GENOTYPE X ENVIRONMENT INTERACTION IN GRASS PEA
(*LATHYRUS SATIVUS L.*) LINES

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genotype x environment interaction, Lathyrus sativus L., multivariate analyses, stability

Eight grass pea lines grown in three different seasons were evaluated for the stability of seed yield, 100-seeds weight, flowering time, plant height and biomass. Significant differences existed among years, lines and lines x years interaction for all traits except for 100-seeds weight. Two methods of multivariate analysis, cluster and principal components, were utilized to determine: firstly, whether a pattern existed among lines in their response across years and secondly to examine the relationships among them. In both analysis each line was presented as a vector whose elements were given by the performance of lines in each year. The analyses used arranged the lines into groups that were differentiable in terms of performances and stability.
STRESS INDUCED MODULATION OF WAX BIOSYNTHESIS IN MAIZE AND ARABIDOPSIS

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cuticular waxes, stress tolerance, Zea mays, Glossy1

The cuticular wax layer covering the aerial surfaces of land plants provides protection against the deleterious effects of light, temperature, osmotic stress and pollution. These external stimuli modulate wax biosynthesis in both qualitative and quantitative terms. Little is known however on the signal transduction pathways which translate perception of such abiotic stresses into wax accumulation on epidermal cells.

To start elucidating the molecular events underlying stress induced wax biosynthesis, mRNA steady-state levels of wax related genes were assessed under drought, cold and salt stress. These analyses pointed out that in maize and Arabidopsis only few structural genes involved in key steps of the wax biosynthetic pathway are regulated under stress. Particularly interesting was the finding that, in contrast to its Arabidopsis orthologue, the maize Glossy1 gene turned out to be repressed in response to conditions which stimulate wax synthesis. In order to elucidate the significance of Glossy1 down regulation under stress and shed light on its biochemical function, drought induced changes in cuticular wax composition of maize seedlings were analyzed. In addition, a gain-of-function approach was attempted to gain direct information on Glossy1 activity.
CHARACTERIZATION OF COLD INDUCED GENES FROM CYPRESS

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*Cupressus sempervirens, mRNA, abiotic stress, gene regulation, low temperature

Cold is one of the most important and studied abiotic stresses. In fact, about two thirds of the world’s perennial landmass is subjected to winter temperatures below the freezing point, and about half of it must cope with temperatures below -20°C. It is therefore not surprising that many attempts have been made to improve cold resistance in plants, either by classical breeding or by gene transfer. This molecular study on cypress (*Cupressus sempervirens*) is focused to the isolation and cloning of sequences differentially expressed during the exposure of the plant to low temperatures. For this purpose a subtractive approach has been used, based on the method “PCR-Select”, using the Clontech kit. Two different genotypes of cypress were grown under controlled growth-chamber conditions for 21 days at 22°C and then the temperature were lowered at 2°C for 15 days. Samples of leaf were extracted before the treatment (control) and after 1, 2, 3, 7 and 15 days from the beginning. Samples of mRNA were extracted and used as starting material to obtain cold-regulated genes. With this method a large number of cDNA fragments were obtained, cloned into a plasmid vector and sequenced. cDNA sequences were analysed and compared with DNA and protein databases using the BLAST server at NCBI. A total of 108 gene were analyzed, obtaining 90 unique sequences. Of these 59 resulted to have good homology with known sequences. The greater part of these showed high homology with genes that in other plant species have been found to be regulated by cold or oxidative stress.

A Real-Time PCR experiment was performed on 24 sequences in order to confirm that in cypress these genes are cold regulated. The results showed a clear induction of different genes and in some cases a distinction between the two genotypes used. Even if the trend is always similar, the two different genotypes showed a quantitatively different expression.
EXPRESSION ANALYSIS OF GRAPE RESPONSE TO DIFFERENT SOILS AND ROOTSTOCKS

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gene expression, grapevine, rootstocks, soil

Grapevine is an ancient culture that constitutes one of the most economically important fruit species worldwide. The production can be qualitatively and quantitatively different depending where the plants are grown. Soil conditions and rootstocks are among the main causes that can influence quality and wine production. In this work was analyzed the transcriptomic responses of the grapevine variety Pinot noir (clone 115) to different soils and to different rootstocks. The grapevine plants were grown in three different soils: sand, turf and a typical vineyard soil from the Asti DOC region. The plants were grafted on February 2005 on two rootstocks with contrasting features: 101/14 a “weak” rootstock and 1103 Paulsen a “vigorous” one. Leaf samples were collected on September 2006 taking three replicates for each condition/treatment.

The total RNA of the eighteen samples were extracted, subjected to quality control and afterward the corresponding c-RNA were synthesized and labelled. The c-RNA samples were then subjected to array analysis with the Vitis vinifera GeneChip® array from Affymetrix carrying about 17,000 probe sets from grape genes. The poster will present the preliminary data on genes and pathways up or down regulated in the samples grown under different grafting and soil conditions.
TOLERANCE OF *POPULUS ALBA* TO ULTRAVIOLET–B RADIATION


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global change, UVB, Poplar, microarray, physiology

The Earth’s atmosphere is getting polluted by anthropogenic pollutants such as chlorofluorocarbons. As a result, the stratospheric ozone layer is depleting, causing an increase in solar ultraviolet-B radiation on the Earth’s surface leading, possibly, to morphological and biochemical alterations in plants. Despite genome stability of all living organisms, the DNA is subject to damage by chemical and physical environmental agents. Fresh air (oxygen) and sunshine (UV) are undoubtedly the two main genotoxic environmental agents for most organisms, and plants are obliged to be exposed to both of these mutagens. UV-B radiation of sunlight penetrates and damages their genome by inducing oxidative damage and cross-links (CPD) that affect growth and development. Nevertheless, plants have developed protective mechanisms to cope with potentially harmful effect of UVB such as screening the solar radiation through the production of UV-absorbing compound, reflecting UV radiation by epicuticular waxes and cuticular structures, scavenging ROS through enzymatic and non enzymatic processes and repairing DNA damages.

The present study, based on a combined approach of gene expression profiling (microarray, qRT PCR) and physiological evaluation of whole plant response (photosynthetic efficiency, morphological parameters, fluorescence microscopy, pigment content, etc.) shows that UVB radiation penetrates plant tissues and induces the down expression of the replication, transcription and translation functions and the slowing down of other molecular processes such as photosystems turnover and electron transport chain. Morphological and physiological parameters strongly support gene expression data. The results here brought show that, close to a situation of total block of cell metabolism (cellular cycle) after an artificial UVB treatment showing a damage additive effect, the cell responds with the activation of different post-translational events that overcome the immediate damage (ROS scavenging) and stimulate modification of leaf ultrastructure and leaf reflective properties (accumulation of phenylpropanoid compounds) up to the reactivation of the same photosynthesis with an overall meaning of acclimation to the stress.
CIS REGULATION OF GENES INVOLVED IN UVB STRESS IN POPLAR

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cis -DNA, UVB stress, Populus alba, stress tolerance genes

In the last decade, poplar has become the plant model for forest trees. The availability of the sequenced genome of *Populus trichocarpa* makes it attractive for studies of the mechanism of gene regulation. cis and trans regulatory changes contribute to divergent gene expression and by that modulate phenotypic variation. However their respective contributions remains largeley unknown.

It has been suggested that cis regulatory variation is the primary substrate for the evolution of the species and that the non-coding regulatory DNA evolves faster than coding regions proposing it as the genetic basis of phenotype adaptation to different environments.

We estimated frequencies and magnitudes of cis-acting regulatory variation in *P. alba* in order to investigate the molecular mechanism of transcriptional regulation of genes involved in the mechanism of UV-B stress tolerance since studies in maize and *Arabidopsis* demonstrated that cis-acting regulatory variation is also relevant to abiotic stress response. In addition we characterized the 5’-flanking region of these genes to search for the possible polymorphisms in the proximal promoter region that could cause cis-variation.

To detect cis-acting regulatory variation we used a method that has been developed and widely used in our lab in maize. The method involves the study of two alleles of a gene in heterozygous individuals and the comparison of the transcript expression associated with each allele. This allows recognition of cis-acting variation without the identification of specific regulatory variants. SNP markers (Single Nucleotide polymorphisms) in the transcript itself are used to distinguish between transcripts derived from one of the two alleles. To study the regulation of genes involved in UV-B stress resistance we isolated and retrotanscribed RNA from the petiole-induced calli after UV-B treatment, as well as from their respective controls. We found that expression pattern of UV-B resistance related genes changes upon UV-B treatment: PHR1 expression was induced, while ATM was repressed by UV-B exposure. Out of 8 genes tested only ATM and qUVR10 showed differential allelic expression in some individuals. It is interesting to note that the qUVR10 variation in allelic-expression was induced by UV-B stress. Upstream region of ATM and qUVR10 were analysed to identify putative cis regulatory motifs using web resource such as Phylogenetic footprinting, NSITE-PL and PLACE programs. This study will help us not only to appreciate the extent of functionally important regulatory variation but also to focus on candidate haplotypes with differential expression in order to characterize specific polymorphisms involved in the UVB response.
INVOLVEMENT OF A WALL-ASSOCIATED KINASE AND A WRKY TRANSCRIPTION FACTOR IN TWO *POPULUS* SPP. OZONE STRESS RESPONSE

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ozone stress, *Populus*, WRKY transcription factor, Wall-associated kinase

As a result of anthropogenic activities, concentrations of tropospheric ozone (O_3) have increased during recent decades. Ozone is now considered to be the most phytotoxic of all the common air pollutants. Plants exposed to environmental changes due to pollution undergo suitable changes in gene expression. Depending on its concentration and plant species, ozone causes two different types of responses commonly referred to as acute and chronic. The acute ozone response is considered an excellent tool to study ozone tolerance and susceptibility among model plants that is important to identifying tolerant genotypes.

To improve the knowledge about the molecular mechanisms of acute ozone stress response and tolerance at the level of gene expression in two hybrid poplar clones (*Populus deltoides x maximowiczii*, Eridano clone, and *Populus x euoramericana*, I-214 clone, sensitive and tolerant to O_3, respectively) a gene identification study was previously performed using suppression subtractive hybridisation (SSH). Several differentially expressed cDNA were isolated and sequenced transcripts were subdivided in seven main functional categories such as signal transduction, disease/defence, metabolism and secondary metabolism, energy, cell cycle and DNA processing, protein synthesis and fate and unknown genes.

We obtained interesting data from expression analysis of transcripts belonging to signal transduction category (Wall associated kinase, Ft32C-WAK, Calmodulin-like protein, Ft33B-CaBP, WRKY transcription factor, Ft312B-WRKY and Leucine-rich repeat protein, Fs23A-LRP). Particularly, we observed that the steady state level of Ft32C-WAK transcript was increased by O_3 treatment only in tolerant poplar plantlets. Time course expression analysis shows that Ft32C-WAK up-regulation occurs after 2 h of O_3 exposure and continues since 5 h O_3 in tolerant poplar. On the contrary, expression analysis performed on O_3 treated sensitive poplar plantlets shows a weak Ft32C-WAK up-regulation after 5 h of O_3 treatment. Intriguingly, Ft312B-WRKY transcript shows the same Ft32C-WAK expression behaviour in tolerant poplar, suggesting a link between the two protein functions. According to these observations the hypothesis of an interaction between Ft312B-WRKY transcription factor and Ft32C-WAK promoter region was considered. WAK is a sub-family of plant receptor-like protein kinases (RLKs) involved in signal transduction during stress response. Given that the promoter regions of several RLK family genes contain W-box, which represents a DNA sequence motif specifically recognized by WRKY superfamily members, Ft32C-WAK upstream region was isolated.
These investigations will allow to understand if the activation of Ft32C-WAK during defence response to O₃ stress in tolerant poplar is Ft312B-WRKY dependent and to clarify the different behaviour of the two transcripts in sensitive versus tolerant poplar species.
A GENETIC CONSTRUCT FOR IMPROVING HEAVY METAL PHYTOEXTRACTION IN HIGH YIELDING CROP PLANTS

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phytoextraction, heavy metals, transgenic plants, polyhistidine

The environmental contamination by heavy metals is becoming an intriguing topic. Despite the presence of hyperaccumulators the phytoremediation cannot be based only on these particular species, mainly because of their very low growth rate and very specific tolerance to only one or few metals. On the other hand most contaminated soils are enriched with several metals. Furthermore environmental conditions and the presence of different metals affect negatively the efficiency of hyperaccumulators.

In order to improve the use of high yielding crop plants in phytoextraction we have chosen a genetic transformation approach using Nicotiana tabacum var. Xanthi as model plant. We designed and inserted into tobacco explants a powerful genetic construct coding for a polyhistidine (polyhis) tag with chelating properties. The polyhis is able to bind a variety of bivalent cations such as some heavy metals: Cd, Pb, Hg, Cr, Zn. The beta-D-glucuronidase (uidA) was used as scaffold protein. This enzyme has been widely used in genetic transformation because of its expression stability and absence of side effects on plant metabolism. The correct folding of the protein can be expected to improve the action of the polyhis tag. Moreover the gene construct included a modified 5’-UTR sequence (patent pending) that allows a higher expression activity. The construct is driven by a double enhanced CaMV 35S promoter.

In preliminary experiments we found that the expression of the polyhis in cell cytoplasm may interfere with correct plant development, probably because of polyhis involvement in metabolic pathways. For this reason a sequence for a signal peptide (S.P.) was added in the gene construct between the 5’-UTR and the uidA-polyhis gene. This S. P. derives from a human immunoglobulin and it’s expected to guide the heterologous protein through the endoplasmic reticulum towards the apoplast. The new location of the heterologous peptide is expected to significantly reduce the toxic effect on the genetically modified lines of tobacco.

The comparison of genetically modified lines with control plants are in progress.
MAPPING AND MOLECULAR CHARACTERISATION OF PARALOGS OF CANDIDATE GENES FOR CADMIUM TOLERANCE IN BLACK POPLAR


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candidate genes, paralogs, Populus nigra, cadmium tolerance

Emissions of wastes from variable origins into the environment has led to soil contamination in many sites, mainly by heavy metals. The phytoremediation is emerging as a cost-effective and environmentally friendly technology to remove these pollutants. Poplar is a suitable candidate for phytoremediation because of its rapid growth, ease of propagation, and high biomass production. Moreover, poplar presents a high genetic variability inter- and intra-species and its genome has been entirely sequenced. The availability of the poplar genome sequence is a great progress for the identification of the genetic determinants of environmental adaptation.

The objective of this work is the identification of the allelic variation associated with the tolerance to heavy metals. The strategy is based on the comparison of two highly divergent Populus nigra genotypes that also present a different tolerance to cadmium stress. A mapping pedigree was obtained by controlled cross between these genotypes and used for the construction of genetic maps. The candidate genes characterised in this study are involved in metal sequestration (metallothionein 2a), ion transport (vacuolar H⁺-ATPase), and response to oxidative stress (ascorbate peroxidase 2, glutathione reductase, glutathione S-transferase). In a previous experiment, these genes presented a differential expression among two genotypes of Populus alba submitted to salt stress. They are likely involved in the intra-specific variability of stress tolerance. As the Populus genome is highly duplicated, for each candidate gene the presence of paralogs was checked in the Populus trichocarpa genome database. When paralogs were found, specific primers allowing their discrimination were designed in order to amplify and sequence a fragment of each gene copy. The sequences were used to identify allelic polymorphisms (SNPs), develop SNP markers, and map the genes by allele-specific PCR. The different gene paralogs were individually mapped on the expected linkage groups according to the Populus trichocarpa genome sequence. This strategy aims at obtaining a genetic map rich of candidate genes to dissect cadmium tolerance by QTL analysis. The next step will be to investigate the functional role of the candidate genes in response to cadmium stress. The availability of paralog-specific markers will allow to analyse separately their expression in the two Populus nigra genotypes under stress.
EFFECTS OF CADMIUM ON THE ROOT GROWTH OF THE ELITE WHITE POPLAR CLONE ‘VILLAFRANCA’


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cadmium, poplar, root, tolerance, apoptosis

Cadmium is one of the most toxic heavy metal pollutants in nature. Extensive studies have been carried out to explain the cellular effects of Cd in animal cells both in vivo and in vitro whereas some aspects of its effect on plants are still under discussion (Lakimova et al., 2005; Vassilev et al., 2002). Poplars and willows have an high potential to clean-up sites contaminated by heavy metals (Vassilev et al., 2002). For this reason we transformed an elite white poplar clone ‘Villafranca’ with a gene (trx) encoding an artificial metallothionein to evaluate his tolerance to cadmium by in-vitro and in-vivo experimental trials. We tested several Cd concentrations and determined the Cd-content in roots, leaves and stems by ICP–MS. When the plants were grown in vitro, no statistically significant differences were observed between the root dry weight of the plants cultivated on medium treated with 180 µM Cadmium Sulfate and the root dry weight of the controls. The plants grown on 120 µM Cadmium sulfate showed, on average, a root dry weight significantly higher than the plants of the other treatments. The Cd-content was higher in the roots than in the leaves and stems for each treatment and the highest content of all was found at 120 µM. A significant increase of the dry weight of the roots at 120 µM Cadmium Sulfate had also been observed in a previous Cd-tolerance test on the P. alba clone ‘Villafranca’. At increasing Cd concentrations (0, 20, 50 and 100 ppm) no statistically significant differences were observed among the dry weight of the roots after six months of growth in pots containing soil enriched with cadmium nitrate. However for two of the lines tested, the dry root weight was higher at 50 and 100 ppm Cd. The Cd-content in roots increased significantly with the growing level of Cd. The subcellular localization of cadmium, carried out using EELS connected to the TEM, on the root cells of a transgenic and a control line grown on 100 ppm Cd, showed the presence of Cd in the nucleus. No cellular apoptosis phenomenon was observed. Schützendübel et al. showed that a low concentration of Cd (5 µM) stimulates root growth in poplar but higher concentrations lead to a drastic reduction of root growth (Cosio et al., 2006). At cellular level, Cd induces oxidative stress and apoptosis in both plant and animal cells (Hamada et al., 1997; Lakimova et al., 2005). Hamada et al. demonstrated that apoptosis following Cd exposure in mammalian cells is associated with intracellular movement of Cd and metallothioneins. On the basis of our results we speculate about the role of metallothioneins in the mechanisms of Cd-tolerance in poplar roots. Further studies are currently in progress to investigate the role of metallothioneins in Cd translocation into the nucleus and Cd-induced dose-dependant apoptosis.
IDENTIFICATION OF ANALYTICAL AND MOLECULAR DESCRIPTORS FOR HEAVY METALS DECONTAMINATION IN POPLAR SPECIES: A GENOMIC APPROACH

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heavy metals, cadmium tolerance, genetic variability, molecular markers, phylogenetic analyses

The dispersal of trace elements, heavy metals included, and of organic contaminants into the environment is a relevant factor in environmental contamination capable of compromising soil and water utilisation. The use of particular plant species has been designed to decrease or remove the contaminants in polluted sites: a range of strategies and technologies, collectively defined as "phytoremediation". At the moment 45 species of plants are described as hyperaccumulating plants for different metal and naturally they grow on metal-rich soils whereas accumulation capacities towards other metals as Cd have not been clearly demonstrated [1, 2]. The practical application of hyperaccumulators to phytoremediation is however made difficult by their physiological features, such as little biomass at maturity and reduce growth rates, for these reasons now the researchers are considering plants more important from an economical point of view and with higher biomass such as willows and poplars. A particular approach for studying genetic variability is the analysis of the so called "useful variation" [3], that means the evaluation of the genetic diversity related with specific phenotypic features of the individual. The rationale is that in genotypes, which are tolerant towards contaminants, or able to accumulate them, natural selection has brought to a genetic modification which leads to an increased adaptation of individuals to specific soil characteristics. The genetic modification may be particularly relevant in “candidate genes”, i.e. those genes whose function is directly involved in coping with the contaminant. The evaluation of "useful" genetic diversity is based on the search for polymorphisms through the utilisation of molecular markers related to “candidate genes”, directly or indirectly involved in adaptation and response to contaminants. We considered 11 Poplar clones and we analysed 9 “candidate genes” choose between genes related to Cd uptake, translocation, sequestration, and chelation on A. thaliana and Thlaspi sp databases. Primers for gene sequencing in poplars were designed on genomic DNA for Populus trichocarpa nisqually 1 present among our set of plant samples and whose genome is available on-line. The obtained data were analysed to identify SNPs (Single Nucleotide polymorphism). During this phase, other molecular markers, as short nucleotide polymorphisms were found. A further branch of analysis in silico of the SNPs has been carrying on to evaluate aminoacid variations due to point mutations and the consequent effect at the level of protein structure. These data will be correlated with physiological and biochemical analyses to choose the best traits for phytoremediation purposes.

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THE BRASSICA JUNCEA TRANSCRIPTION FACTOR BjCdR15 ENHANCES CADMIUM TOLERANCE, ACCUMULATION AND TRANSPORT TO THE SHOOT IN TRANSGENIC PLANTS

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Brassica juncea, bZIP transcription factor, Cadmium tolerance, phytoremediation

Phytoremediation is considered as a cost effective and environmentally friendly technology to remove toxic metals from polluted soils and waters. Molecularly, our knowledge about metal tolerance and hyperaccumulation in plants remain limited, and the investigation is still needed to understand the genetic mechanisms responsible for metals accumulation and detoxification.

Previous analysis showed that BjCdR15, a gene isolated from B. juncea, is up-regulated in plants treated with cadmium. Hence, the aim of this study was to investigate whether BjCdR15 is involved in Cd tolerance and accumulation. This gene shows high similarity to the Arabidopsis TGA3 bZIP transcription factor (nucleotides and amino acids identity of 89% and 95% respectively) therefore, tga3 mutant line was also analysed. Expression analysis showed that BjCdR15 is immediately induced after Cd, Ni and Pb exposure indicating a general function in response to heavy metals. Moreover, cell harboring BjCdR15::dsRED fusion protein indicated that BjCdR15 is a nuclear-localized protein and in situ localization showed that BjCdR15 transcript is mainly present in epidermis and vascular tissues.

Arabidopsis and tobacco plants over-expressing BjCdR15 were greener than control plants and showed enhanced tolerance, measured as shoot fresh weight and chlorophyll content when exposed to Cd. Furthermore, BjCdR15-over-expressing plants accumulated more Cd in shoots than control plants. It was also found that in tga3 mutant line the long-distance transport from root-to-shoot is inhibited and Cd accumulates in the roots; when the function of TGA3 is restored by BjCdR15 the level of Cd in shoots and roots is comparable to the accumulation of the over-expressing lines. Since phytochelatins are rapidly synthesized in response to toxic level of heavy metals, the expression of the Arabidopsis phytochelatin synthase (AtPCS1) was measured in 35S::BjCdR15 and control lines. It was observed that, as expected, Cd induced an increase of AtPCS1 in control lines whereas its level is not affected by Cd treatment in transgenic lines. This study provides several lines of evidence to implicate BjCdR15 in Cd tolerance in plants and accumulation to the shoots and demonstrates the biotechnological potential of removing heavy metals from contaminated soils by growing transgenic plants with enhanced capacity to accumulate toxic metals in their above ground.
CHARACTERIZATION OF AtMYB59 TRANSCRIPTION FACTOR

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Myb transcription factor, Cadmium, RNA Interference

Heavy metals and metalloids such as Hg, As, Cd and Pb are extremely toxic, and pollution caused by these metals is a major environmental concern. Cadmium, in particular, is also strongly toxic to plants, causes growth inhibition, and may cause plant death by interfering with important biochemical pathways. Our work is focusing on the molecular characterization of an *Arabidopsis* Myb transcription factor induced by Cd.

AtMyb59 encodes a transcription factor belonging to the R2R3MYB family and it is present in three splicing variants (Myb59-1, Myb59-2 and Myb59-3). Northern analysis showed the expression of Myb59 in all plant organs. Furthermore, Real Time-PCR analysis allowed to measure transcription levels of the three Myb59 variants. Promoter analysis showed that Myb59-2 is expressed only at a particular stage of microspore development whereas Myb59-3 gene product was found in roots and vegetative tissues. The ectopic expression of Myb59 had an effect on vegetative growth: plants transformed with 35S::Myb59-1 showed an increased leaf area compare to control plant.

Furthermore, transgenic tobacco and *Arabidopsis* plants overexpressing Myb59 were able to tolerate up to 200 µM Cd(NO₃)₂ in solidified agar medium without showing chlorosis symptoms or growth reduction, while WT plants were severely affected by Cd treatment. Since this transcription factor is modulated by Cd, the effect of other abiotic stresses was also studied. It was observed that Myb59 is also induced by ABA, cold and drought treatments. Myb59 mutans were not available, therefore, plants transformed with a construct for RNA Interference-Induced silencing are under investigation.
CADMIUM-INDUCED CELL DEATH IN ARABIDOPSIS CELL CULTURES

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cadmium, phytochelatin, nitric oxide, cell death, cell cultures

Cadmium is a toxic pollutant. Plants react to cadmium exposure by producing phytochelatins, a class of metal-chelating oligopeptides. Arabidopsis cell suspension cultures were exposed to 20-150 µM cadmium, and growth and cell viability were measured at different days after treatments. In presence of cadmium, cells reduced their growth and eventually died, in a dose-dependant fashion. At lower cadmium concentrations, Arabidopsis cells blocked their growth and died only if a pre-treatment with the phytochelatin-synthesis inhibitor buthionine-sulfoximine (BSO) was performed. Cadmium triggered a senescence-like process, as the nuclear condensation and the expression of the senescence-specific genetic marker SAG12 suggested. Cadmium-induced cell death was shown to involve both nitric oxide (NO) and reactive oxygen species (ROS) as intermediates. Prevention of NO synthesis reduced or delayed ROS production and eventually cell death, indicating that this molecule is an important actor in the toxicity of cadmium. Phytochelatin contents were quantified and a correlation with cell death observed.
Plants tolerate excess of heavy metals through mechanisms acting at two different levels: metal exclusion in the root or metal accumulation followed by compartmentalisation or sequestration of metal ions within lignified structures or complexation with organic chelates [1, 2]. The effects of metal ions (microelements and toxic metals) on plant proteomes are still mostly unexplored even if proteomic profiling has been used to study the effects of several biotic and abiotic stress factors in plants. In this work we utilised a proteomic approach in the effort to understand the functional modifications occurring in *Thlaspi caerulescens* (a natural Zn/Cd/Ni hyperaccumulator) subjected to different Ni treatments. In particular, seeds of plants collected at three different sites of Monte Prinzera (Parma Province, Italy) a serpentine hill rich in Ni, Fe and Co were grown in hydroponics with 0 or 10mM Ni. Mineral analysis was performed on soil samples from different sites and on leaves and roots of these plants that showed also in control conditions phenotypically distinct characters. Proteins’ fingerprintings of leaves and roots of two months old plants were compared to underline the executors’ differences occurring in *Thlaspi caerulescens* grown on different sites of Monte Prinzera.

2D liquid chromatography technique was utilised in spite of the classical 2DE analysis. Qualitative and quantitative differences between protein profiles of treated and untreated samples were evidenced by DeltaVue Software (Eprogen). Proteins more differently expressed in various conditions will be further characterised by MALDI-TOF/MS to infer on their possible role in metal response.

The results will be discussed with the aim to understand the different processes at the basis of hyperaccumulation, tolerance of metal and the plant-environment interaction.

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STUDY OF AN ARABIDOPSIS ABC1-LIKE GENE

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ABC1domain, cadmium

In previous work cDNA-AFLP technique was employed for the identification of genes modulated by cadmium. The Arabidopsis At3g07700, classified as a putative Abc1-like was selected, among the genes modulated by cadmium, for further investigation. It has an open reading frame of 695 amino acids and the protein sequence shows similarity to ABC1 protein family (activity of bc 1 complex) and with ABC transporters (ATP-binding cassette). The amino acids sequence contains an ABC1 domain and two transmembrane regions. In wild-type plant this Abc1-like gene is induced immediately after cadmium, nickel and selenium exposure. Its expression is not modulated by other abiotic stresses and hormonal treatments (ABA and IAA). Promoter study using the GUS gene as a marker showed GUS activity in cotyledons, leaf hydathodes and tricomes, and roots. In flowers GUS staining was observed in sepals and anthers. We identified mutant line (SALK_020431) harbored a T-DNA in the coding region and transcript analysis indicated a complete loss of the gene function. Under standard growth conditions, mutant plants showed normal phenotype, however when plants were grown in vitro, seedlings of mutant plants presented roots longer than wild-type. In addition, seed germination and stomatal response to light were different in mutant and wild-type. Over-expression of At3g07700 and mutant complementation are under investigation.
TRANSCRIPTIONAL REGULATION UNDER SULFUR STARVATION IN ARABIDOPSIS AND MAIZE


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sulphur starvation, RT-PCR, microRNA

Sulfur is an essential macronutrient for plants growth; it is required for stress-related metabolites and protein biosynthesis. Sulfur assimilation is a multi-step pathway whose first step consists in the uptake of inorganic sulfate from soil. Under S-starvation, early uptake response is mediated by high-affinity sulfate transporter (ST1;1), and then sulfate is activated to adenosine 5'-phosphosulpate (APS) by ATP-Sulfurylase. Genes coding for these enzymes are key point of regulation in sulfur uptake. After activation, sulfate is either reduced for biosynthesis of cysteine or phosphorilated to produce secondary metabolites such as flavonoids. The family of sulfate transporters comprises many isoforms, belonging to 5 groups, according to their cellular localization and function. Arabidopsis and rice have 14 and 15 isoform respectively, whereas only the sequence of the ST1;1 isoform is known in maize.

Aim of this work is to relate transcription regulation of key genes of sulfur metabolism to S-Starvation. We analyzed maize inbred lines and their hybrid to investigate heterosis at gene expression level in sub-optimal growth conditions such as low macronutrient availability. We set out to determine whether gene expression levels of key genes were differently expressed at specific sulfur concentration of growth between inbred lines and hybrid by Real Time PCR. Data clearly show that the F1 hybrid responds much faster to stress condition than either parental lines.

We tested and identified different arabidopsis ecotypes tolerant or susceptible to S-Starvation. Given the accurate annotation of arabidopsis genome, we were able to study the expression levels of many sulfur transporters (ST1;1, ST1;2, ST2;1, ST5;2) and ATP-Sulfurylase by Real Time PCR in different ecotypes.

The existance of epigenetic regulation in these genes was also investigated. Northern analysis and NESTED-RACE-PCR suggest that miR395 regulates ATP-Sulfurylase in maize and arabidopsis.
TRANSCRIPTIONAL ANALYSIS OF GENES PUTATIVELY INVOLVED IN CADMIUM ACCUMULATION IN RICE

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rice, cadmium accumulation, ZIP genes

Heavy metals, including cadmium, are important soil and water pollutants, deriving from the use of ammendants, poor quality irrigation water and, in particular, mineral fertilizers. Besides being toxic to the plants themselves, cadmium may be adsorbed through the roots, translocated to the seedling and finally accumulated into edible parts such as kernels, with negative consequences on human health. In this regard rice is particularly at risk, due to the peculiar agricultural practices, the frequent proximity of pollution sources and its elevated translocation ability. In order to characterize the genetic basis of cadmium accumulation in rice and, in the long term, to reduce Cd uptake and storage in the grain, we have undertaken the transcription profiling of rice genes putatively involved in the transport of the metal into edible parts. So far, we focused our attention on a family of metal transporters coding genes, the ZIP family, involved in the transport of a variety of cations including cadmium, zinc, iron and manganese. In rice 10 ZIP genes have been reported. Here we present the first expression analysis, performed by Real Time RT PCR, of three of them, OsZIP1, OsZIP3 and OsZIP4, in roots and leaves of control and Cd treated plants of five cultivars; three high- (Loto, Nembo and Gladio) and two low- (Roma and Volano) cadmium accumulators. In all cultivars, in control plants, ZIP1 was expressed in roots but barely or not at all in leaves. Viceversa for ZIP3 and ZIP4. In the competent tissue, the response to Cd was gene-cultivar specific: in Gladio, Nembo and Volano ZIP1 was induced while ZIP3 and ZIP4 were downregulated. On the contrary, in Roma and Loto ZIP1 was downregulated whereas ZIP3 and ZIP4 were induced. No correlation between the expression of the three genes, both in control and Cd treated plants, and the metal accumulation characteristics of the different cultivars was observed. Thus, at present, no conclusions can be drawn about a possible role of these ZIP genes in the transport/accumulation of cadmium into rice grains. To better understand the mechanisms subtending cadmium adsorption, transport and accumulation in the grain, additional genes of the ZIP family as well as genes from other metal transporter families are being investigated.
WASTEWATER PHYTOREMEDIATION: GENOMIC ANALYSIS AND SCREENING OF GREEN MICROALGAE SPECIES FOR EXTRACELLULAR LACCASE ACTIVITY


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microalgae, laccase, ABTS, phytoremediation

Phytoremediation deals with the use of plants, or other green photosynthetic organisms, to reduce organic or inorganic pollutant in the environment, mainly waters and soils (Pivetz. B.E. 2001. EPA /540/S-01/500). Our research group is involved in studies regarding the use of plants for soil phytoremediation (Galante et al., 2005. Proc. SIGA Congress, L04) and, more recently, of unicellular green algae species (green microalgae) for wastewater treatments. Some microalgae species have been recently tested to degrade an array of pollutants such as phenols, polyphenolic aromatic compounds (PAH) and even hormones (Pollio et.al, 1994. Phytochemistry, 37:1269-1272;. Pinto et.al., 2003. Biotechnol Lett., 25:1657-1659). It is worthy to note that about 2,500 species belong from Chlorophyceae, seldom living in contrasting habitat under severe environmental conditions. So far, a little has been done to exploit this genetic biodiversity bonanza; thus, few reports have been published on enzymes implicated in their degradative action (Semple et.al., 1996. Appl. Envr. Micr, 62:1265-1273).

Since a wide collection of green microalgae species are available at the University of Naples, Department of Biological Science, recently we have started a research aimed to (a) find algae species with extracellular phenoloxidase enzymatic activity; (b) identify extracellular enzymes able to degrade xenobiotic like synthetic dyes and other PAHs; (c) clone and overexpress genes producing phenoloxidases in homologous and in heterologous systems, in order to use these enzymes primarily for phytoremediation of milling oil wastewaters. Among phenoloxidaes, we focused our interest on laccases (EC 1.10.3.2) that are phenol-oxidoreductases able to catalyze the oxidation of various aromatic compounds (particularly phenols) with the concomitant reduction of oxygen to water.

Selected algae strains were grown in liquid culture at 22°C under continuous light conditions, starting with an inoculum of 0.1 OD. After ten days, the algal growth was measured as optical density at 600 nm. A screening was performed by detecting the laccase activity in the broth medium culture, deprived of algae cells, in the presence of 2,2-azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) at 420 nm. The laccase activity was referred to the polyphenol oxidase activity of Trametes versicolor; thus, each positive strain was assayed on industrial azo-dye Remazol Brilliant Blue R (RBBR) and on the natural phenol compound syringaldazine by kinetic analysis. Preliminary results, obtained comparing different species, showed a wide variation both within the same substrate and among the different microalgae.
Microalgae strains able to produce and secrete laccase enzymes were further chosen for more
detailed genetic studies. To clone phenoloxidase genes from those species, we have started a bio-
informatics approach, on the basis of highly conserved coding sequences of laccases already
isolated and sequenced from several higher plants. Primers drawn on the alignment of those
sequences have been used to amplify genomic DNA.
MOLECULAR MAPPING FOR SALINITY TOLERANCE IN RICE

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molecular mapping, salinity tolerance, QTLs, SSR

It is estimated that saline soils cover from 400 to 950 million hectares of Earth’s surface. Accumulation of salt in the soil causes deleterious effects and leads to a reduction in plant production. Improvement of salt tolerance is one of the most important objectives of rice breeding-programs in coastal areas. The broad aim of the current study is the identification of genes for salt tolerance in rice in order to develop salt tolerance genotypes. 300 genotypes were randomly chosen in a F2 population from the cross(TCCP266-1-2 x Giza177) (TCCP266-1-2 = Indica Tolerant variety) (Giza177=Japonica Sensitive variety) and grown in summer 2006 to produce F3 seeds. DNA was extracted from each of the F2 and used to construct a linkage map, using 185 out of 272 SSR markers that showed polymorphism between the two parental genotypes. Plots of F3 plants are presently in the field for phenotyping.
TRANSCRIPTIONAL CONTROL OF ASCORBATE METABOLISM IN SALT STRESSED ARABIDOPSIS THALIANA

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ascorbate, phenolics, reactive oxygen species, salt stress, transcriptional profiling

Antioxidants, such as ascorbate and phenolics, play a crucial role in controlling the level of reactive oxygen species (ROS). Stresses cause an increase in antioxidant demand, leading regulatory mechanisms to be exploited to enhance antioxidant biosynthetic flux. Ascorbate is synthesized in plant through alternative pathways, which may differentially contribute to its pool size according to the adaptation to specific environmental stresses.

The aim of this work is to profile transcriptional modifications within ascorbate metabolism as a useful approach to identify both key genes and their controlling relationship driving the stress response. Four-week old Arabidopsis plants were supplied with 300mM NaCl and leaves were sampled 6 hour and 12 hour after the application. Interestingly, phenols concentration significantly increased whereas stressed plants showed ascorbate pool to significantly inflect.

The level of transcription of ascorbate genes in stressed plants compared to the placebo treatment was assayed by qPCR approach using a library of 48 validated primer pairs. The salt stress application dramatically affected the expression of many genes. Particularly, putative ascorbate peroxidase, ascorbate oxidase and dehydroascorbate reductase genes showed a 10-fold increase of the transcription level. In addition, a D-galacturonate reductase transcript displayed a 100-fold increase suggesting a preferentially induction of the D-galacturonate pathway of the ascorbate biosynthesis following salt stress application. In order to identify co-regulation patterns within ascorbate transcripts, a hyerarchical clustering was used to group genes according to their regulation. Putative phosphodiesterase, D-galacturonate reductase and ascorbate peroxidase descreased their over-espression 6 hour to 12 hour post application.

In conclusion, the transcription profiling of genes putatively involved in the ascorbate metabolism revealed key transcriptional control points of specific pathways. Further profiling in plants treated with different stresses may help to better understand the antioxidant response to the adaptation demand.
THE OVER-EXPRESSION OF SYSTEMIN IN TOMATO IMPROVES THE AGRONOMIC PERFORMANCE OF PLANTS GROWN UNDER SALT STRESS CONDITIONS


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cross tolerance, Lycopersicon esculentum, prosystemin

Plants undergo continuous exposure to several different biotic and abiotic stresses in their natural environment. To survive under such conditions, they have evolved intricate mechanisms to perceive external signals and develop optimal responses to environmental conditions. To date, the molecular mechanisms that are involved in plant response to different stresses have been studied independently, while only recently novel studies have revealed the existence of cross-tolerance among the plant responses to different stresses. A relationship between the reaction to salt-stress and to mechanical damage such as wounding has been demonstrated in tomato. Plants exposed to salt stress accumulated proteinase inhibitors and activated other wound-related genes. Similarly, mechanical wounding increases salt-stress tolerance through a mechanism that involves the signalling peptide systemin and calmodulin-like activities.

We investigated the physiological and growth performance of tomato plants over-expressing the systemin peptide grown under salt stress (20 and 40 mM NaCl in the irrigation water). Systemin over-expressing plants (BBS) had a decreased stomatal conductance in absence of stress. Nevertheless, upon salinization, the stomatal conductance was more reduced in the wild type controls (WT) than in BBS plants. It is likely that such effect is a result of a higher hydration state of BBS plants whose leaf water potential was slightly higher than WT plants. Consistently, the leaf concentration of ABA and proline, two molecules that typically accumulate in response to salt stress, were lower in stressed BBS plants compared to WT. The result of such performance may explain the higher biomass production observed in BBS plants grown under salt stress. Exogenous applications of proline (5 mM in the irrigation water) enhanced the salt tolerance of BBS whereas it caused some detrimental effect in WT plants. Comparative gene expression profiling has been performed to highlight possible cross talk between genes involved in wounding and osmotic stress adaptation pathways in tomato.
ARRAY ANALYSIS OF DROUGHT RESPONSE IN BREAD AND DURUM WHEAT AT GRAIN FILLING STAGE


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Triticum durum, drought resistance, array analysis, drought responsive genes

Drought tolerance is a complex trait where many morpho-physiological mechanisms are involved in the response/adaptation processes are supported by a complete reorganization of the cell transcriptome. To provide a global study of the changes in gene expression we have investigated the transcriptome of bread (T. aestivum cv Chinese Spring and its deletion line CS-5AL) and durum wheat (T. turgidum, cvs. Creso and Trinakria) subjected to two different levels of water stress (mild and severe stress) at the grain filling stage. Expression study was conducted with the Affymetrix 61K wheat chip on three biological replicates of mRNA extracted from flag leaves and glumes. The t-test comparing analysis between control and stressed samples have shown more than 1,000 genes up or down –regulated in each genotype. When the stress responses of tetraploid and hexaploid wheat were compared we have found that under mild stress conditions the molecular response involved much more genes in the hexaploid than in the tetraploid wheat. Data-mining on the expression data have identified some known drought response pathways (i.e. biosynthesis pathway of ABA, proline, sorbitol, glycine betaine) as well as a number of transcription factors whose expression can be associated to stress resistance. The higher number of expressed genes detected in the hexaploid bread wheat compared to the tetraploid durum wheat allowed the identification of sequences putatively located on the D genome. In the same way the comparison between Chinese Spring and CS-5AL allowed the finding of putative sequences belong to long arm of 5A chromosome. The poster will present data on genes and pathways up- or down–regulated in the different specie and cultivars and clustering organization of the genes depending on the intensity of the water stress applied.
EVALUATION OF DURUM WHEAT BIODIVERSITY FOR PRODUCTIVITY IN ENVIRONMENTS WITH DIFFERENT WATER SUPPLIES


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durum wheat, water stress, yield stability

The Mediterranean region is characterised by drought stress limiting grain yield in quantitative and qualitative terms. A general tendency toward decreasing mean rainfall has been observed and suggest that plant more tolerant to water stress are needed in order to limit yield decrease in the light of future climate changes. Furthermore, drought events are irregular and unpredictable, therefore the durum wheat ideotype for this environment should be able to produce in water limiting conditions but also to take advantage from a possible water supply.

In order to study the effects of different levels of water availability during the whole life cycle on plant production, a field trial was performed with a collection of durum wheat genotypes including modern varieties with high productivity value, local populations, old cultivars and genotypes selected in arid environments. The selected genotypes have been grown for three years in three locations, Foggia, Catania and Fiorenzuola, characterised by different water regimes. In Foggia, an environment characterised by a mild water stress, the trial was carried out under rainfed conditions or with supplementary irrigation.

The phenotypic characterization based on the analysis of yield performance and other agronomics traits under the different tested conditions allowed to identify genotypes with good yield and a minimal G x E interaction, so as genotypes with both high yield potential and high yield stability. These genotypes could be considered very close to the durum wheat ideotype for environments characterised by a mild water deficit, so as those of Mediterranean Europe, where durum wheat is traditionally grown.
WATER USE AND WATER USE EFFICIENCY IN TWO DURUM WHEAT CULTIVARS USED AS PARENTS IN A MAPPING POPULATION


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drought, durum wheat, stomatal conductance

The aim of this work was to analyse the response to water stress in two durum wheat cultivars (“Ofanto” and “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. Gas exchange and mass accumulation were measured throughout the experiment. “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”. The different behaviour of the two genotypes was consistent with the results of field experiments conducted at Foggia (Southern Italy) in rainfed conditions and with supplementary irrigation. Leaf temperature measured at anthesis on flag leaf using an infrared thermometer showed a trend for lower leaf temperatures in “Ofanto”. Carbon discrimination (D), analysed in the grains harvested from the field trials as a measure of the integrated WUE during the kernel development (Farquahar and Richards, 1984 Aust. J. Plant Physiol. 11: 539-552, 1984), was significantly higher in “Ofanto” than in “Cappelli”, in both rainfed and irrigated trials. The overall results agree in suggesting a constitutive difference in stomatal responses in “Ofanto” and “Cappelli”, with consequence for water use efficiency. These traits together with other physiological and yield associated traits will be studied in the segregating population to verify their association and their contribution to yield stability in presence of water stress.
CANDIDATE GENE ASSOCIATION MAPPING OF DROUGHT AND SALINITY RESISTANCE IN DURUM WHEAT

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association mapping, stress resistance, durum wheat

Association or linkage disequilibrium (LD) mapping is a strategy to identify associations between the alleles or haplotypes present in natural populations or collections of germplasm and the trait of interest. Association studies exploit recombination events accumulated over many generations, therefore only markers in LD with the causal allele will show statistical associations with the trait of interest, allowing to improve the mapping resolution. Marker-trait associations can be found either saturating the genome with markers (whole genome scan) or looking at variation in specific genes (candidate gene association mapping). We carried out a candidate gene association study to identify genes that are involved in the genetic control of drought and salinity resistance in durum wheat. Candidate genes were selected among those involved in the control of the trait of interest in Arabidopsis and rice. To verify the presence of polymorphic sites in the candidate gene sequence, genes were preliminarily sequenced on 12 inbred lines, that greatly differ in terms of genetic diversity and stress resistance. This preliminary analysis revealed the presence of polymorphic sites in 26 out of 72 genes sequenced resulting in a SNP frequency of 0.17%. Moreover, no significant decay of LD was observed in the sequenced regions. The polymorphic genes were then sequenced on the rest of the inbred lines that were characterized phenotypically during the project (87 inbred lines in total). In the association studies correction for population structure is essential to avoid spurious associations. The analysis of 20 SSR loci revealed the presence of 5 subpopulations in the germplasm considered and the estimates of population structure were incorporated in the association tests. We performed the association tests between 35 polymorphisms able to distinguish the haplotypic variations in the germplasm and 8 phenotypic traits (among others, yield, a thousand seeds weight and heading date). The LD mapping method allowed to detect statistical significant associations and to identify genes putatively responsible for the phenotypic variability. We present and discuss results of the association tests and of the further characterization of the genes associated with the phenotype, including the determination of their position and of the extent of the associated region.
QTLs FOR DROUGHT-RELATED TRAITS IN A DURUM WHEAT RIL POPULATION EVALUATED IN THE MEDITERRANEAN BASIN UNDER DIFFERENT WATER AVAILABILITY


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A better knowledge of the genetic basis of the developmental processes involved in tolerance to drought will allow breeders to more effectively improve yield in drought-prone environments, i.e. the vast majority of the durum wheat production area in the Mediterranean basin. Resistance to drought stress, especially in terms of yield stability under the various stress conditions, is a main objective for durum wheat improvement. In the frame of the IDuWUE (Improving Durum wheat for Water-Use Efficiency) project, a mapping population (249 RILs developed from the cross between the Italian cv. Svevo and the US cv. Kofa) was evaluated for yield (GY), its components and morpho-physiological traits in 16 field trials carried out in Mediterranean environments (Italy, Spain, Morocco, Tunisia, Syria and Lebanon) characterized by different water availabilities and yield potential. Large differences among trials were evident for the length of the cycle and water input with GY ranging from 0.5 to 5.8 ton ha⁻¹. A broad variation was shown by the RILs for most of the investigated traits; heritability values calculated across environments varied greatly (e.g. 0.95, 0.68 and 0.28 for heading date, GY and fertile tillers per m², respectively). Two quantitative trait loci (QTLs on chromosomes 2BL and 3BS), out of the 16 that affected GY, showed significant effects in eight and seven environments, with $R^2$ values equal to 21.5 and 13.8% (mean data of all 16 environments), respectively. In both cases, the LOD profile for GY overlapped with those for other traits such as kernel weight and plant height. Three major QTLs for heading date (chr. 2AS, 2BL, and 7BS) and the major QTL for plant height (chr. 1BS) showed limited or no effects on GY
and its components. Noticeable epistasis between the 2BL and 3BS QTL clusters was consistently detected across traits and environments. Epistasis favored the parental genotypes and negatively affected the performance of the recombinant genotypes. Trait-specific QTLs were also identified for all the considered traits. The effects of the 2BL and 3BS QTLs were fully validated in a set of 11 trials conducted in 2006. In view of the relevance and consistency of their effects on grain yield and other agronomically valuable traits, the 2BL and 3BS QTLs are being isogenized in order to proceed with their fine mapping and, on the basis of their effects on plant height and peduncle length, their positional cloning.
FUNCTIONAL ANALYSIS OF SATO, A DROUGHT STRESS RESPONSIVE GENE IN POTATO AND ARABIDOPSIS

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water stress tolerance, nuclear RNA-binding protein, gene expression and phenotypic analysis

Water deficit is one of the major limiting factors for crop productivity. Osmotic stress conditions affect many plant functions, including growth and development. Plants respond to stress signals by altering the expression of many genes, whose products have important reparative and protective functions. A previous transcriptome analysis (Ambrosone et al. 2006) indicated that distinctive changes in gene expression occur during adaptation to long term water stress. Among the genes identified as up regulated we found the EST AW906734 coding for gene sato, acronymous of SAlt TOlerance.

The EST AW906734, isolated from a potato stolon cDNA library, covers the 30% of the TC126154, composed by 9 EST with 90% of identity with a putative transcriptional factor of Vicia faba (GenBank O04273) and similarly to sato2 of B. vulgaris (AJ313093). Sato2 encode for a protein conferring salt resistance by complementation of a yeast defective mutant (Ros et al. unpublished) and contains a conserved RNA-binding protein domain. BlastP search reveals that the protein is highly conserved with more than 70% homology in several species as B. vulgaris, V. faba, S. olearia and A. thaliana.

The expression of sato gene was confirmed to be regulated by water stress which induced 2, 6 and 10 fold increase of sato transcript level in potato cells, leaves and roots respectively.

In order to get insights into the functional role of this gene in cellular response to osmotic stress, a preliminary characterization of the sato hortologous gene in Arabidopsis (Atsato, At4g16830) was carried out. At4g16830 is a nuclear RNA-binding protein of 355 aa containing a RGG box with unknown function and putatively localized in the nucleus and/or in the cytoplasm (www.arabidopsis.org). In Arabidopsis cells, sato was up-regulated by different stress treatments, in particular the expression was induced by 50 uM ABA, 150 mM NaCl and 10% PEG. A preliminary phenotypic analysis showed that seed germination of Atsato knock out (Atsato KO) was severely affected by ABA, NaCl and PEG treatments. Root elongation of Atsato KO was inhibited in presence of 0,2 uM ABA, 80 mM NaCl and in PEG treatments compared to wt Columbia (N60000) genotype. These results suggest a possible role of sato in ABA-mediated water stress response.

The generation of sato overexpressing /RNAi plants, the promoter analysis and protein localization are in progress to establish the biological role of sato and its contribution to water stress tolerance.
NEW INSIGHT INTO ROLD, A GENE THAT AFFECTS ROOTS AND AXILLARY BUDS GROWTH IN ARABIDOPSIS


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Arabidopsis, rolD, root, axillary bud meristem, SPS

rolD oncogene from A. rhizogenes induces strongly flowering potential in both day-neutral tobacco and tomato plants. (Mauro et al. 96, Bettini et al. 2003.)

In tomato, rolD could lead to an increased competence for defense response, as shown by toxin tolerance and increased expression of the systemic acquired resistance (SAR) marker gene PR-1. (Bettini et al. 2003).

In the arabidopsis plants transgenic for rolD we have previously show sign of a slight early flowering time and of an enhanced proliferation and growth of the basal leaf axillary buds due to cell meristematic post embryonic activity. Moreover primary rolD roots are longer and quite hairy, compared to the controls, meanwhile the number of basal hypocotyl roots is reduced.

In this progress we show that in transgenic rolD plants the SUPERSHOOT gene (SPS) is down-regulated during the juvenile phase. The SPS is a member of cytochrome P450 gene family that negatively regulates lateral buds induction and development due to an altered concentration of cytokinin and auxin.

We also report the analysis of rolD transgenic seedlings treated with cytokinin and auxin hormones and, in turn, with NPA, an inhibitor of auxin transport. Suggestions of data obtained will be discussed.

The analysis of the arabidopsis plants containing DR5-GUS and rolD genes are currently in progress to examine the auxin localization on plant organs and tissues.

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GENETIC AND BIOCHEMICAL ANALYSES TO EVALUATE INTERACTIONS BETWEEN BACTROCERA OLEAE (ROSSI) AND OLEA EUROPAEA L.


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In the Mediterranean basin the olive fruit fly Bactrocera oleae (Rossi) is the most dangerous pest of olive crop production. It is an insect strictly associated with the genus Olea and most of the O. europaea cultivars show high levels of susceptibility.

This work aims to characterize the fruit metabolites involved in the B. oleae defence response and to evaluate the genes involved in the resistance/tolerance mechanisms.

A set of new olive genotypes derived from reciprocal crosses between ‘Picholine’ and other six cultivars, under evaluation for B. oleae resistance, were chosen for biochemical and genetic analyses. Two groups, characterized by high and low susceptibility to olive fly, were identified and evaluated for fruit metabolic profiles and differentially expressed genes.

Metabolic analyses have been conducted on olive fruits from field-growing plants collected at five ripening stages, from August to November. HPLC-DAD-MS analyses have allowed the identification of different classes of phenolic compounds specific to high and low susceptible olive genotypes. Metabolites common to all samples were quantified at 280 and 330 nm in order to identify quantitative differences between the two groups of genotypes.

cDNA-AFLP approach was used to identify differentially expressed transcripts between low and high susceptible genotypes and to identify genes putatively involved in insect-plant interactions. We isolated 109 transcript-derived fragments (TDF) that were sequenced and compared to the GeneBank database using BlastX. Among them, 43 TDFs showed high similarity to known proteins, some of them being involved in disease resistance, stress response and signal transduction in defence mechanisms.
STUDY OF TRANSCRIPTIONAL CHANGES ASSOCIATED TO PLASMOPARA VITICOLA INFECTION IN RESISTANT AND SUSCEPTIBLE GRAPEVINE THROUGH CUSTOM ARRAYS SYNTHETIZED BY THE COMBIMATRIX TECHNOLOGY


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microarray, Plasmopara viticola, Vitis vinifera

In the newly developed Combimatrix platform at Verona University, we produced a Grapevine chip carrying 24562 specific probes (35-40 mers) in triplicates from assembly of Tentative Consensus of the last TIGR Vitis vinifera Gene Index release 5.0 and from non redundant genomic sequences produced by the genome annotation in the International Grape Genome Project. Combimatrix technology is characterized by an exclusive in situ oligo synthesis driven by electrochemistry and by the reusability of the same microarray up to four times, all factors that confer high flexibility to the system and reduce drastically the costs of microarray analysis.

Downey mildew is one major grapevine disease caused by Plasmopara viticola, an obligate biotrophic oomycete pathogen. A comprehensive analysis of transcriptional changes associated to the infection process of P. viticola in susceptible (Vitis vinifera cv. Pinot Noir) and resistant (Vitis riparia cv. Gloire de Montpellier) grapevine genotypes was undertaken by microarray analysis, at different time points after infection.

Leaves of resistant and susceptible in vitro plants were infected with the pathogen, or treated with water as a control, and sampled at 12, 24, 48 and 96 hours post-inoculation. RNA deriving from three biological replicates of the experiment was used for hybridisation in Cy5 channel. Data were normalized with a global normalization. Differentially expressed genes were selected using the multi experiment Significance Analysis of Microarray test, and gene clustering was performed using Genesis software.
GENETIC DETERMINANTS OF DOWNY MILDEW RESISTANCE IN GRAPE

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resistance genes, plant disease, Vitis vinifera, Plasmopara viticola

Downy mildew is caused by the biotrophic oomycete Plasmopara viticola. P. viticola attacks all green organs of the European grapevines (Vitis vinifera), moving from the primary site of infection, which is the substomatal cells of the leaf blade, to berries where it causes yield loss and deterioration of fruit quality. P. viticola is native to North America and spread to other continents not earlier than the 1850s. Resistance sources for breeding purposes were preferentially searched in the wild germplasm of southeastern US where the centre of distribution of downy mildew was located.

This study was aimed to understand the genetic bases of downy mildew resistance in a variety currently cultivated in some countries of Eastern Europe, named ‘Bianca’, which was bred through recurrent cycles of interspecific hybridisation and backcrosses to Vitis vinifera. ‘Bianca’ was selected in Hungary in the 1960s. The pedigree of ‘Bianca’ includes ancestors from a number of North American Vitis species such as V. labrusca, V. rupestris, V. berlandieri, V. lincecumii from which is expected to have inherited various mechanisms of fungal resistances.

Genetic mapping of the major determinants of disease resistance has been initiated, using an F1 population derived from the controlled cross between ‘Bianca’ and the susceptible cultivar V. vinifera ‘Chardonnay’. A linkage map has been constructed following a pseudotestcross strategy, currently incorporating 305 microsatellite markers heterozygous in ‘Bianca’. The backbone of the SSR-based map of ‘Bianca’ has been integrated with the placement of 33 Resistance Gene Analog (RGA) markers for candidate resistance genes of the NBS-LRR class.

Phenotypic scoring of downy mildew resistance has been carried out using 125 F1 individuals based on field observation of vines grown in absence of spraying and on leaf disks artificially inoculated. The level of whole plant sporulation was scored in the field using two vegetatively duplicated vines per genotype. Two leaves per biological replicate and seven disks per leaf were inoculated by soaking the disks in a 50,000 spores/ml suspension, incubated onto wet paper in Petri dishes at 20°C, 16 h of light, until complete sporulation of susceptible controls. Two disks per leaf were stained with aniline blue three days post inoculation and the area colonised by fungal mycelium was estimated at fluorescence microscope. Five disks per leaf were scored for the area colonised by sporangiophores and the number of sporangia per unit volume of a suspension was counted by a hemocytometer after soaking the disk surface with 1 ml of water.

The progeny displayed a continuous variation for the level of disease resistance. By combining the phenotypic scores, a major locus has been identified on the linkage group 18 of
‘Bianca’ which explained the absence of sporulation in the field experiment and a reduced level of sporulation in the leaf disk tests. This chromosomal arm on LG 18 is extremely rich of NBS-LRR genes. Screening with markers on LG 18 of the whole F1 population currently consisting of 1,700 seedlings is in progress aiming to find recombinants in the target region. A ‘Bianca’ BAC library is also being constructed to help the positional cloning of the trait-controlling gene/s.
IDENTIFICATION OF BACTERIA LIVING IN THE VETIVER ROOT BY MOLECULAR APPROACH


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*Vetiver root, essential oil, endophytic bacteria, amplified 16S rRNA*

_Vetiveria zizanioides_ (L.) Nash (the Vetiver) is a graminaceous plant native to India, growing wild, half wild or cultivated in many tropical and subtropical areas. In particular, selected germlines of this plant species have long been cultivated for their odorous roots that contain the essential oil of Vetiver, used extensively in perfumery and cosmetics (Maffei, 2002).

Vetiver oil is one of the most complex mixtures of sesquiterpene alcohols and hydrocarbons, and also one of the most viscous oils with an extremely slow rate of volatility.

Electron microscope analysis of Vetiver cells of the root glands demonstrated the presence of intracellular bacteria into lysigen lacunae in association with essential oil. The close relationship between these bacteria and the essential oil stimulated the hypothesis of a direct involvement of the symbiotic bacteria in the essential oil metabolism (Massardo et al., 2004). In addition, recent data evidenced that a Vetiver cleansed of bacteria (presumed to be normally associated with field-grown Vetiver) produced only trace amounts of oil and strikingly different composition compared to the oils from non-cleansed Vetiver plants. Using a culture-based approach, a number of root-associated bacteria have been recently identified. Most of these microorganisms belong to the g subdivision of Proteobacteria. The objective of the present study was to analyze the root-associated community of _Vetiveria zizanioides_ (L.) Nash by molecular approach. An amplified 16S rRNA pool was used to generate a library in pGEM®-T Easy Vector. 100 independent DNA clones were subjected to RFLP analysis (Restriction Fragment Length Polymorphisms) with Hinf1 and EcoRI endonucleases that cleave polymorphic sites in 16S rRNA genes, grouped on the basis of the restriction profile and finally sequenced. This analysis not only confirmed the presence of all cultivated strains, but it also demonstrated the existence of additional bacteria that eluded identification by culture-based approaches.

The present molecular approach led to identification of six additional taxa of Proteobacteria belonging to a, b, and g subdivisions, in addition to a single member of the Fibrobacter/Acidbacteria group. When the mix of amplified 16S rRNA samples from the isolates was enriched with the mix of the DNA clones, a pattern almost identical to that of the 16S rRNA pool from the Vetiver root was reproduced in SSCP (Single-Strand Conformational Polymorphism) experiments. This finding suggested that our analysis was nearly exhaustive. Work is in progress to analyze the capacity of those bacterial isolates to degrade the Vetiver essential oil.


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**COMPUTATIONAL DISCOVERY OF PATHOGEN RESISTANCE GENES IN SOLANACEAE FAMILY USING THE SRG DATABASE**


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*R Genes, bionformatic, Solanaceae, gene discovery*

Plant disease resistance genes (R-Genes) are an important class of genes which are well characterized at the molecular level. These genes play a key role in the recognition of the products of avirulence (Avr) genes from pathogens and in the activation of plant defence responses. In Solanaceae, extensive plant-pathogen interaction data has been recently generated by using various methods. In order to obtain the maximum benefit, a public archive of these data was created. The Solanaceae resistance genes database (SRG) provides a collection of resistance genes, defensive genes, markers and other sequences involved in plant-pathogen interaction in the Solanaceae family. It represents the first bioinformatic resource that provides a comprehensive R-genes overview in plants. The dynamic structure of this platform will allow new insights to be achieved in plant-pathogen studies. A Computational analysis was performed using the Soolanaceae reference genes recorded in SRG database in order to identify and dissect genes involved in plant pathogen interactions. Annotation carried out for each single sequence fish out in NCBI unigene dataset revealed important features of major resistance genes and defense related genes.
EARLY ACTIVATION OF BIOCHEMICAL NETWORKS IN ASYMPTOMATIC LEAVES OF THE AUTONECROTIC TOMATO MUTANT LINE V20368

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lesion mimic mutant, tomato, oxidative stress, polyamines

Lesion mimic plant genotypes, developing disease symptoms in absence of pathogen challenge, are useful tools for dissecting the molecular, biochemical and genetic pathways underpinning the plant response to biotic and abiotic stresses. Upon attainment of its 6-7th true leaf stage in vedril tunnels, the autonecrotic tomato (Solanum lycopersicon L.) line V20368 spontaneously develops acropetal necrotic lesions in response to the natural increases in air temperature and light irradiance occurring along the growth season. The molecular basis of such a syndrome is thought to be the interaction between the protein product(s) of the pathogen resistance gene Cf2 (introgressed from S. pimpinellifolium) and the protease inhibitor encoded by Rcr3esc.

From V20368 and from its non-necrotic control line MP22, leaf samples from basal, central and apical plant sectors were collected at four different developmental stages, i.e. 5-6th (I), 6-7th (II), 7-8th (III), 8-9th (IV) true leaves. On these leaf samples, the expression of genes associated with the generation of the oxidative burst, MAPK signalling, antioxidant plant protection, ethylene production, polyamine and γ-aminobutyrrate metabolism, and with the development of the hypersensitive response (HR), was studied.

In V20368, the expression pattern of Rcr3esc confirmed that the interaction with Cf-2 takes place early, when the plants are green and asymptomatic (stage I) or when chlorotic areas on the basal leaves begin to develop (stage II). At these same early stages, mitochondrial function might be affected in V20368, but not in MP22, at least by judging from the differential expression pattern of the alternative oxidase gene AOX1b and by the strong activation of the b-cyano-alanine synthase gene, whose encoded enzyme detoxifies the HCN formed during the last step of ethylene biosynthesis.

We also observed that, in V20368, the gene coding for NADPH oxidase, a key enzyme in the triggering of oxidative burst, is actively transcripted starting from stage II (i.e. following the Cf-2/Rcr3esc interaction occurring in stage I) and is expressed in the apical (relatively younger) leaves during stages III and IV. In V20368, the oxidative burst counteracting appears be entrusted to a battery of enzymes which are often expressed at these latest stages, and just in the apical leaves. Only a few antioxidant enzymes, such as phospholipid-hydroperoxide peroxidase and monodehydroascorbate reductase, seem to be activated early in V20368, but their expression is again limited to the apical leaves.
Finally, differential expression patterns between the mutant and its control for genes linked to the polyamines metabolism and for HR marker genes (*HIN1, LeHSR203J* and *LePirin*) were observed soon after the *Cf-2/Rcr3esc* interaction or when the necroses began to spread.
A LTR COPIA RETROTRANSPON AND MUTATOR TRANSPOSONS INACTIVATE PGIP GENES IN WILD AND CULTIVATED WHEATS

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PGIP, wheat, LTR retrotransposon, mutator transposon, genome evolution

Polygalacturonase-inhibiting proteins (PGIPs) are plant cell wall glycoproteins of many plants that inhibit fungal endopolygalacturonases (PG) and modulate their activity favouring the accumulation of elicitor-active oligogalacturonides.

PGIP belongs to the leucine rich repeat (LRR) family protein, containing 9-10 LRRs flanked by conserved N- and C-terminal regions.

Wheat (Triticum spp.) tissues contain PGIP activity and two pgip-like genes, Tapgip1 and Tapgip2, have been isolated. Southern blot analyses localized these genes on the short arm of homoeologous chromosome group 7B and 7D, respectively, and did not show any clear hybridization fragments from the A genome.

In order to investigate the origin of this lack, whether due to gene loss or to a marked sequence rearrangement that could strongly reduce the capability of the probe to detect the target sequence, we performed PCR analysis, by using a number of conserved primer pairs spanning the complete coding regions of both Tapgip1 and Tapgip2, on the wild diploid relatives with A genome (T. urartu and T. monococcum), and screened a T. turgidum ssp. durum Bacterial Artificial Chromosome library.

We identified pgip sequences from the genome of both wild relatives and isolated two BAC clones. Southern blot and DNA sequence analyses demonstrated that one BAC clone contains a pgip-like gene, Tdpgip1, almost identical to Tapgip1, whereas the other one contains a pgip-like gene, Tdpgip3, interrupted by a LTR copia retrotransposon. Genome-specific PCR analyses on ditelosomic wheat lines demonstrated that Tdpgip1 and Tdpgip3 are located on short arms of chromosome 7B and 7A, respectively.

A PCR screening on a number of diploid and polyploidy wheat species, including T. turgidum (BBAA) and T. timopheevii (GGAA) showed the absence of the retrotransposon insertion on the A genome of T. monococcum and confirmed its presence on T. urartu and in all the polyploidy wheats assayed. It was concluded that the insertion took place after the divergence between T. monococcum and T. urartu but before the formation of the polyploid wheats. This result represents also an additional evidence that the A genome of emmer and common wheats derived T. urartu.

During our mapping analysis of the wheat pgip locus, we identified also a Class II transposable element, belonging to the Mutator superfamily, that interrupted the Tdpgip1 gene in T. turgidum ssp.dicoccoides. Since no similar sequences were found in the Triticeae Repetitive (TREP) database, nor annotations were available for the other copies found in the sequence databases, the insert was considered as a new element and was called Vacuna.
PCR analysis of the pgip1-like gene on wild and cultivated wheats identified an additional accession of *T. turgidum* ssp. *dicoccoides* interrupted by an element showing 78% identity to the Vacuna transposon. Since this additional insertion interrupt pgip1 in a different position than Vacuna, we suggest that they belong to different insertion events.
THE GENETICS OF WHEAT POWDERY MILDEW RESISTANCE EXPRESSED AFTER THE INTROGRESSION OF CHROMOSOME 6V IN T. AESTIVUM


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Pm21, powdery mildew, major gene introgression, Dasypyrum villosum, gliadin

Powdery mildew is an important wheat disease that cause grain yield loss in Europe and in other areas where wheat is grown under cool temperate conditions. The use of resistance genes is the most effective way to combat the spread of this disease. One such gene was reported to be located at locus \textit{Pm21} on the short arm of chromosome 6V#2 introgressed in \textit{T. aestivum} from \textit{Dasypyrum villosum}. Previous attempts to study the genetic basis of the resistance were unsuccessful and prevented the preparation of a genetic map of 6V#2 and the identification of molecular markers closely linked to the \textit{Pm21} locus. Only a deletion map for four groups of RFLP markers and one SCAR marker is available for 6V#2. The CSxV63 disomic addition (DA) line of chromosome 6V#4 introgressed into \textit{T. aestivum} ‘Chinese Spring’ (CS) chromosome complement from a \textit{D. villosum} population collected in Latium-Italy, showed immunity to powdery mildew. This line has been crossed to the susceptible DA6V#1 obtained by E. R. Sears and an \textit{F}_2 population has been produced for assessing the genetic basis of the immunity to powdery mildew and preparing materials (\textit{F}_3 lines) to be used in conventional genetic mapping procedures to locate and mark the \textit{Pm21} locus. The DA state for 6V was checked in both parents and randomly selected \textit{F}_2 seedlings using GISH. The caryopses of parents and of individual \textit{F}_2 seeds were cut in half and the embryonic side was used to obtain the two-leaf seedling that were infected with a powdery mildew isolate in the greenhouse, while the endosperm end was used for seed storage protein electrophoretic analyses. Three rounds of infections on seedlings from different subsamples of about 120 \textit{F}_2 caryopses were performd to test segregation for powdery mildew resistance. The level of powdery mildew symptom was estimated visually examining the number and size of micelia spots using a 0-to-4 scale, 0= no micelia, 4= dense and large micelia spots. All \textit{F}_2 plants were transplanted to produce \textit{F}_2:3 seed (\textit{F}_2-derived \textit{F}_3 lines) for progeny testing and molecular genotyping. Segregation for resistance to powdery mildew fit a 3:1 monogenic dominant inheritance pattern. The segregation was assumed occurring at the \textit{Pm21} locus and the resistance allele on 6V#4 was designated \textit{Pm21-R} and the susceptible allele on 6V#1 as \textit{Pm21-S}. Segregation for gliadin protein subunits encoded at the \textit{Gli-V2} locus on 6VS also segregated according to a 3:1
monogenic codominant inheritance pattern. Co-segregation of the alleles at the Pm21 and Gli-V2 loci indicated substantial linkage, giving the first information on the genetic map for the region surrounding the Pm21 locus and confirming their location on 6VS. The genetic map is being enriched with polymorphic molecular markers that differentiate alleles on the 6V#4/6V#1 homologs.
FUNCTIONAL ANALYSIS OF ORYZA SATIVA WRKY TRANSCRIPTION FACTORS IN PLANT DEFENCE RESPONSE


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functional genomics, transcription factors, rice, plant-microbe interaction, pathogen resistance

WRKY are large families of transcription factors, characterized by the presence of one or two ~60 aa consensus sequences with a WRKYGQK highly-conserved motif and a novel zinc-finger domain that provides DNA-binding properties. WRKY proteins were shown to be implicated in plant response to abiotic and biotic stresses in tobacco, barley, *A.thaliana* and rice. *At*WRKY factors have been extensively studied, whereas in rice, apart from annotation studies, few reports investigated biological function of the 101 *Os*WRKY genes. *Os*WRKY71 and *Os*WRKY3 were shown to function as transcriptional regulator in rice defence signalling pathway and *Os*WRKY71 and *Os*WRKY51 may mediate the cross talk of GA and ABA signalling. A comprehensive expression analysis of *Os*WRKY family by RT-PCR indicated that several genes are differentially regulated upon challenge with pathogen and SA-JA treatment. The main objective of our project is to investigate *Os*WRKY function in resistance to pathogen and environmental constraint and to assess the potential role of few of them in the crosstalk between biotic and abiotic stresses. We performed an exhaustive transcriptome analysis of the whole *Os*WRKY family in stress conditions, by a custom 60-mer oligo microarray following inoculation with host and non-host strains of *Magnaporthe grisea* and osmotic stress treatments. A relevant part of *Os*WRKY genes showed high expression levels in all experiments, whereas the remaining appear to be expressed in a tissue-/condition-specific manner. Interestingly, few WRKY genes were found to be regulated upon biotic and abiotic stimuli, suggesting a key role in both signalling pathway. Phylogenetic analyses revealed the existence of six major groups (1C, 1N, 2A-2B, 2C, 2D-2E and 3) and pointed out the existence of a rice-specific WRKY group of 18 genes (3C). Results will be presented of the ongoing systematic expression study by Q-PCR and phenotypic characterization of WRKY mutant lines to assess the role in resistance to biotic and abiotic stress of a selected set of rice WRKY genes.
TOWARDS SINGLE-CELL TRANSCRIPT PROFILING OF VITIS VINIFER L. INFECTED BY ERYSIPHE NECATOR

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oidium, grape, laser microdissection

Erysiphe necator is the ascomycetes casual agent of powdery mildew (oidium) in grape (Vitis vinifera L.). In grapevine, powdery mildew mainly infects leaves, but it can also infect the fruit and other young tissue including flowers, shoots and petioles. During infection, the fungus grows its hyphae on the surface of the leaf and the hyphal tip (haustorium) penetrates into epidermal cells from which the fungus drags nutrients.

With the aim of contributing to the elucidation of the complex molecular mechanisms controlling grape leaf interactions with this fungal pathogen, we intend to exploit the potentiality of laser microdissection (LMD) technology, in order to characterize the specific transcriptome of infected cells. In many plant-pathogen interactions there are several possible situations caused by the pathogen attacking susceptible plant cells. For instance, an attack by oidium on an upper epidermis cell of a grape leaf may succeed in infection and formation of a functional haustorium (susceptible cell), whereas a neighboring cell, at the same time, may resist fungal penetration reinforcing its wall, producing the so called papillae (resistant cell); in the same leaf other upper epidermis cell do not interact with the pathogen at all (uninfected cell). By laser microdissection technology all these different cells can be directly harvested from the same histological preparation and mRNA can be subsequently isolated for further characterization. Our main interest is to apply such a technology to identify i) genes that are specifically regulated in infected cells and presumably involved in fungal establishment and ii) genes involved in host defense. Here we present our preliminary results on the application of laser microdissection technology for the isolation of mRNA from single cells derived from leaves of grape. The minimum number of cells to be harvested by LMD to perform gene expression analysis by RT-PCR has been established, together with protocols for the amplification of housekeeping genes (actin), tissue-specific genes (epidermis and mesophyll) and fungal specific genes.
THE MAIZE RIBOSOME-INACTIVATING PROTEIN B-32: GENETIC ENGINEERING FOR MAIZE PLANT PROTECTION AGAINST FUSARIUM VERTICILLIOIDES ATTACK


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Zea mays L., ribosome-inactivating-protein, resistance gene, b-32, Fusarium verticillioides

The development of improved maize genotypes with increased resistance to fungal pathogens is one of the major objectives of breeding biotechnology strategies. *F. verticillioides* attacks maize, causing root, stem, and ear rot diseases, and produces mycotoxins (fumonisins), their presence in feed and foods is often associated with mycotoxicoses in livestock and also in humans.

In maize endosperm a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins. Although, the role of b-32 in maize endosperm remains unclear, this protein has homology with several previously characterized Ribosome-Inactivating Proteins (RIPs). It was found that b-32 is a functional RIP and shows anti-fungal activity by *in vitro* and *in vivo* experiments.

Research is in progress in our laboratories to verify if maize plants expressing b-32 in various organs and tissues have an increased defence against fungal pathogens in comparison with plants expressing b-32 only in the kernel. For these purposes transgenic plants were obtained through genetic transformation using the vector *pSC1b32* containing the b-32 coding sequence clone under the constitutive promoter *35SCaMV* and the cassette *ubi1-bar* for Basta herbicide resistance as selectable marker. A set of homozygous progenies southern and western-b32 positive and a negative control were raised to maturity into a containment-greenhouse and used, at flowering stage, for a detailed analysis of b-32 expression in leaves and for pathogenicity tests.

A differential b-32 expression in the leaves of various progenies was recorded.

Proteomic experiments on protein leaf extracts have been set up. The 2DE map matching clearly showed the presence of additional spots in a progeny b-32 western positive, in comparison to a progeny Basta-sensitive and b-32 western negative. These spots have been cut and digested with trypsin to achieve protein identification by MALDI-TOF MS. Both induced b-32 and herbicide resistance in multiple spots have been successfully identified. The identification of progenies with a differential b-32 expression in the leaves was useful for setting up pathogenicity experiments, in order to evaluate a possible differential response to *Fusarium* attack in leaf tissue colonization bioassays. Plants were raised to maturity into a containment-greenhouse. The negative control was the most susceptible to *F. verticillioides* attack, in comparison to all the progenies expressing b-32.
Experiments are in progress to extend pathogenicity tests to other plant tissues and to evaluate the specificity of b-32 role in the defence against other fungal pathogens (i.e., *Aspergillus*, *Penicillium*).
DEVELOPMENT OF SCREENING ASSAYS TO DETERMINE MAIZE PLANT SUSCEPTIBILITY TO *Fusarium verticillioides* INFECTION*

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plant disease, resistance, *Fusarium verticillioides*, fumonisins, *Zea mays* L.

The availability of reliable methods for the screening and evaluation of maize plants for improving tolerance to *Fusarium* attacks is an invaluable tool in breeding programmes to increase crop protections against fungal infection. 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.

The proposed 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). One important goal of the current study, is to i) evaluate the effectiveness of inoculation and selection techniques to study variability in fungal disease susceptibility among genotypes tested, ii) determine the most efficient and reproducible plant inoculation method. For this purpose, during 2006 commercial maize hybrids were used as experimental material and tested for fungal pathogen resistance in two separated artificial inoculation experiments by applying i) the non-wounding Silk Channel Inoculation Assay (SCIA) technique applied to each primary ear, and ii) 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 (region within the husk between the tip of the cob and tip of the husk where the silks emerge) length was recorded for each maize genotype. At maturity, ears were manually harvested. For husk cover visual rating 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. After hand de-husking; the severity of ear *F. verticillioides* attack was evaluated using rating scales based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth. 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. Fumonisin content was evaluated using enzyme-immunoassay-ELISA kit. Furthermore, each entry tested in the artificial inoculation experiments, was evaluated in field tests at different locations in North Italy in order to compare the response of hybrids in different environmental conditions.

Correlation analyses between visual ear rot ratings, internal kernel infection evaluation, fumonisin content, silk channel length at pollination, husk cover ratings, are in progress.

*Research developed in the Program MICOCER
EVALUATION OF MAIZE FOR TOLERANCE TO ASPERGILLUS FLAVUS*

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Aspergillus flavus, Zea mays L., artificial inoculation, aflatoxin

For maize production there is concern about aflatoxin contamination for its potent potential carcinogenicity. In this crop, resistance against A. flavus infection and aflatoxin accumulation is a complex trait influenced by genotype, agronomic practices, and environmental conditions. Beneficial secondary traits such as husk covering and tightness, and drought or heat stress tolerance are factors contributing to aflatoxin resistance. The availability of reliable methods for the screening and evaluation of maize genotypes for improving tolerance to Aspergillus attacks is an invaluable tool in breeding programmes to increase crop protections against fungal infection.

The aim of research was to evaluate and compare maize hybrids for A. flavus resistance and for aflatoxin accumulation in field trials, carried out in 2005 and 2006. The test included: i) self pollinated non–inoculated ears, ii) self-pollinated inoculated (A. flavus) ears. iii) open-pollinated non inoculated ears (in 10-20 different locations). At pollination, silk channel (region within the husk between the tip of the cob and tip of the husk where the silks emerge) length was recorded for each genotype. Ten hand pollinated plants per plot were inoculated with a fresh spore suspension (mixture of five A. flavus isolates from Northern Italy), by the non-wounding silk channel inoculation technique (SCIA). Controls were non-inoculated and sterile water-inoculated plants. At maturity, ears were manually harvested. For husk cover visual rating ranging from 1 (good tight long husks extending beyond the tip of the ear) to 5 (poor: loose short husks with exposed ear tips) was recorded. After hand de-husking; the severity of ear A. flavus attack was evaluated using rating scales based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth. After visual inspection ears were dried and shelled. The kernels were bulked within plots. To evaluate internal kernel infection fifty kernels were randomly chosen from each sample, were surface-disinfected and plated on potato DRBC agar. Content of Aflatoxin B1 in the control, inoculated and open-pollinated non inoculated materials, has been evaluated using enzyme-immunoassay-ELISA kit. The aflatoxin content in the inoculated ears resulted higher than in the controls and in the open-pollinated non inoculated materials; this indication confirm that the A. flavus isolates used for the inoculum procedure were successful in accumulating mycotoxin in grains. Variability was found among the genotypes under study. Correlation analyses between visual ear rot ratings, internal kernel infection evaluation, aflatoxin content, silk channel length at pollination, husk cover ratings, in the two field seasons, are in progress. Furthermore, the genotypes tested in field experiments with SCIA on the adult plants and were tested by an in vitro bioassays (KIA-Kernel Inoculation Assay) to follow the progression of A. flavus infection in inoculated maize germinating seeds. The results of the in vivo and in vitro experiments were compared to evaluate if the in vitro bioassays reflect in vivo plant response to Aspergillus attack.

*Research developed in the Program AFLARID
DEVELOPMENT OF A LINKAGE MAP TO DISSECT THE GENETIC BASES OF LEAF RUST RESISTANCE IN DURUM WHEAT


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leaf rust resistance, durum wheat, genetic linkage map

Leaf rust, caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*) is one of the most important diseases for wheat, causing significant yield losses annually in many wheat-growing regions of the world. The utilization of resistance genes is the most viable and economical strategy to minimize the yield losses. Many studies on genetic resistance were carried out on hexaploid wheat (*Triticum aestivum*) in which more than 60 resistance genes and QTLs have been described but little is known regarding leaf rust resistance in durum wheat (*Triticum turgidum*).

We are currently developing a genetic linkage map on a RIL population derived from a cross between two durum wheat varieties, Creso and Pedroso, for the dissection of the genetic bases of leaf rust resistance. Creso is a durum wheat cultivar whose resistance has been postulated to be durable and Pedroso is a susceptible cultivar. 123 F9 Recombinant Inbred Lines are now available from this cross and more than 400 biochemical and molecular markers (SSR) with known map position have been tested on the parental lines, resulting in 125 polymorphic markers.

Diversity Array Technology (DArT) can detect and type DNA variation in several hundred genomic loci in parallel and it can be effectively applied to genetic mapping in wheat. The DArT has been utilized to develop the map, resulting in about four hundred markers positioned on durum wheat genome.

The phenotypic characterization of the RIL population for hypersensitive response and durable resistance to leaf rust at different growth stages will allow to identify genetic determinants of qualitative and quantitative aspects of resistance to leaf rust in durum wheat.
CYTOLOGICAL AND MOLECULAR ANALYSIS OF THE BARLEY-LEAF STRIPE INTERACTION


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defense gene, embryos, barley, leaf stripe, gene expression analysis

Leaf stripe of barley (Hordeum vulgare L.) is caused by the seed borne fungus Pyrenophora graminea. We investigated at the microscopic and molecular levels the reaction of barley embryos to leaf stripe infection. In the resistant genotype NIL3876-Rdg2a, abortion of fungal growth occurred at the level of a embryo position defined by the scutellar node, while in the near isogenic line (NIL) Mirco-rdg2a no fungal growth arrest was observed and the pathogen colonized the embryo structures. A quantitative difference between resistant and susceptible NILs was observed for the level of tissues reacting with autofluorescence under UV light excitation. This response correlated with a positive reaction to toluidine blue staining, indicating accumulation of phenolic compounds in the resistant line. Suppression subtractive hybridisation (SSH) and cDNA-AFLP analyses were used to identify genes differentially expressed in barley embryos in response to leaf stripe infection and low abundant sequences of fungal origin. SSH led to the isolation of genes involved in generation of reactive oxygen species and detoxification mechanisms. Microarrays containing the entire set of cDNA-AFLP fragments and 100 genes selected from publicly available databases were used to study gene expression changes at 7 and 14 days after inoculation (dai) in the resistant and susceptible NILs. Gene clusters grouping 238 significantly modulated genes were identified. Genes that responded to leaf stripe infection included pathogenesis-related (PR) genes, genes involved in oxidative stress generation and protection, jasmonate (JA) synthesis and several other different pathways such as vesicle trafficking, protein ubiquitination and signal transduction. Interestingly, we observed induction of several genes involved in secondary metabolic pathways and cell wall reinforcement, supporting microscopic observations and suggesting that this may represent a major defense response of barley embryos infected with leaf stripe. In addition, during the cDNA-AFLP analysis we identified six differentially expressed fungal genes that are likely involved in pathogenicity.
PLANT CELL LOCALIZATION OF TnBVANK1, AN IKB-LIKE GENE FROM TOXONEURON NIGRICEPS BRACOVIRUS

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IkB, Ankyrin, Nicotiana tabacum, immunolocalizationToxoneuron nigriceps Bracovirus TnBV is a polydnavirus associated with Toxoneuron nigriceps an endophagous parasitoid of larval stages of the tobacco budworm Heliothis virescens that injects the viral DNA into the host with the egg at the ovideposition. Viral genes play an important role in the suppression of the host immune reaction and in the development of a severe alteration in the hormonal balance of parasitized larvae. Viral genomes have been sequenced and evidenced the presence of a gene family characterized by the presence of two ankyrin repeats showing high similarity to the IkB-like proteins involved in NfkB signalling pathway (Falabella et al., 2007). These proteins play a key role in the negative regulation of immune system in mammals and insects. The conservation in planta of a IkB-NFkB like pathway and its possible involvement in plant response to pathogen attack, supported the expression of one of the gene of this family, TnBVank1 in tobacco in order to study its possible role in plant defence. Transgenic plants constitutively expressing TnBVank1 gene where fully characterized by RT-PCR and western blotting which confirmed the expression of the recombinant protein often complexed with other proteins through bounds possibly mediated by the ankyrin domains. Here we report on the cellular localization of the recombinant protein in transgenic tobacco protoplasts and discuss its implications with defence mechanisms.
THE CONSTITUTIVE PRODUCTION OF SYSTEMIN IN TOMATO PLANTS MODIFIES THE EXPRESSION OF GENES INVOLVED IN THE VOC PATHWAY

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plant insect interactions, terpenes, PTGS

Plants and insects have coexisted since very long time and have developed several relationships that concern the organisms at all levels, from basic biochemistry to population genetics. Although some of these relationships between the two phyla are mutually beneficial, such as pollination, the most common interactions are antagonistic and involve insect predation of plants and plant defences against herbivorous insects. The latter may be carried out by chemical and physical defences that influence pest performance and attract natural enemies of herbivores. These mechanisms are termed direct and indirect defence, respectively. Direct defence includes the production of anti-nutritive and poisonous compounds for plant-feeding insects, while indirect defence typically involves the production of Volatile Organic Compounds (VOC), which are used by parasitoids and predators to localize their victims. After herbivore attack, plants release complex bouquets of volatile that include terpenes and C6-volatiles playing a key role in tritrophic interaction. Great interest is presently oriented to the identification of major genes involved in VOC production in response to herbivores attacks. We focused our attention on genes involved in terpenoid biosynthetic pathway. In order to study gene expression we used transgenic tomato plants that were transformed to constitutively express the tomato prosystemin gene as these plants in the absence of herbivores attacks, accumulate defence proteins similarly as occurs in control plants damaged by larvae feeding (McGurl et al., PNAS: 1994; 91:9799-802). The results of the expression analysis, carried out by SYBR-Green Real Time PCR, indicated that herbivory up-regulates genes involved in late steps of the VOC pathway such as FPS1, responsible of the production of Farnesyl pyrophosphate and LeSST1-2 possibly involved in the production of b-caryophyllene in planta, while downregulating LeCCD1A and LeCCD1B genes involved in carotenoid cleavage that produces volatile terpenoid such as b-ionone and geranylacetone. No differences were observed in the expression of early genes of the biosynthetic VOC pathway (DXS and DXR). These results encouraged the production of transgenic tomato plants in which LeSST1-2 genes were silenced through PTGS approach in an attempt to identify the role of such genes in the indirect defence mechanism.
INTRACELLULAR TRANSPORT AND TOXICITY OF A TYPE I RIBOSOME-INACTIVATING PROTEIN

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antiviral protein, translocation efficiency, signal peptidase

Ribosome-inactivating proteins (RIPs) are potent inhibitors of protein synthesis that accumulate in different tissues of many plant species. These proteins are N-glycosidases that are able to remove a specific adenine present in a universally conserved region of 23S/25S/28S rRNA, thus blocking protein synthesis. Several of these proteins have been shown to be endowed with antiviral and/or antifungal activity. According to the local cell death hypothesis, compartmentalized RIPs would reach the cytosol upon viral infection causing inactivation of host cell ribosomes and thus blocking virus replication and spreading through the plant. Several attempts have been therefore made to exploit RIPs for the production of transgenic plants resistant to viral or fungal infection. However, constitutive expression of these proteins has often been found to be toxic toward host cells.

Here we have studied the intracellular trafficking of saporin (a type I RIP from *Saponaria officinalis*) when expressed in tobacco protoplasts, and characterized the mechanism of toxicity toward host cells. We find that saporin expression is extremely toxic to tobacco protoplasts, causing a drastic reduction in protein synthesis. By expressing active site mutants we could determine that saporin behaves as a secretory protein, accumulating in the incubation medium of transfected tobacco protoplasts. Still, the toxicity associated with saporin expression indicates that a fraction of the synthesized polypeptides accumulates in the cytosol. This could be due either to inefficient targeting to the endoplasmic reticulum, to retrotraslocation from the endomembrane system, or to re-uptake of the toxin from the incubation medium. Our data indicate that toxicity is not due to endocytosed saporin and that the signal peptide has the potential of controlling the toxicity of any saporin precursor that fails to be targeted to the endoplasmic reticulum. In addition, we find that mutations interfering with signal peptide cleavage reduce the toxicity associated with saporin expression. All together these data indicate that toxicity is due to the release in the cytosol of polypeptides that have transiently been exposed to the action of signal peptidase and suggest potential mechanisms by which RIP-expressing cells may control the access of these enzymes to the cytosol upon viral infection.
STRATEGIES FOR THE EXPRESSION IN TOBACCO OF THE PROTEIN TYROSINE PHOSPHATASE 7 GENE FROM THE POLYDNAVIRUSES OF THE PARASITOID TOXONEURON NIGRICEPS

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viral gene, plant transformation, parasitoid, resistance, inducible system

The PTP7 gene from the polyDNAvirus of the parasitoid Toxoneuron nigriceps (Hymenoptera, Braconidae) codes for a Protein Tyrosine Phosphatase. The PTP7 protein is probably involved in the underphosphorylation of regulatory proteins of the prothoracicotropic hormone signal transduction pathway, which culminates with a translational block of protein synthesis in the parasitized H. virescens larvae (Falabella et al. 2006). The PTP7 displays an high similarity to well characterised protein tyrosine phosphatases of the Meg2 subtype, which can negatively or positively regulate diverse signalling pathways in eukaryotes. Here we report on the expression of the PTP7 gene in tobacco aimed to evaluate its potential utility for crop protection and investigate a possible role in planta. To this aim, we constructed a chimaeric gene encoding a fusion protein, in which the PTP7 is fused to SP1 signal peptide of the tobacco Pathogenesis-Related protein-1. Furthermore, an ER-retention signal peptide was added at the C-terminus. These signal peptides should ensure an efficient translation of this viral gene in tobacco while minimising the risk of putative detrimental effect. Stable genetic transformation of tobacco originated putative transgenic plants without obvious phenotypic abnormalities. Out of 67 plantlets, 56 regenerants were PCR positive. These plants were screened by RT-PCR to verify the transcription of the PTP7 gene and by Western to verify the expression of this protein in plant cells. Only 7 plants transcribed the transgene and accumulated the PTP7 protein in very low quantity. Concurrently, the PTP7 gene was cloned in a binary vector of a new chemically-inducible transgene expression system based on the used pOp/LhG4 transcription activation system and stable transformation of tobacco is presently in progress.
POPULATION OF MYCORRHIZAL AND ENDOPHYTIC FUNGI COLONIZING ROOTS RICE GROWN IN SIMIL-UPLAND CONDITION

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In the last few years, different experiments were designed at CRA - Istituto Sperimentale per la Cerealcoltura in Vercelli (Italy) to find well performing-rice varieties in dry land condition. As parallel study, a survey of the presence of colonizing fungi in rice roots was performed. Therefore, the populations of arbuscular mycorrhizal (AM) fungi and of endophytic fungi were studied in the roots of twelve different rice varieties grown in simil-upland condition.

AM fungi provide to plants a partnership in which a complex system of intra-radical and extra-radical hyphae is involved. The symbiotic system contributes to the uptake of water and nutrients and creates a modified rhizosphere, which confers protection to the plant under stress conditions. Endophytes live asymptomatically within plant tissues and may have effects on their host such as enhancement of stress-, insect- and disease-resistance and herbicide activity. Endophytes usually occur in above-ground plant tissue, but they are also occasionally found in roots.

Rice roots were observed under optical microscope after staining with Cotton blue. The observations showed the presence in all the varieties of fungal structures, as hyphae, vesicles, spores. Some structure, like arbuscules and vesicles could be assigned to AM fungi, which were present with a percentage of arbuscularisation between 19% and 62%. Other structures belonged probably to other kind of endophytic fungi, which were isolated after plating roots homogenate on malt extract medium. All the fungal isolates obtained were tentatively grouped based on morphology on MEA medium. Some isolates from each morphological group were chosen for identification by means of molecular analysis. All the varieties, except one, resulted colonized by endophytic fungi, though at different extent. About 300 fungal isolates were obtained, belonging mainly to *Penicillium, Plectosphaerella, Fusarium, Acremonium* genera. The possible role of these fungal isolates on the rice plant biology will be discussed and tested in *in vitro* experiments for pathogenic fungal competition and/or growth promotion.