Fr-H2 is either the result of a single CBF gene, or the combined effect of a subset/all the CBF genes, or an effect of other sequences independent from the CBF genes. As a first step towards Fr-H2 physical mapping we have generated a large mapping population derived from the freezing tolerant genotype ‘Nure’, and the freezing susceptible ‘Tremois’, in order to both fine map the Fr-H2 interval, and to generate recombinants between the different CBF genes. Screens for recombinant individuals from F2 populations consisting of 2,849 plants, and their subsequent phenotypic evaluation in F4 lines provided an estimated refined genomic interval of 4.6 cM for Fr-H2. Recombinants between seven out of the 14 CBF genes under Fr-H2 have been identified and showed that the CBF gene cluster spans 0.81 cM on barley chromosome 5H.

A positional cloning effort of Fr-H2 has been undertaken. A genomic BAC library of barley (cv. ‘Morex’) was screened with a total of six CBF markers mapping in this locus. Using a PCR-based screening strategy the first BAC clone addresses were obtained for all the CBF markers assayed. To create anchor points between the genetic map and a ‘future’ physical map of barley, in this region, a high information content fingerprinting (HICF) of the selected BACs has been performed and then the selected BAC clones have been assembled into contigs.

To close the gaps between the assembled clones, additional BACs belonging to the contigs detected, have been screened with further CBF markers and a total of three BACs were sequenced and assembled using 454 sequencing. The construction of a single physical contig encompassing the Fr-H2 region will be our next purpose. This will provide further information on gene content and structural locus organization and thus provide a fundamental resource for detailed comparative analyses of the genomic organization of the locus in other barley cultivars, like ‘Nure’ and ‘Tremois’.
CANDIDATE GENE EXPRESSION PROFILING DURING CHILLING IN TOMATO

CAFFAGNI A.*, ALBERTAZZI G.*, MILC J.*, RIZZA F.**, FRANCIA E.*

*) Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42100 Reggio Emilia, Italy
**) C.R.A., Genomic and Postgenomic Research Centre, Via S. Protaso 302, Fiorenzuola D'Arda (PC), Italy

Solanum lycopersicum, chilling tolerance, CBFs, candidate genes

Growth of thermophilic plants is impaired for a considerable time by also brief periods of exposure to low, chilling, temperatures. Many species from tropical regions, such as tomato, maize and rice, are unable to acclimate and tolerate freezing, and they suffer chilling injuries when exposed to temperatures in the range of 0 to 12°C. In case of tomato, the phenological phase mostly affected by cold, often together with hypoxic conditions, is the first phase of the crop establishment after the transplantation. Therefore, because of chilling sensitivity, the geographic distribution and the length of the growing season of Solanum lycopersicum species are limited, and too anticipated sowing (transplantation) in field-grown tomatoes often leads either to yield reductions, or to longer lasting early vegetative stages in the field, without any yield advantage. Genetic variation for chilling tolerance exists between the cultivated S. lycopersicum and its related wild species, but it has not been thoroughly investigated and exploited intra-specific variation to improve such tolerance. The present work was aimed (1) to test a tomato germplasm collection of wild and cultivated genotypes for their chilling tolerance by means of both electrolytic leakage and Fv/Fm parameter and (2) to examine changes in gene expression occurring between two tomato cultivars selected as contrasting for the trait, in response to chilling stress, by using RT-PCR assays.

We report here results of genetic variation in the tomato germplasm for chilling tolerance, after a stress treatment given at 0-1°C. Two tomato cultivars resulted contrasting in behaviour, as one tolerant (Albenga) and one susceptible (San Marzano) to low temperature exposure (1°C for 24h) in terms of both electrolytic leakage and Fv/Fm fluorescence ratio.

Fourteen candidate genes induced by chilling and/or with putative roles in abiotic stress-response pathways have been then identified. These fourteen genes comprise both transcription factors and effector genes. EF1α and RPL2 genes have been chosen for quantitative RT-PCR normalization because in previous studies, these two genes had been shown to be the most stable during cold stress. We present here the first results of the modification of candidate gene expression in the two contrasting cultivars, and in different chilling and control situations.
COLD-INDUCED CHANGES OF TRANSCRIPT PROFILES CORRELATED TO SUCROSE METABOLISM IN SUGAR BEET

PACIFICO D., MANDOLINO G.

CRA-Agriculture Research Council - Research Institute for Industrial Crops (C.I.N.), Bologna, Italy

Sugar beet, cold stress, sucrose metabolism, real-time PCR, Beta vulgaris

Many studies showed that plants have inducible and multilevel networks of cold response and acclimation. However little is still known in sugar beet, a crop of relevant economic importance in Europe. Cold modulation of expression of several key genes involved in sucrose metabolism was investigated in cultivars adapted to different climatic areas: the autumnal cv. Franca and the spring cv. Bianca. The cold-induced transcriptional profiles were compared in order to extend our knowledge of low temperature adaptation mechanism, especially in relation to sucrose metabolism because of its economic importance. Real-time PCR analysis of the temporal expression of 12 genes involved in sucrose quality and yield during and after stress condition came after an accurate ESTs research and analysis in BVGI (Beta vulgaris Gene Index) and a reference gene selection in order to normalize the Ct raw data. 24 DAP plantlets grown in hydroponic culture were stressed for different hours (from 3 hrs up to 8 hrs) at different temperatures (4°C, 0°C and -2°C).

The results highlight that the turning point in transcriptional changes was the exposure of the young plantlets at 4°C chilling temperature, as showed by the induction of fructose 1,6 biphosphatase in root tightly linked to “hardening” exposition.

In addition, an organ-specific variability and stress-modulated transcription of some genes analyzed (SBSS and SPS) was recorded and the response of the different cultivars was showed.

The cold responsive genes identified can provide the bases to improve cold tolerance in sugar beet during the developmental phase.
THE TRANSCRIPTIONAL PROFILE OF RED ORANGE FLESH AS CHALLENGED BY LOW TEMPERATURE STRESS: THE FLAVONOID BIOSYNTHETIC PATHWAY

LO PIERO A. R., CRIFÒ T., PUGLISI I., PETRONE G.

Department of Agronomic, Agrochemical and Animal Production Sciences, University of Catania, Via S. Sofia 98, 95123 Catania, Italy

red orange, cold stress, anthocyanin, subtractive hybridization

Anthocyanin pigments belong to the diverse group of ubiquitous secondary metabolites known as flavonoids. It has been shown that low temperature induced the increase of the anthocyanin levels in red orange juice vesicles which is accomplished by the stimulation of the expression of biosynthetic enzymes acting in the early step (phenylalanine ammonia lyase), as well as those catalyzing the later steps of the pathway (chalcone synthase, dihydroflavonol 4-reductase, UDP-glucose 3-O-glucosyltransferase and glutathione transferase) (Lo Piero et al., *J. Agr. Food Chem.*, 53, 9083-9088, 2005). As a consequence, the anthocyanin biosynthesis in orange flesh can be considered as *cor* (cold regulated) pathway, and, it might represent an excellent subject for characterizing plant molecular response to low temperature. The expression of a variety of genes, indeed, is cold-induced starting from those involved in the signal transduction network originating during the perception of stressful conditions, up to those conferring either stress tolerance or response. In our work, a transcriptomic study based on subtractive hybridization was performed in order to emphasize the overall changes in gene expression after prolonged exposure of red orange fruit flesh ([Citrus sinensis] L. Osbeck) cv. Sciara] to cold stress conditions (4°C for 75 days). The stressful status was verified by monitoring the anthocyanin accumulation, whereas the induction of gene expression was confirmed by real time PCR. From the analysis of the isolated EST sequences emerged out that red orange fruit flesh undergoes to intense metabolic modifications under low temperature stress. A certain number of ESTs encoding lipid desaturating enzymes, Δ12-fatty acid desaturase and ω6-fatty acid desaturase, were found to be induced by cold, along with lisophosphatidic acid acil transferase and diacylglycerolacil transferase, all of them accounting for a rearranging activity of the membranes in order to increase the extent of fatty acid desaturation; pathways involved in the defence mechanism against oxidative damage and in the synthesis of compatible solute to adjust the cell osmotic potential are also specifically activated. In particular, the data confirms that chilling activates the expression of genes involved in anthocyanin biosynthesis as previously reported (Lo Piero et al., *J. Agr. Food Chem.*, 53, 9083-9088, 2005). Interestingly, two ESTs encoding respectively the 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase and the chloroplastic isoform of chorismate mutase, both of them involved in the phenylalanine synthesis (shikimate pathway), were found specifically induced in cold treated samples. Moreover, the expression of many ESTs encoding enzymes catalyzing biochemical reactions leading to substrates that feed the phenylalanine biosynthesis, such as citrate lyase, phosphoenolpyruvate/Pi-translocator and phosphoenolpyruvate carboxykinase, are sharply induced under low temperature stress thus indicating that pathways functioning very upstream the anthocyanin biosynthesis probably play a crucial role in cold acclimation.
TRANSCRIPTIONAL ANALYSIS OF THE REPRODUCTIVE BEHAVIOUR OF THE PHYTOPHAGOUS PEST INSECT, *CERATITIS CAPITATA* (MEDFLY)


*) Dept Animal Biology, Univ. Pavia, Pavia, Italy
**) Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

insect pest, Ceratitis capitata, microarray, reproductive behaviour, immunity

The medfly is an invasive agricultural pest that has become a model insect for the development of biological control strategies. These strategies depend on knowledge of the behaviour, physiology and molecular genetics of reproduction. The recent availability of medfly ESTs has permitted the development of microarrays for mass-gene expression profiling.

A microarray-based approach was used to compare the adult head transcriptomes of each sex at differential physiological stages, sexually immature and mature virgin and mated individuals. Particular attention was placed on transcripts involved in reproduction, behaviour, olfaction, and the immune system. Compared to the massive transcriptional changes during the maturation of the female, post-mating changes were modest, suggesting that mating does not trigger extensive transcriptional changes. Mating in the medfly does not appear to trigger changes in gene expression to the extent seen in *Drosophila*.

Apart from increasing our understanding of the molecular machinery behind these biological processes, the genes implicated may represent important targets for control programmes aimed at controlling populations of this pest species.