HIGH THROUGHPUT SCREENING TECHNOLOGY FOR AGROCHEMICAL IDENTIFICATION

G. COLUCCI

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biotechnology, agrochemicals, High Throughput Screening (HTS)

Arterra Bioscience srl is an early stage Italian biotechnology company that started operations in 2004 to develop new and highly specific agricultural products. The company focus is on crop protection against abiotic and biotic stresses with the ultimate goal of making food production sustainable in the 21st century. Arterra adapts high-throughput technologies used for drug discovery to identify chemicals of interest for crop protection.

Technology development at Arterra focuses on signal transduction pathways of plants, pathogens and pests. Interference with signal transduction pathways has multiple outcomes: death of the organism, inability to reproduce itself, as well as protection against stresses. By expressing modified signal receptors in cellular systems it is possible to develop High-throughput assays that enable the rapid screening of thousands of chemicals that act as agonists, antagonists or reverse agonists. This results in the identification of candidate agrochemicals that will kill pathogens and pests or enhance plant growth under abiotic stress conditions.

Arterra's scientific team has an extensive experience in target identification, bioassay development and HTS platform development.

In January 2005 Arterra signed a 5 years research agreement with Isagro Ricerca srl to develop a new generation of agrochemicals that mitigates plant stress for the purpose of improving plant health.

Arterra Bioscience is located at the School of Medicine of the University of Naples. The company occupies laboratories in the state of the art facility of the CEINGE (Centro Ingegneria Genetica), which opened for occupancy in 2004.
GENOTYPE BY ENVIRONMENT INTERACTION ANALYSIS FOR GRAIN YIELD IN BARLEY (HORDEUM VULGARE L.) GROWN IN MEDITERRANEAN ENVIRONMENTS

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barley, genotype by environment interaction (GEI), additive main effects and multiplicative interactions (AMMI) analysis

Mediterranean regions are characterized by the unpredictable timing, duration, frequency and intensity of the annual rainfall and of drought stress. Crop production may therefore vary erratically over the years owing to the interactions between the genotypes and environments (GEI), and in the harsher environments, crop failures may occur. From this, the importance of GEI analyses emerges, particularly in relation to research activities aimed at improving crop performances in diverse and difficult environments.

According to this perspective, our study was carried out to evaluate the yield performances of 24 barley genotypes across 6 different environments in Sardinia, which is characterized by a Mediterranean climate. The genotypes were chosen from three different groups: Sardinian barley landraces (SBL), improved varieties (VAR) and recombinant inbred lines (RILs), obtained from the cross between two pure lines extracted from Sardinian landraces and one improved variety.

Additive main effects and multiplicative interactions (AMMI) analysis was used to investigate the major GEI effects. AMMI analysis has shown that: i) environment and GEI effects were the main causes of variation for yield levels, explaining 67.1% and 26.7%, respectively, of the model sum of squares (P≤0.001); ii) genotype performances mainly differed according to their group of origin; and iii) RIL groups showed the more stable yield performance across Sardinian environments; performance of the VAR group was superior in the favourable environments, while the SBL group showed the best performance in the adverse environments.
FLAVOHAEMOGLOBIN HMPX FROM *ERWINIA CHRYSANTHEMI* CONFERS NITROSATIVE STRESS TOLERANCE AND AFFECTS THE PLANT HYPERSENSITIVE REACTION BY INTERCEPTING NITRIC OXIDE PRODUCED BY THE HOST


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flavohemoglobin, nitric oxide, hypersensitive response, plant-pathogen interaction, Erwinia chrysanthemi

Host cells respond to infection by generating nitric oxide (NO) as a cytotoxic weapon to facilitate killing of invading microbes. Bacterial flavohaemoglobinins are well known scavengers of NO and play a crucial role in protecting animal pathogens from nitrosative stress during infection. *Erwinia chrysanthemi*, which causes macerating diseases in a wide variety of plants, possesses a flavohaemoglobin (HmpX) whose function in plant pathogens has remained unclear. Here we show that HmpX consumes NO and prevents inhibition by NO of cell respiration, indicating a role in protection from nitrosative stress. Furthermore, infection of *Saintpaulia ionantha* plants with a HmpX-deficient mutant of *E. chrysanthemi* revealed that the lack of NO scavenging activity causes the accumulation of unusually high levels of NO in host tissue and triggers hypersensitive cell death. Introduction of the wild-type *hmpX* gene in an incompatible strain of *Pseudomonas syringae* had a dramatic effect on the hypersensitive cell death in soybean cell suspensions, and markedly reduced the development of macroscopic symptoms in *Arabidopsis thaliana* plants. These observations indicate that HmpX not only protects against nitrosative stress but also attenuates host hypersensitive reaction during infection by intercepting NO produced by the plant for the execution of the hypersensitive cell death program.
EXPRESSION PROFILE OF OZONE INDUCED TRANSCRIPTS AFTER AN ACUTE TREATMENT IN TWO *POPULUS* SPP.

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ozone stress, *Populus*, suppression subtractive hybridisation, ozone-associated genes

Ozone (O<sub>3</sub>) is now considered to be the most phytotoxic of all the common air pollutants. O<sub>3</sub> is toxic to plant and animal because it is a powerful oxidating agent, which is able to react directly with lipids and proteins. Such reaction and the decomposition of O<sub>3</sub> in the plant apoplast can lead to the production of other reactive oxygen species (ROS) that can act as signal transduction molecules when generated in a controlled, localized and transient manner. Such an oxidative burst has been implicated as an early step in plant responses to ozone stress and lead to a change in the expression of several genes.

In order to identified differentially expressed genes after an acute ozone treatment in two hybrid poplar clones (*Populus deltoides* x *maximowiczii*, Eridano clone, and *Populus x euoramericana*, I-214 clone, sensitive and tolerant to O<sub>3</sub>, respectively) a gene identification study was previously performed using suppression subtractive hybridisation (SSH), obtaining four subtractive cDNA libraries. Sequenced transcripts were subdivided in six functional categories such as stress/defence signalling, stress/defence response, stress/related proteins, other/protein, putative protein and unknown protein.

The results presented here refer to the characterisation of the temporal gene expression changes of transcripts belonging to stress/defence signalling category (wall associated kinase, Ft32C clone, Calmodulin-like protein, Ft33B clone, WRKY transcription factor, Ft312B clone and Leucine-rich repeat protein, Fs23A clone) after an acute O<sub>3</sub> treatment both in sensitive and tolerant poplar hybrids. Time course expression analysis shows that in tolerant poplar clone transcript level of all four genes increases soon after the end of treatment, decreases until 24 h, and increases again at 48 h since the end of treatment. Except for Fs312B clone that presents a biphasic behaviour like in tolerant poplar, other clones show a quite different but monophasic response to acute O<sub>3</sub> exposure in sensitive poplar clone, with a variable peak between 0 h and 24 h. These results are very intriguing, particularly for the comprehension of how different patterns of gene expression modulation are linked to the different O<sub>3</sub>-responses in tolerant and sensitive poplar clone.

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CHARACTERIZATION AND EXPRESSION PATTERN OF GERANYLGERANYL HYDROGENASE GENE IN OLEA EUROPAEA L.

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geranylgeranyl reductase, fruit quality, Olea europaea L, defense gene

The geranylgeranyl hydrogenase enzyme reduces free geranylgeranyl diphosphate to phytil diphospfate, providing the side chain to chlorophylls, tocopherols and plastoquinones.(1). Tocopherols and plastoquinones are antioxidant compounds which shield plants from ageing and photo-oxidative processes (2). They also constitute vitamins E and K, respectively, and affect nutritional value of fruits, their stability in the post-harvesting cold chain and shelf live. Therefore, the gene involved the synthesis of these compounds, such as CHLP may be useful tools as expressed markers in breeding programmes or genetically manipulated to improve traits of fruit quality. (3).

In the present work gene coding for CHLP has been characterized in Olea europea L. as an ORF of 1395bp, encoding a deduced polypeptide of 464 amino acid. The deduced protein OeCHLP was 51,2 kDa and exhibited the maximal identity with Nicotiana tabacum.

Gene expression analysis by RT-PCR showed that OeCHLP transcripts are present in photosintetic organs such as green fruits and leaves at different developmental stage. Notably, gene expression was detected also in prefloral buds suggesting a gene function not exclusively linked to photosynthesis. According to this hypothesis, analyses by semi-quantitative RT-PCR showed that transcripts levels of OeCHLP increase in fruit injured by pathogens as compared to intact ones. These results are discussed in view of OeCHLP function in defense response mechanism.


DIFFERENTIAL GENE EXPRESSION INDUCED BY OZONE STRESS IN THE MEDITERRANEAN EVERGREEN SHRUB PHILLYREA LATIFOLIA L.


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ozone, air pollution, oxidative stress, cDNA-AFLP, Mediterranean forest

In most densely-populated areas of the world, tropospheric ozone (O₃) is one of the major phytotoxic pollutants causing damage to both cultivated plants and natural vegetation. Over the last decades, forest species native of cold or continental climates have been most intensively studied, whereas the peculiar features of the Mediterranean climate, as they affect O₃ uptake and responsiveness in indigenous plant communities, have comparatively received much less consideration. In a previous study, physiological and biochemical measurements indicated *Phillyrea latifolia* L. as the most tolerant among three Mediterranean evergreen broadleaf species exposed to realistic O₃ regimes, and suggested that such tolerance might overlap with an inborn adaptability to drought. To understand the molecular basis of such tolerance, we studied differential gene expression in two-years old seedlings of *P. latifolia* exposed to either 0 or 110 nL L⁻¹ for 90 days, 5 h each day, from March to May in a greenhouse. The expression profiles of the transcripts from leaves of ozonated and control plants were compared by a modified cDNA-AFLP technique. The analysis of about 300 amplification products detected 70 differentially expressed transcripts, 45 of them induced and 25 suppressed by O₃. The nucleotide and deduced amino acid sequences of 34 out of 45 O₃-induced clones, whose preferential expression in ozonated plants was verified by RT-PCR analyses, were compared with the online databases of DNA and protein sequences. On the basis of their presumed functions, 16 out of 34 O₃-induced clones were assigned to seven groups: I) energy (4 clones); II) metabolism (1); III) cell rescue/defense (3); IV) cellular organization/biogenesis (4); V) signal transduction (2); protein degradation (2). At the present preliminary stage, we can tentatively argue that, in general, the pattern of gene induction detected in phillyrea is rather logically related to the suite of known molecular responses to O₃. Eleven of the 16 isolated clones, found to match genes with known functions, have been previously recognized as O₃-responsive genes in *Arabidopsis*. The observed O₃-induced accumulation of transcripts coding for heat- and salt-inducible proteins, for a pathogenesis-related protein and for a putative mitogen-activated protein kinase, orthologous to the *Arabidopsis* AtMPK3, could lend further support to the accepted notion that O₃ behaves as an abiotic elicitor of defence responses in plants, i.e. that molecular plant- O₃ interactions might have much in common, in terms of signal perception, transduction, gene expression and defence, with the reaction cascades elicited by a variety of other biotic and abiotic environmental stressors. In contrast with previous studies from others, the transcription of genes coding for subunits of photosystem I or potentially important for the synthesis of chlorophyll was activated by the exposure to a realistically elevated O₃ level, protracted over the annual period of the most active vegetative growth in the field. This would suggest in *P. latifolia* a sort of compensative ability in coping with an accelerated turn-over of photosystems components in the presence of chronic O₃. The analysis of differential gene expression in *P. latifolia* appears as a promising approach in deciphering molecular responses to realistic O₃ dosages in natural plant communities belonging to Mediterranean ecosystems.
GENETIC AND PHYSIOLOGICAL ANALYSIS OF THE GLOSSY1 GENE OF MAIZE

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cuticular waxes, glossy genes, Zea mays, water deficit, light response

The cuticle is a lipidic water-repellent layer covering the aerial surfaces of land plants. It consists of a polyester matrix, the cutin, interspersed and overlaid by waxes, which are the main determinants of the cuticle functions. In spite of its importance as a preformed defence against several environmental stresses, genetic analysis of cuticle biosynthesis has been very limited and focussed mainly on two model species, maize and Arabidopsis.

Several wax deficient mutants (glossy or gl) have been isolated in maize but only three genes involved in cuticular wax biosynthesis have been cloned and characterized: Glossy1 (Gl1), Glossy2 (Gl2) and Glossy8 (Gl8), all conditioning wax accumulation in the juvenile stage of maize development. Mutations in the Gl1 gene have a pleiotropic effect affecting wax production, trichome development and cuticle membrane formation, though the GL1 protein appears to be a metabolic enzyme belonging to a class of membrane bound hydroxylases-desaturases widespread in both prokaryotes and eukaryotes.

To determine whether the different effects of the gl1 mutations are due to the impairment of a single biosynthetic step or depend on a multifunctional nature of the enzyme, we analyzed a collection of 20 stable independent mutations of the Gl1 locus all impairing wax biosynthesis. Sequence analysis of the transcripts, when present, gave indications of some functional domains involved in wax biosynthesis whereas analysis of trichome morphology and distribution in the same mutants did not support the hypothesis of the presence of multiple catalytic domains.

To analyze the regulation of the wax biosynthetic pathways in maize seedlings, Gl1, Gl2 and Gl8 expression was assayed in plant subjected to environmental stresses (drought and high salinity) and in response to light. All these factors are known to cause an increase of wax load on leaf surfaces. Contrary to Gl2 and Gl8, Gl1 transcription was down regulated by water stress and light indicating the presence of multiple regulatory pathways in the control to wax biosynthesis or an indirect involvement of Gl1 in this metabolic process.
RELATIONSHIP BETWEEN DNA METHYLATION PATTERN AND STRESS CONDITIONS IN PLANTS OF *POSIDONIA OCEANICA* (L) DELILE

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DNA methylation, antropic stress, bioindicator, *Posidonia oceanica* L.

*Posidonia oceanica* (L) Delile is an important marine phanerophyte of the Mediterranean area. In the last years a regression of *Posidonia oceanica* meadows has been caused by the increasing anthropization and *Posidonia* has been proposed as an effective bio-indicator to monitor sea environments (1).

DNA methylation is a fundamental mechanism for gene expression regulation and plant development (2,3). In this context, we investigated the putative relationship between DNA methylation and stress conditions in plants of *Posidonia* derived from anthropic-stressed and intact meadows respectively, during three different periods of the year. In addition, the analysis of DNA methylation pattern was extended to plants grown in aquarium under controlled stress conditions (Cd 10 mM). DNA methylation of shoot apical meristems and young leaves was monitored by immunocytological and Methylation-Sensitive Amplification Polymorphism analysis (MSAP). A set of morphometric and nucleotipic features were also investigated. The plants of anthropized meadows showed cytosine hypermethylation and chromatin re-modelling with respect to those of intact areas. Notably, we observed similar changes in Cd-stressed plants as compared to those of controls. Moreover, MSAP signal-band profiles revealed a clear polymorphism between antropic stressed and well preserved meadows, thus suggesting that the methylation pattern in *Posidonia* may be used as a molecular marker of altered environmental condition.


THE SHADE AVOIDANCE RESPONSE IN ARABIDOPSIS THALIANA


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Arabidopsis thaliana, shade avoidance response, vascular development, ATHB-2, auxin

Plants undergoing shade exposure, as an effect of close proximity of taller plants that absorb most of the Red (R) component and reflect a Far Red (FR)-enriched light, try to adapt to this energetically adverse condition by acting the so-called shade avoidance response. In several plants, this has been described as a re-direction of the energy towards stem elongation and flowering to the detriment of leaf expansion and root growth. In the model plant Arabidopsis thaliana, however, this process has been limitedly described (i.e. elongation response of petioles and stems, effects on flowering time). Here, we report the first comprehensive description of the shade avoidance response in Arabidopsis. The hypocotyl and petioles significantly increase their length, while cotyledons and leaves strongly slow down their expansion. The root system undergoes a reduction in growth rate upon exposure of the plant to shade, affecting both the primary and the lateral roots. At bolting, plants grown in shade are dramatically less developed with respect to plants grown in normal light conditions, including a reduced number of leaves. In addition, flowering is significantly accelerated in shade. Interestingly, we observed for the first time that shade specifically affects the development of the vascular system in leaves. By promoting the differentiation of the provascular and mesophyll cells, shade simplifies the final vascular network. We are currently exploiting molecular markers in order to study the effect of FR-rich light on leaf development. Moreover, the role of the phytohormone auxin and of the homeobox gene ATHB-2 in this aspect of the shade avoidance response will be discussed.
EXPRESSION STUDY OF A GENE FOR A DEHYDRATION-RESPONSIVE TRANSCRIPTION FACTOR IN DURUM WHEAT

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hydric stress, durum wheat, dehydration responsive factors, molecular assisted breeding

The repeatability of dry years makes now necessary a new orientation of selection activity of cultivation species, characterized by high yield to meet population increase and industry demand, considering the climatic tendencies. An effective merging of classical breeding techniques with modern plant biotechnologies is foreseen to increase agricultural productivity.

It is known that the DREB genes, firstly isolated from Arabidopsis genome, are the key-genes conferring resistance to water stress, high salinity and cold, in the ABA-independent pathway. These DREB genes codify for transcription factors that control the expression of several target genes involved in the mechanism of tolerance to the above mentioned stresses. In a previous studies, we isolated and characterized a gene for a factor responsive to dehydration, DREB-related, in durum wheat (TdDRF1: Triticum durum Dehydration Responsive Factor 1) and this gene is highly homologous to the barley gene HvDRF1. The primary transcript of this gene produces three mRNAs by alternative splicing, two of them (TdDRF1.1 and 1.3) codifying transcriptional activators, involved in the genic ABA-mediated regulation through their AP2 DNA-binding domain.

We have investigated the expression profiles of this gene in different moments of hydric stress and our results indicate that TdDRF1.1 and 1.3 transcripts are very low abundant when water is available to the plant and their quantity increase when it is not available, while TdDRF1.2 transcripts seems to be always expressed.

We preliminarly used this gene as a marker and analized the expression levels of the three messangers of TdDRF1 throught Real-Time RT-PCR in several durum wheat varieties, having different features concerning drought tolerance, yield and other agronomic traits. Two set of experiments were carried out both in controlled greenhouse and in open experimental fields.

After an improved understanding of the role of TdDRF1 in hydric stress adaptation, this gene, or the quantity of these transcripts, could be selected as tolerance indicator to hydric stress. Dehydration-responsive molecular markers will permit a selection of adapted durum wheat varieties to be used in Molecular Assisted Breeding (MAB). The future application consists in the generation and production of new durum wheat varieties (genotypes) drought-resistant, either by assisted breeding or by transgenesis.
A GUARD CELL-SPECIFIC MYB TRANSCRIPTION FACTOR REGULATES STOMATAL MOVEMENTS AND PLANT DROUGHT TOLERANCE

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guard cells activity, regulation of transcription, drought tolerance, Arabidopsis thaliana

Stomatal pores located on the plant epidermis regulate the uptake of CO₂ for photosynthesis and the loss of water by transpiration. The opening and closing of the pore is mediated by turgor-driven volume changes of two surrounding guard cells. These highly specialized cells integrate internal signals and environmental stimuli to modulate stomatal aperture for plant survival under diverse conditions. Modulation of transcription and mRNA processing play important roles in controlling guard cell activity, even though the details of these levels of regulation remain mostly unknown. We have identified the AtMYB60 gene of Arabidopsis, as the first transcription factor involved in the regulation of stomatal movements. AtMYB60 is specifically expressed in guard cells and its expression is negatively modulated during drought. A null mutation in AtMYB60 results in the constitutive reduction of stomatal opening and in decreased wilting under water stress conditions. Transcript levels of a limited number of genes are altered in the mutant, many of which involved in the plant response to stress. Our data indicate that AtMYB60 is a transcriptional modulator of physiological responses in guard cells and open new possibilities to engineering stomatal activity to improve plant survival to desiccation.
FUNCTIONAL GENOMICS TO DISSECT DROUGHT SIGNAL TRANSDUCTION IN CEREALS BY USING A. THALIANA AS MODEL SYSTEM


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Our work present a functional genomics approach to dissect drought signal transduction in cereals by using A. thaliana as model system. We have analysed four clones, named 6H8, 6g2, 1C1 and 10d10, previously isolated in durum wheat in response to drought using a suppression subtractive library. They showed sequence similarity with genes in A. thaliana never reported to be involved in stress response: a putative transmembrane protein belonging to the UPF0016 family, a RING-FINGER protein, a farnesylated protein and an E2-ligase involved in sumoylation pathway. To identify the function of these genes two approaches are currently in progress: 1) analysis of the knock-out T-DNA mutants via a reverse-genetics approach, and 2) protein-protein interaction analysis using yeast two-hybrid system.

The isolated T-DNA mutants were studied under green house and laboratory conditions to test both their phenotype and stress resistance. The knock-out mutants showed a particular phenotype in control condition (20°C, 8h light, 150µE) with red leaves and trichomes. In literature is reported that the same phenotype was shown by the wild-type in high light condition, revealing that the red pigmentation, due to anthocyanins, is caused by ROS accumulation. To test the level of stress-tolerance of these mutants we measured chlorophyll fluorescence (Fv/Fm) in response to photoinhibition (1h at 2000µE and 10°C). The mutants showed a lower Fv/Fm than the wild-type plant, suggesting a higher sensitivity to light stress. We have also found that the mutants flower later than the wild-type plants only in short day condition. The future aim is the characterisation of the mutant plants in drought and cold stress conditions for understand the particular phenotype and the resistance.

The 6g2 and 10d10 genes are putatively involved in sumoylation pathway and a protein-protein interaction study via yeast two-hybrid system has been started.
INFLUENCE OF GROWTH STAGE ON MOLECULAR RESPONSE OF DURUM WHEAT TO WATER STRESS

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drought, durum wheat, growth stage

Physical stresses, particularly drought, place major limit on cereal productivity in Mediterranean environments which are undergoing a continuous decrease of mean rainfall.

The exposure of plant to abiotic stresses determines a physiological and molecular response depending, in initial phases, on a complex cascade of stress signal transduction in which few regulatory genes control many downstream genes that contribute to stress tolerance.

Many studies describe the expression profile of stress-related genes in early developmental stages, nevertheless the plant can react to stress in different ways if different phenological stages are considered, depending on the developmental processes in course.

In this work a set of 15 stress-related genes has been analysed for expression, in terms of transcript amount, in response to water stress in different tissues and phenological stages from the third leave to the physiological maturity (dough stage) in the Ofanto variety of durum wheat.

The tested genes were selected by means of two strategies based on an in silico and an experimental approach. In the first case, genes coding for transcription factors involved in abiotic stress response have been selected among wheat ESTs available in public databases. Different members of DREB/CBF family and a WRKY transcription factor have been assessed with specific primer couples.

In the second case, genes with a putative role in regulation of abiotic stress response on the basis of sequence homology and early expression following exposure to stress, have been chosen among those isolated from a subtractive library in which the oxidative stress, a common aspect to different abiotic stress types, was imposed by treating durum wheat plantlets to low temperature (3°C) in presence of light. The selected genes could be involved in the response to different stresses, included dehydration, in which the oxidative damage plays a key role.

Among the selected sequences some of them are putatively involved in the regulation of gene expression at transcriptional level, coding for zinc finger transcriptional factors, as well as at in post-transcriptional (RNA-binding proteins) or post-translational (ubiquitination and sumoylation pathways) control.

All tested genes appeared to be induced by stress in booting and flowering stages, while only some of them showed a clear response to the applied stress also in early stages and/or at physiological maturity, these results indicaticing a strong influence of developmental stage on gene responsiveness to water stress.
NUCLEOTIDE DIVERSITY AND LEVELS OF LINKAGE DISEQUILIBRIUM IN CANDIDATE GENES FOR DROUGHT AND SALINITY RESISTANCE IN DURUM WHEAT

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association mapping, stress resistance, durum wheat

The genetic factors that underlie complex quantitative traits can be identified by using linkage disequilibrium (LD) mapping. Such an analysis allows to exploit natural populations to map the genetic determinants of a trait of interest. In this way it relies on many more informative meioses (i.e., all those occurred in the history of the genotypes) than those contained in a traditional mapping population. Association analysis has the potential to identify a single polymorphism within a gene that is responsible for the phenotypic variation. In this study we used the candidate gene approach to localize the genes that contribute to the drought and salinity resistance in durum wheat (Triticum turgidum ssp. durum). An initial set of 100 candidate genes was determined by choosing genes with known function in Arabidopsis and rice. This set comprises stress-inducible transcription factors belonging to many different classes, including the bZIP, MYB, ERF/AP2, WRKY and zinc finger families. Primers have been designed on the basis of the wheat ESTs homologous to these genes. In the case of co-amplification of more than one locus, the primers have been redesigned from the single sequences obtained by cloning the PCR product. 30 genes have been analysed so far with this procedure. For a number of genes more than one locus per gene was amplified and sequenced. In total 46 loci have been sequenced in a subset of the 12 lines that are representative in terms of genetic diversity and stress resistance of the whole set of 88 lines that will be characterized phenotypically during the project. Overall, we found a low SNP frequency (equal to 0.20%, i.e. one SNP every 510bp) and, as a consequence, a very low nucleotide diversity in most loci (on average equal to 0.00066). It has been observed that in the sequenced regions LD does not decay significantly within physical distances of 1kb. This approach will potentially allow to establish statistically significant associations between nucleotide diversity at the candidate loci and the phenotypic variation for the traits of interest and, thus, to identify the genes responsible for such variation to use in durum wheat breeding programs.
GROWTH DYNAMICS OF THE SEAGRASSES *POSIDONIA OCEANICA* (L.) DELILE UNDER STRESS CONDITIONS

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*Posidonia oceanica*, lepidochronology, bioindicator

The seagrass *Posidonia oceanica* (L.) Delile represents one of the most important primary producers of the Mediterranean coastal zone. *P. oceanica* forms meadows with a multifunctional role: produce and export biomass and energy, host diversified animal and algal community and stabilise coastal sediments protecting from erosion. However, the plant is sensitive to environmental disturbances and useful as bioindicator for environmental conditions: distribution pattern, shoot morphometry and dynamics of *P. oceanica* meadows are employed to detect an integrated response to disturbances.

In this work we have used the lepidochronology technique (that estimate the age leaf sheath persisting on the rhizomes after the leaf blade fall) as a tool to analyse seagrass dynamic. We analysed two meadows placed along the Dino Island (South Italy) subject to different environmental stress. The results show a decline in the shoot leaf production in *P. oceanica* shoots sampled in the disturbed site vs the preserved one; furthermore the same data provide evidence of the lepidochronology as suitable methods for detecting environmental disturbances.
THE EXPRESSION OF SEVERAL CBF GENES AT THE FR-A2 LOCUS IS LINKED TO FROST RESISTANCE IN WHEAT


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wheat, frost tolerance, Cbf, transcription factor

The C-repeat Binding Factor (Cbf) gene family has been shown to have a critical role in the regulation of low temperature stress response in Arabidopsis. In Triticum monococcum a locus carrying a family of Cbf-like sequences, orthologs of Arabidopsis Cbf genes, map at the frost tolerance locus Fr-A*m2, representing candidates for the differences in frost tolerance mapped at this locus. In this work we show that several of the Cbf genes have dramatically different levels of induction after cold exposure in hexaploid wheat. The Cbf-transcription levels differ between substitution and single chromosome recombinant lines carrying different 5A chromosomes or chromosome segments of the chromosome 5A from frost tolerant and frost sensitive wheat varieties. When the expression of eight Cbf’s mapped at the Fr-A2 was investigated with gene specific primers using real-time quantitative PCR, three Cbf sequences showed a significantly higher relative transcription levels (more than 4 fold change) in lines differing for the Fr-A2 region. Differences in Cbf expression was also associated with a difference in frost tolerance. These results suggest that the amount of some Cbf mRNAs might be the critical factor in determining the level of frost tolerance.
GENETIC ENGINEERING OF MAIZE WITH THE TRANSCRIPTION FACTOR OSMYB4 FOR TOLERANCE TO LOW TEMPERATURE

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genetic engineering, Zea mays, cold tolerance, drought tolerance

A major constraint faced in maize cultivation is the extensive damage caused by late cold events frequently occurring at high latitudes or with very early sowing, especially in condition of high light intensity. Early sowing in maize also allows to reduce the negative effect on grain yield of mid-summer drought episodes. The rice gene Osmyb4 encodes for a transcription factor involved in the cold acclimation pathway. When expressed into Arabidopsis, Osmyb4 increases chilling and freezing tolerance (Vannini et al., 2004, Plant J, 37, 115-127). In this work, Osmyb4 was genetically engineered in corn in order to explore the possibility to improve its level of tolerance to cold. We transformed three maize genotypes (bo21, B73A and GS3) with a biolistic protocol applied to type–II callus obtained from immature embryos. We used a mixture of two plasmid constructs, one carrying the Osmyb4 gene and one carrying the selectable marker (bar) and the reporter (gus) genes both driven by a constitutive promoter (ubiquitin). Two different constructs containing Osmyb4 were prepared with either an inducible (Cor15) or a constitutive (ubiquitin) promoter. The selection for transgenic events was carried out by cultivation of calli in media containing Bialaphos (3 mL/L) for 90 d followed by testing for Gus expression. All Gus-positive calli were then regenerated. Out of several hundreds of independently treated calli, transformation frequency was 1.13% for bo21 and 0.13% for GS3. We did not recover any transgenic event from B73A callus and from the constructs with Osmyb4 under constitutive promoter. The transgenic nature of the regenerants was confirmed by PCR. Southern analysis is in progress to verify the integrity of the transgene in each transformation event. Three out of 20 independent transgenic events were finally selected (R0 plants) based on a check for integrity of the gene and promoter sequences. The corresponding selected R1 progenies were subjected to a further PCR-based check to confirm the integration of the gene. Then the progenies were tested for cold-response, at 2 °C for 4 h, total RNA was extracted and used for RT-PCR to assess the level of transgene expression. Out of the three selected events, only one of them showed a strong and rapid induction of the transgene expression. The selected event (bo21-E-14-2) was further investigated as to its cold response by subjecting the plants to two different cold-stress treatments. Plants (the selected transformed event and the wild type, bo21) were treated either at 2 °C for three days or 2 °C for six days. Plants were allowed to recover at 24 °C for five days with 16 h of photoperiod. The shorter cold treatment did not affect the integrity of the leaf tissues in bo21-E-14-2, while the wild-type showed clear tissue necrosis; the longer cold treatment induced leaf damages in both wild type and bo21-E-14-2, but the damage was more evident in the control. After the recovery period only the transformed plants survived and were able to recover. After exposure to cold, leaf tissue of bo21-E-14-2 showed a substantial increase in sugar and praline content as compared to the control. Preliminary results indicate that bo21-E-14-2 is also able to withstand a drought stress sufficient to cause irreversible damage in control plants. Further experiments have been planned to better ascertain the level of tolerance to drought of bo21-E-14-2.
REAL TIME-PCR AS A TOOL TO ANALYSE GENE EXPRESSION RESPONSE TO TEMPERATURE STRESS IN DURUM WHEAT

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gene expression, real-time PCR, durum wheat, hsp101

Plant have both an inherent ability to survive exposure to temperature above the optimal for growth (basal thermotolerance) and an ability to acquire tolerance to normally lethal temperatures after an initial exposure to mild heat stress (acquired thermotolerance). Relatively little is known about the genetic factors involved in both type of thermotolerance in plants. One of the most studied response is the synthesis of specific proteins named “heat shock proteins” (HSP). Quantitative and/or qualitative variation in HSPs production was suggested to be correlated to the varying capacities of thermotolerant and thermosusceptible strains to acquire thermotolerance. At present in Arabidopsis only the HSP101 has been proven to be related to acquisition of thermotolerance. There are other signalling pathways that could be involved in thermotolerance (basal and acquired) and they depend on signalling molecules such as ABA, ethylene, active oxygen species (AOS), salycic acid (SA). Evidences are accumulating to support the hypothesis that thermotolerance is a complex multigenic process, with different gene sets involved in its development. With the purpose to analyse the expression levels of different sets of gene potentially related with thermotolerance in durum wheat, real-time reverse transcription PCR (RT-PCR) was employed on wild and domesticated durum wheat accessions, classified as sensitive or tolerant to heat stress. To asses the role of HSP101 with regards to thermotolerance, a molecular analysis was performed to elucidate the composition of HSP101 gene family in durum wheat. Different cDNA fragments cloned from two durum wheat cultivars, Creso and Ofanto, classified as sensitive and tolerant to heat stress, confirmed the presence of two distinct genes encoding HSP101. To understand the role of each gene product in the thermotolerance trait, a multiplex Real-Time PCR reaction was performed, targeting the polymorphic regions of each gene. The expression of these genes in the two cultivars, exposed to different thermal regimes, was subsequently compared. By this approach significant differences in the expression profile of HSP101 genes between the cultivars, in response to the same stress conditions, are evidenced. The expression of several ESTs related with thermotolerance, isolated from a wild durum wheat accession, was evaluated by real-time RT-PCR and the results obtained confirmed the multigenic determination of the thermotolerance trait.
EXPRESSION ANALYSIS OF HEAT SHOCK PROTEINS 70 (HSP70) IN
SEVERAL CULTIVARS OF MEDICAGO SATIVA AFTER HEAT SHOCK
TREATMENT

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HSP70, alfalfa, Medicago sativa, heat shock, stress tolerance

Heat shock proteins represent a class of multi-gene proteins that is highly conserved through all kingdoms
of living organisms. They are transiently activated in response to several stimuli such as environmental
stress or physiological stress. Their principal role in vivo is to act as molecular chaperones and help
folding of nascent proteins, refolding of denatured protein and transport of irreversibly damaged proteins
to degradative cell compartments and proteasomes. Recently mammalian HSPs have been shown to have
strong potential in improving immune response to pathogen attack and against tumours. Besides they
have strong adjuvant property.

Plants HSPs are typically activated transiently after heat shock and their expression level remains high up
to several minutes after induction. The activation of HSPs machine in plant can be correlated to their
tolerance to heat or cold stress helping the selection of plant cultivars tolerant to specific environmental
stress.

In this work we studied the expression and accumulation of HSP70 in five alfalfa cultivars and in
Arabidopsis (as model plant) in response to treatments to heat and cold stimuli. Heat shocked leaves were
harvested and frozen. cDNA obtained after heat shock was analysed by Real time PCR using specific
probes for HSP70 constitutive and inducible form. Cytosolic proteins were extracted and analysed
through SDS-PAGE and Western blot.

The response of different varieties to heat shock treatment both at RNA and protein level will be
discussed.
EXPRESSION PROFILING IN RESPONSE TO HIGH TEMPERATURE DURING TOMATO FLOWER DEVELOPMENT


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gene expression, cDNA microarrays, heat stress, tomato flower development, fruit set

In tomato, high temperature severely impairs fruit set when it occurs during flower-to-fruit transitions, often decreasing yield in this crop species by 70%.

In this study, the effect of heat stress (HS) on tomato was flower development was investigated in two genotypes with high (cv Saladette) and poor (cv Pullrex) fruit set ability under HS using expression profiling and the TOM1 cDNA microarray.

Plants of the genotypes Pull and Saladette were grown under controlled temperature conditions, in greenhouse at 26°C/20°C day/night (optimal condition) and high temperature (36°C/25°C day/night). Heat stress was imposed on flowers at different developmental stages, including gametogenesis, fertilization and anthesis, all of which are known to be very HS sensitive. cDNA targets used to probe the TOM1 array were obtained from RNAs from control and HS floral buds of three developmental stages.

Statistical analysis of the obtained profiles of three biological replicas for each stage, allowed us to identify several sequences differentially and significantly regulated by HS (~400 clones, t-test p-value < 0.05) during flower development in the two different tomato genotypes.

To the regulated ESTs belong sequences corresponding to genes coding for transcription factors, signal transduction factors, protein related to flower and gamete development, enzymes involved in primary or secondary metabolism and in cell wall structural changes, ribosomal proteins, heat shock proteins and other stress-responsive gene products.

Preliminary results indicate the anthesis as the developmental flower stage where major transcriptional changes occur under heat stress.

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QUALITATIVE PROPERTIES OF BOUND WATER ARE ASSOCIATED WITH BETTER WATER STATUS OF DURUM WHEAT LEAVES

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drought tolerance, bound water, free water, mutants

Much is known of the Bound Water (BW) role in desiccation tolerance of seeds, by contrast little is known of BW role in drought tolerance of vegetative tissues. Results of physiologic studies performed to investigate the adaptive value of BW in tolerance to moderate dehydration stress are shown in this poster. Leaf samples showing large variation for BW content were obtained using three durum wheat (Triticum durum Desf.) cultivars: Capeiti 8, Creso, Trinakria and three mutants (108, 364, 290) that differ for tissue affinity for water. By construction of adsorption Isotherm (AI) curves, we analysed the qualitative and quantitative properties of water that is bound with different strength to ionic, polar or hydrophobic sites of macromolecules. Three parameters related to the amounts of the weakly and strongly-bound water (quantitative BW properties) and five parameters related to tissues-binding strength for the same water fractions (qualitative BW properties) were determined. Pressure-Volume curves were also constructed on fresh tissues to determine the amount of the non-osmotic BW fraction, free water Relative Water Content at turgor loss point and osmotic potential at full turgor. Leaves with high quantity of strongly BW had also great content of weakly BW, but not necessarily large affinities for the same water fractions, suggesting that the quantitative and qualitative BW properties are probably affected on different factors. Qualitative properties of bound water are related to the water status of living leaves, in fact the higher the affinity of leaves for BW, the lower the tissue water content at turgor loss point and the larger the quantities of free water. These results support the idea that exists a continuum transition among the different tissue water fractions and suggest that the state of BW is an important adaptive mechanism not only for tissues that withstand extreme desiccation, but also for vegetative tissues exposed to drought stress.
EXPRESSION PATTERN OF TWO AQUAPORINS-ENCODING GENES IN POSIDONIA OCEANICA AFTER SALT STRESS


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Posidonia oceanica, aquaporins, salt stress

Seagrasses of the genus Posidonia are marine phanerogams of the Potagemotonaceae family and they are diffused in the Mediterranean basin and in Australia. Posidonia meadows represent a very important food substrate for many marine organisms and it’s a useful bioindicator for environmental conditions (Pergent et al., 1995). However we are observing a progressive extension reduction of the Posidonia meadows due to natural and antropic factors as well (Marbà et al., 1996). Like other seagrasses, i.e. Zostera e Cymodocea, Posidonia plays an important role in maintaining marine environments. However, basic understanding of seagrass molecular physiology is still limited (Fukuhara et al., 1996; Giordani et al., 2000) and it needs to improve such knowledge in order to planning right conservation projects.

In this work we have investigated the expression domains of two genes encoding two Aquaporins called respectively PoPIP1;1 and PoTIP1;1 isolated in P. oceanica plants (Maestrini et al., 2004). Aquaporins belong to a highly conserved group of membrane Major Intrinsic Proteins (MIPs) playing an important in the water transport through cell membranes. Aquaporins have been studied at molecular, phylogenetic, biochemical and biophysical levels and are likely to be important both for the whole plant (for water transport to and from vascular tissues) and for the cells (for buffering osmotic fluctuations in the cytosol). We have verified, by in situ technique, the expression domains of these two genes in different organs of P. oceanica both in vivo as well as after salt stress. The experimental results showed that PoPIP1;1 is preferentially localized in the apical meristem and in the rhizome, whereas PoTIP1;1 is localized in the root; both the aquaporins are expressed in the leaf mesophyll, suggesting an important role of these aquaporins in the uptake and water homeostasis in P. oceanica plant.

PERGENT et al.,1995. - Mésogée, 54, 3-27
Anthocyanins are water soluble pigments belonging to the flavonoids compounds family in nature involved in a wide range of functions; since these pigments impart much of the colour and flavour of fruits and vegetables they are considered components of human diet not exclusively as food products but also as therapeutic agents; in this respect the anthocyanins have been suggested to protect against oxidative stress, coronary heart diseases, certain cancers and other age related diseases (Ross et al., Annu. Rev. Nutrition, 22:19-34, 2002). Nowadays, the anthocyanin’s biosynthesis pathway has been almost completely elucidated and most of the structural genes encoding the enzymes responsible for each steps have been isolated from different sources (Holton and Cornish, The Plant Cell, 7:1071-1083, 1995). The activity of the anthocyanins biosynthetic genes is largely regulated at transcriptional level and consequently the pigmentation pattern must be specified by the expression patterns of the regulatory genes. Moreover, several environmental stimuli can activate the transcription of anthocyanins biosynthetic genes such as light, osmotic and cold temperature stresses (Chalker-Scott, Photochemistry and Photobiology, 70:1-9, 1999). The cold induction of pigmentation has been studied in flower development and related to activation of the expression of anthocyanin biosynthetic genes, including phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), dihydroflavonol 4 reductase (DFR) and anthocyanidin synthase (ANS) (Martin and Gerats, The Plant Cell, 5:1253-1264, 1993). As regards tree fruits, studies on low temperature induced anthocyanins accumulation have been only conducted on apple skin (Reay, Scientia Horticulture, 79: 113-119, 1999) and peach seedlings (Leng and Qi, Scientia Horticulture, 97:27-39, 2003); therefore, the knowledge of low temperature effects in regards to the fruits eatable portions is still missing. In this work, we studied the impact of a low temperature (4°C) during a moderately long storage period (75 days) on sweet orange (Citrus sinensis L. Osbeck, cv Tarocco) anthocyanins production and on the expression of structural genes involved in their biosynthesis such as PAL, CHS, DFR and UFGT whose partial cDNA clones have been previously isolated (Lo Piero et al., J. Plant Biochem. Biotech., 14: 1-6, 2005). Moreover, the above mentioned parameters have been also monitored in orange samples in which cold treatment was extended only for 45 days being they subsequently placed at 25 °C for further 30 days. A third oranges group was stored at 25 °C for the entire experimental period representing the control samples. Our results showed that low temperature induced anthocyanins accumulation in sweet orange juice vesicle reaching after 75 days values eight times higher compared with those kept at 25 °C. Besides, real time RT-PCR showed that expression of PAL, CHS, DFR and UFGT whose partial cDNA clones have been previously isolated (Lo Piero et al., J. Plant Biochem. Biotech., 14: 1-6, 2005). Moreover, the above mentioned parameters have been also monitored in orange samples in which cold treatment was extended only for 45 days being they subsequently placed at 25 °C for further 30 days. A third oranges group was stored at 25 °C for the entire experimental period representing the control samples. Our results showed that low temperature induced anthocyanins accumulation in sweet orange juice vesicle reaching after 75 days values eight times higher compared with those kept at 25 °C. Besides, real time RT-PCR showed that expression of PAL, CHS, DFR and UFGT was strongly induced during low temperature exposure since levels of all transcripts increased at least 40 fold with respect of control samples. Interestingly, oranges fruits subjected to a brief low temperature exposure (45 days) still maintained higher levels of anthocyanins than those registered in control samples even after a subsequent 30 days storage period at 25 °C, thus suggesting that 45 days exposure was sufficient to get at least a two fold increase. Concordantly, expression of CHS, DFR, and UFGT was always much higher in samples subjected to brief induction than in the control samples. Only PAL transcripts rapidly decreased due to the temperature change 4°C to 25 °C indicating that “early” and
“late” gene implicated in anthocyanins biosynthesis might have been affected by different regulation mechanisms.
DIFFERENTIAL EXPRESSIONS OF POPLAR SUPEROXIDE DISMUTASE AND ASCORBATE PEROXIDASE GENES IN RESPONSE TO DROUGHT IN CAMBIUM AND LEAVES OF *POPULUS ALBA V. VILLAFRANCA*

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*antioxidant gene, APX, drought, Populus alba, SOD*

In this study, we investigated in poplar (*Populus alba v. villafranca*) the effects of drought on expression genes encoding for SOD and APX. These enzymes play an important role in the defence against cell oxidation induced during stress conditions and in plants the SOD and APX genes are known to be spatially and developmentally regulated.

Trees of *Populus alba v. villafranca*, were grown under well-watered conditions and then subjected to a moderate stress treatment by the 50% reduction of optimal water requirement. To evaluate physiological responses to drought at the whole-tree level, we used as indicator the radial growth of cambium determined by a non destructive automated system. After 23 days of moderate stress a growth difference of 37% due to the cambial activity was observed. At this critical time samples were taken for RNA extraction. The expression profiles of the components of SOD and APX gene families were analysed in cambium and leaves. Transcript analysis was carried out by means of relative quantitative RT-PCR (RQ RT-PCR) using specific primers for each gene and tubuline as housekeeping gene. The data evidenced that the transcription levels of antioxidant genes were higher in leaves than in the cambium, furthermore interesting differences were observed among the SOD genes. In particular, the increase of *Mnsod* transcripts in stressed tissues suggested a strong involvement of mitochondria in the drought response.
NUTRIENT SOLUTIONS CAN INFLUENCE THE (POLY)PHENOLIC COMPOSITION OF TOMATO FRUITS?

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(poly)phenolic compounds, tomato fruits, posidonia, nutrient solution, electric conductivity

During recent years the contents of (poly)phenolic compounds in plant foods have received much attention because of their biological properties and potential human health benefits. Epidemiological studies have shown that consumption of fruits and vegetables rich in phenolic compounds is associated with lower risk of cardiovascular diseases and cancer mortality. Natural phenolic compounds occur as free compounds, in glycosidic linkage or in some instances as acylated derivatives.

The contents of (poly)phenolic compound in various tomato varieties were characterized as flavonoids (naringenin, rutin, quercetin and kaempferol) and hydroxycinnamic acids (chlorogenic, caffeic, ferulic and coumaric). The most abundant hydroxycinnamic acid was chlorogenic acid.

The (poly)phenolic compound contents of tomatoes depend on genetic, and environmental (temperature, light, water availability) factors, and on the agricultural techniques used (cultivars, plant growth regulators, date of harvest, etc).

In recent years increasing interest has been focused on the use of soilless cultivation systems using posidonia (*Posidonia oceanica* (L) Delile) as substrate and nutrient solution containing several salt types (*Ca(NO_3)_2\cdot4H_2O*, KNO_3, KH_2PO_4, MgSO_4\cdot4H_2O, K_2SO_4, HNO_3) at various concentration. This production method presents together with some disadvantage, such as high installation cost and dependence on the electricity automation of the system, several advantages. The main advantages are: 1) possibility of using areas not suitable for traditional agricultural systems, 2) greater productivity per area, 3) possibility of several harvests during the year, 4) less consumption of fertilizer and water, 5) greater hygiene an less possibility of contamination with microorganisms, nematodes, insects inherent to the soil, 6) less manpower needed, and 7) high quality control.

It has also been shown that the cultivation condition can be modified so as to increase the concentration of desiderable constituents. As example water or nutrient solutions containing moderate to high concentration of salts are frequently supplied to improve the taste of tomato fruits. Unlike growth and yield studies, few experiments have been conducted on the influence of NaCl salinity on nutraceutic properties of tomatoes such as the (poly)phenolic compound contents. The aim of this study was to investigate the influence of three levels of electric conductivity (by varying the NaCl concentration) of the nutrient solution on the (poly)phenolic compound contents of tomato fruits (cv. Kabiria) harvested at four ripening stages.

Three level of electric conductivity (EC) were investigated: low (L), high (H) and medium (M). Results show that the main phenols presents in Kabiria fruits in all the ripening stages and EC levels are
chlorogenic acid, rutin and naringenin. Caffeic and coumaric acid are present at very low concentration (< 1 mg/kg f.w.). The (poly)phenolic content seems to be related to the salinity and to the ripening stage.
NaCl EFFECTS ON IN VITRO TISSUE CULTURES OF DENDRANTHEMIUM (CHRYSANTHEMUM MORIFOLIUM)

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salt-stress, cell cultures, chrysanthemum spray

The conditions for a salt-stress (NaCl) were simulated in vitro using calli of chrysanthemum spray, obtained a number of subcultures of callus derived from the explants of 2 genotypes.

In the Reagan A and Reagan D genotypes, explants are derived from internode by plantlets grown in vitro. The callus culture was carried out on MS modified. The calli were cultured in presence of NaCl (2000 ppm)-MDI added in the basic medium-MD0.

The experiment was carried out according to an experimental factorial design with 6 repetitions, submetting, on the conditions of salt-stress, the callus of 2 genotypes of Dendranthemium (Chrysanthemum morifolium) to 2 lengths of time (7 and 14 days).

On the callus treated fresh weight and dry weight were measured, leaving the callus at 65°C for 48 hrs.

The effects on the growth and development of callus of chrysanthemum spray under saline conditions and selective ions accumulation have been observed.
SUSCEPTIBILITY TO BACTERIAL SPECK OF TOMATO CULTIVARS HARBOURING RESISTANT GENE PTO: DIFFUSION OF AVRPTO IN PSEUDOMONAS SYRINGAE PV. TOMATO STRAINS ISOLATED FROM THESE CULTIVARS

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resistance gene, avrPto, bacteria, plant disease

Resistance of tomato genotypes to the causal agent of bacterial speck (Pseudomonas syringae pv. tomato) (PST) is monogenic dominant and is due to Pto gene that interact with a “gene for gene” manner with the avirulence gene avrPto. Pto gene that originate from Lycopersicon pimpinellifolium is linked to Fen gene that confer susceptibility to the insecticide fenthion. The susceptibility to fenthion of tomato lines is used than inoculation with bacteria, when breeders sorting their materials for resistance to PST. A lot of PST resistant genotypes are commercially available. In the year 2002, heavy attack of bacterial speck were observed in an experimental field in Southern Italy. At least six out twenty resistant tomato genotypes showed spots of bacterial speck. From four resistant tomato cultivars (Coimbra, Talent, Fastel and Alange) resulted susceptible in the field, isolations of PST were made from single spots of the fruits and the presence of avrPto was investigated by PCR. The typical amplification product of avrPto was generally observed on agarose gel, confirming large presence of this effector gene in the natural infections of PST in the experimental field. The results strongly support the hypothesis that loss of resistance of these tomato cultivars was due to mutation or loss of Pto gene. Further investigation on these tomato genotypes are needed.
ROLE OF AVIRULENCE GENE AVRRPT2 ABOUT VIRULENCE OF PSEUDOMONAS SYRINGAE PV. TOMATO ON TOMATO

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effector proteins, bacteria, plant disease, virulence

Avirulence gene avrRpt2 of Pseudomonas syringae pv. tomato (PST) encode an effector protein secreted into tomato plant cell by type III secretion system of the bacterium. This effector gene is a lot of diffused in PST populations and, recently, has been demonstrated that it contribute to virulence on tomato. In this work, the role of avrRpt2 about virulence on tomato plants is further investigated by inoculation on three tomato cultivars of a mutant derivative of a virulent strains of PST that carries a disruption in avrRpt2. After two weak, the mutant gave significantly reduction of symptoms on all tomato cultivars with respect to virulent wild strain of PST, confirming the role of avrRpt2 to reduction of symptoms on tomato. Nevertheless, inoculation of the mutant strain with avrRpt2 reintroduced into bacterial cell by plasmid, gave slowly symptoms. It is possible that a different expression of avrRpt2 from the plasmid with respect to expression from the bacterial chromosome is responsible for our inability to observe complementation. It is also possible that we introduced an additional modification during mutant construction. Further investigation is in progress.
SECONDARY METABOLITES PRODUCED BY TRICHODERMA SPP AND THEIR ROLE IN THE INTERACTION OF THIS FUNGUS WITH PLANTS AND OTHER MICRORGANISMS


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secondary metabolites, Trichoderma, antibiotics, biopesticides, systemic resistance

Trichoderma strains are among the most studied fungal biocontrol agents and are successfully used as biopesticides and biofertilizers in greenhouse and field plant production. These applications are related to their ability to control plant diseases and promote plant growth and development.

Biocontrol Trichoderma strains show different antagonistic mechanisms towards fungal pathogens. These include the production of a variety of antibiotics, mycoparasitism or hyperparasitism, competition for nutrients or space and cell wall-lytic enzymes activity. In addition, these fungi may induce systemic resistance in plant which prevents further attacks by pathogens. Secondary metabolites play an important role during biocontrol, although their modes of action towards microorganisms and plants have not been fully elucidated.

In this work, we isolated the major secondary metabolites from three biocontrol strains of T. harzianum (T22, T39 and A6) and one of T. atroviride (P1). Seven compounds were extracted and characterised from fungal culture filtrates: two new molecules, a T22 azaphilone (3) and a T39 butenolide (5), and five already known secondary metabolites [1-hydroxy-3-methyl-anthraquinone (1), 1,8-dihydroxy-3-methyl-anthraquinone (2), harzianolide (4), 6-n-pentyl-6H-pyran-2-one (7) and harzianopyridone (5)] were characterized. The compounds isolated showed different levels of antibiotic activity against the pathogens Gaeumannomyces graminis var. tritici, Rhizoctonia solani and Pythium ultimum, and may have specific modes of action and roles in the antagonistic activity of Trichoderma.

In order to investigate the potential involvement of secondary metabolism in the interaction of Trichoderma with plants, the ability of the newly isolated compounds to induce systemic resistance was evaluated. Preliminary results showed a reduction of disease symptoms in canola seedlings treated with the fungal metabolites and inoculated with the pathogen Leptosphaeria maculans.
\[ \text{1 } R = \text{H} \\
\text{2 } R = \text{OH} \]
DEVELOPMENT OF A “FILTER ARRAY” FOR THE DETECTION OF VIRAL RECOMBINANTS IN PLANTS

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viral recombination, macroarray, tomato spotted wilt virus (TSWV), cucumber mosaic virus (CMV)

Viral recombination between different virus and viral recombination in virus resistant transgenic plants play an important role in their variability and evolution, this is particular for RNA virus. These phenomena happen with low frequency and it is necessary to use very sensitive methods to detect them. Among different molecular techniques currently used, we preferred macroarray methods for different reasons:

a) simple genome virus necessities a low density of spotted genes compare to microarray;
b) detection is based on $^{33}$P;
c) it is possible to repeat hybridisation on the same array at several time;
d) low cost.

The aim of this research was to optimize an array and to study the limit of sensitivity in detecting viral recombinants. For this study two important tomato phytoviruses, one cucumber mosaic virus (CMV) and one tomato spotted wilt virus (TSWV), were chosen. The viral genome organization was checked to determine possible similarities and homologies between CMV and TSWV sequences by NCBI and EMBL bank. One hundred primers pair were designed and used to amplify whole viral genome. Polymerase chain reaction (PCR) products were spotted on filter by multifunctional station GeneTAC G3 (Perkin Elmer). Artificial recombinants (in vitro syntetized), made by parts of CMV and TSWV genome, were used as $^{33}$P probe to check array sensitivity. In order to mimic a natural scenario, artificial recombinant transcript (used in decrescent concentration) was mixed to total RNA extracted from tomato leaves. Different concentration of transcript (100, 10, and 1 ng) were mixed by maintaining total RNA quantities. Filter array sensitivity, observed by filter hybridisation, was lower than 1 ng of transcript per 100 ng of total RNA. The signal was so intense to reach lower sensitivity than 1 ng. The lower limit of sensitivity is currently being elaborated.
TRANSCRIPTIONAL PROFILING OF TOMATO PLANTS INFECTED WITH THE TOMATO YELLOW LEAF CURL SARDINIA VIRUS BY LONG SERIAL ANALYSIS OF GENE EXPRESSION

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plant-pathogen interaction, tomato, geminivirus, transcriptional profiling, long-SAGE

The geminivirus Tomato Yellow Leaf Curl Sardinia Virus (TYLCSV) is one of the agents of the tomato yellow leaf curl disease, which causes dramatic crop losses in the Mediterranean and sub-tropical regions. The viral genome is a 2.7 kb single stranded circular DNA; two proteins are encoded by the viral strand and four by the complementary one. Although a huge amount of molecular data exist on TYLCSV, little is known about its interaction with the host plant. In order to gain insight into TYLCSV-tomato interactions at molecular level, we decide to analyse the transcriptional profile of TYLCSV infected tomato plants. To do this, Long-Serial Analysis of Gene Expression (Long SAGE) was used; this technique, based on the identification of 20 nt long sequences (TAGs), allows the quantitative analyses of transcripts without the need for any prior knowledge of their sequences.

Tomato plants were infected with TYLCSV in three independent experiments and leaf samples were collected from five plants in each experiment at four weeks post-inoculation; parallel sampling was performed on control healthy plants. Total RNAs were extracted and pooled resulting in two final samples, the infected and the control ones, which were used to construct two Long SAGE libraries.

About 41,000 TAGs were produced for each library, representing a total of 9,433 genes; among these, 5,809 differed in their expression at least two-fold. A preliminary analysis was performed on TAGs whose expression differs at least seven-fold between the two samples. 62 and 50 TAGs were respectively up- and down-regulated by TYLCSV infection. In the up-regulated group, beside viral transcripts, 39 TAGs matched tomato unigenes and 15 were not found in the SGN data bank, representing possible novel genes. In the down-regulated group only 20 TAGs matched tomato unigenes. It is noteworthy that only 37 out of 59 unigenes identified were represented on the TOM1 cDNA chip. Moreover, 10 out of 59 TAGs matching tomato unigenes were in antisense orientation; further analysis will be done to understand the biological relevance of this observation. Regarding the functions of up- and down-regulated genes, TYLCSV infection stimulates expression of genes involved in defense and stress responses, signal transduction and protein metabolism, while it reduces expression of genes implicated in photosynthesis.

These preliminary data suggest that transcriptome analysis by Long SAGE is a powerful tool to study virus-host interactions.
LONG SERIAL ANALYSIS OF GENE EXPRESSION OF TRANSGENIC PLANTS TO DECIPHER TOMATO YELLOW LEAF CURL SARDINIA VIRUS-TOMATO INTERACTION

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plant-pathogen interaction, tomato, TYLCV, virus-derived sequences, LongSAGE

The Tomato Yellow Leaf Curl Sardinia Virus (TYLCSV) is one of the most devastating pathogens that affect tomato (Solanum lycopersicum) crops in a vast sub-tropical region of the world. The infecting agent is a ssDNA virus belonging to the family Geminiviridae. The viral genome is a 2,7 Kb single stranded circular DNA; two proteins are encoded by the viral strand and four by the complementary one. In order to gain insight into TYLCSV-tomato interactions, our laboratory is currently analysing the transcriptional profile of TYLCSV-infected tomato plants by Long-Serial Analysis of Gene Expression (Long SAGE). Long SAGE identifies 20 nt long sequences (TAGs), allowing the quantitative analysis of transcripts without the need for any prior knowledge of their sequences. 5809 genes were differentially expressed (at least two fold) between healthy and TYLCSV-infected tomato plants (see accompanying poster). To further dissect the viral-host interaction we decided to analyse the transcriptional profile changes induced by the expression of single viral products in transgenic tomato by longSAGE. We focalised our attention on a key factor for viral replication: the replication associated protein (Rep). Rep is a 359aa long protein codified by the viral C1 gene; it is a multifunctional factor able to regulate its own gene transcription and to bind and nick the viral DNA during the initiation and termination phase of the rolling-circle replication (RCR). Moreover, it interacts with plant factors involved in DNA replication and cell cycle regulation, such as the plant retinoblastoma-related proteins (pRBR). We produced different lines of transgenic tomato plants stably expressing two truncated forms of the TYLCSV Rep protein (Rep-210 or Rep-130). Rep-210 posses the DNA binding and cleavage domains and the RBR-binding domain whereas Rep-130 retains only the DNA binding and cleavage domains. Both Rep-210 and Rep-130 have the ability to down regulate C1 gene transcription binding the C1 gene promoter. Rep-130 and Rep-210 plants were grown alongside healthy and TYLCSV-infected wt tomato under controlled conditions in three independent experiments. Rep-210 and Rep-130 longSAGE libraries were produced and are currently being sequenced. Transcriptome analysis of Rep-210 and Rep-130 plants will be presented.
ANALYSIS OF VIRUS RESISTANCE IN A GM TOMATO LINE: BIOLOGICAL STUDIES AND MECHANISMS INVOLVED

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Transgenic plants, virus resistance, tospovirus, gene silencing

Tomato spotted wilt virus (TSWV), genus Tospovirus, is an important pathogen of many crops worldwide. We have previously described the production and selection of a TSWV-resistant tomato line (30-4) obtained by genetic transformation with the N gene of TSWV. This line has a true-to-type phenotype and a single integration locus with multiple rearranged transgene copies.

Due to the interesting features of this line, we decided to study the resistance more in depth, using biological as well as molecular tools. Since post-transcriptional gene silencing (PTGS) is often involved in cases of extreme resistance to viruses and is associated with the presence of siRNAs, RNAs were extracted from the transgenic homozygous 30-4 plants and analysed by urea PAGE: following transfer on nylon membrane, hybridizations were performed with transgene-specific dig-labelled probes and small interfering RNAs (siRNA) were detected.

The biological role of these siRNA in the resistance has been studied by grafting experiments. Transgenic scions were grafted on TSWV-infected tomato rootstocks to evaluate whether viral infection could be transmitted through phloem to the transgenic scion. In a second type of grafting, nontransgenic scions were grafted on transgenic rootstocks and, after one week, mechanically inoculated with TSWV. These experiments should indicate whether a “resistance signal” is present in the GM plants, and whether it can be translocated through the grafting junctions, affecting virus replication.

Several plant viruses have been shown to code for silencing suppressors (2b, HC-Pro, p19, p25, AC2, etc.). Experiments are in progress to understand if these viruses interfere with the resistance observed.
THE MOLECULES THAT ACTIVATE ANTAGONIST FUNGI USED AS BIOPESTICIDES

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plant disease, biocontrol genes, biocontrol inducers

Fungi of the genus *Trichoderma* are biological control agents commonly applied against a variety of plant diseases. Pathogens and plants release molecules that are detected by antagonists and induce the expression of biocontrol genes. We used two *Trichoderma* mutants reporter gene systems based on the GFP and a gene encoding for a glucose oxidase under the control of biocontrol-related promoters to select low molecular weight compounds acting as “biocontrol inducers”.

Various compounds capable of producing these inducers were tested singly and in combination: purified *Trichoderma* enzymes (endochitinase, exochitinase, chitobiosidase and glucanase); culture filtrates (CFs) containing extracellular enzymes coming from *T. atroviride* P1 (wild-type and knock out mutants), *T. harzianum* and *T. resei*; CFs of the pathogens *Botrytis*, *Pythium* and *Rhizoctonia*; colloidal crab shell chitin; plant extracts from cucumber and tomato leaves, stems and roots. CFs from chitinase knock-out mutants and cell walls from Oomycete fungi were the less active. The compounds of MW less than 3kDa obtained from the host CW digestion were found to strongly activate *Trichoderma* gene expression as well as stimulate its mycelial growth and spore germination. HPLC-purified fungal host-derived inducers stimulated the production by *Trichoderma* of endochitinase and exochitinase even under repressing conditions in presence of glucose. These compounds assayed *in vivo* were also able to reduce disease symptoms induced by *B. cinerea* on bean leaves enhancing biocontrol effect of P1. Finally, purified inducers added to *T. atroviride* cultures stimulated the production of antibiotics that inhibited *Botrytis* and *Alternaria* spore germination. Mass spectrometry analysis (EMI-MS) of the inducers indicated the presence of hexose oligomers like cellobiose, whereas MS/MS-analysis by selective fragmentation of peaks in the spectrum demonstrated the presence of at least three distinct biologically active compounds.
CHARACTERISATION OF AN ALMOND 9-HYDROPEROXIDE LYASE TARGETED TO LIPID BODIES


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almond, hydroperoxide lyase lipidi body, oxylipins

Oxylipin metabolism represents one of many defence mechanisms employed by plants. The products of the pathway are biologically active both in defence signalling and as direct anti-microbial agents. Some of them are also significant in determining the organoleptic characteristics of food, through their flavour and aroma properties.

Most of the phyto-oxylipins so far identified are synthesised via the lipoxygenase (LOX) pathway. LOXs catalyse the hydroperoxidation of polyunsaturated fatty acids such as linoleic (C18:2) or linolenic acid (C18:3) and produce 9- or 13- hydroperoxides. 9- and 13-hydroperoxides are very reactive compounds and are further metabolised to an array of different oxylipins by the action of the other enzymes downstream located in the pathway, including hydroperoxide lyase (HPL).

HPL converts fatty acid hydroperoxides into aldehydes and oxoacids; in the case of 13-hydroperoxides and 13-HPL the products are 6-carbon aldehydes that are believed to have a signalling function and also play a direct role in plant defence. At present it is unknown if the products of 9-HPL can function as signal molecules and induce the expression of other genes.

Although the subcellular location of the enzymes of the early part of the pathway is well established, there is relatively little information on the subcellular distribution of HPL. In order to investigate on the subcellular localization of HPL, we used an almond HPL cDNA, previously isolated and identified as a 9-HPL, and prepared a set of green fluorescent protein (GFP)-tagged HPL fusions used to transform tobacco protoplasts. Confocal laser scanning microscopy analysis revealed that the almond HPL is targeted to the endomembrane system and to spherical bodies that were selectively stained with Nile Blue A thus indicating that they are strictly related to oil bodies.

This localization has been compared with a GFP based marker which localizes in oil bodies (oleosin-GFP) and with other markers targeted to other compartments, directly associated to endoplasmic reticulum, but independent from oil bodies (GFP-Chi).
ANALYSIS OF METHYLATION PROFILE OF TOMATO GENOME FOLLOWING INFECTION BY *TOMATO YELLOW LEAF CURL SARDINIA VIRUS* (TYLCSV)


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*TYLCSV*, *Lycopersicum esculentum*, MSAP, Real Time PCR, resistance genes

Activation of plant defences following recognition of pathogen attack involves a complex variety of signaling metabolic pathways and includes the transcriptional activation of an array of plant defense-related genes. This control of gene expression includes epigenetic phenomena such as DNA methylation. To date, the role of DNA methylation during viral infection in plants has been investigated to a limited extent. We analyzed the methylation profile of tomato genome during infection by *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV). This DNA virus belongs to the *Geminiviridae* family (genus *Begomovirus*) and possesses a monopartite genome of 2.8 kb. TYLCSV is transmitted by the whitefly *Bemisia tabaci* in a circulative persistent manner and causes severe crop losses in tomato, mainly in the Mediterranean basin. Geminiviruses, analogously to the animal DNA virus, have evolved the capability to interfere with host gene expression and cell cycle regulation, by reprogramming the whole host cell cycle. We assessed the methylation pattern of the tomato genome using an adaptation of the AFLP technique, called MSAP (methylation-sensitive amplified polymorphism). This technique is based on using enzymes (e.g. HpaII and MspI) sensitive to methylation of their recognition sequences.

We compared DNA profiles of infected vs uninfected tomato plants (cv ‘Moneymaker’), by collecting samples at different times following whitefly inoculation (1, 7 and 14 days). Changes in MSAP profiles of genomic DNA were detected and ten polymorphic fragments were excised from gels and sequenced. Sequences were compared with the ones available from the Solanaceae Genomics Network (SGN) website or GenBank database (NCBI). Some of the differentially methylated genes appeared to be involved in the plant defence mechanism, i.e. *cysteine protease, methionine adenosyltransferase*, while others showed similarity with leucine-rich repeat (LRR) proteins. This confirms the efficiency of MSAP in identifying gene sequences potentially involved in the defence of tomato plant to TYLCSV infection.

The expression level of these genes is currently being evaluated with Reverse Transcription-Real Time PCR.
DIFFERENTIAL ANALYSIS OF DURUM WHEAT-THINOPYRUM PONTICUM RECOMBINANT LINES CARRYING THE \textit{LR19} LEAF RUST RESISTANCE GENE BY NBS-PROFILING

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disease resistance (\textit{R}) genes, nucleotide-binding-site motif, DNA and RNA fingerprinting, \textit{R}-gene markers, wheat-alien gene transfer

\textit{Lr19}, one of the few remaining largely effective genes conferring resistance to leaf rust, was transferred from chromosome 7AgL of \textit{Thinopyrum ponticum} to 7AL of durum wheat. To identify specific DNA fragments tightly linked to \textit{Lr19} and to enrich the 7AL/7AgL region with new markers, several recombinant lines with varying amounts of alien chromatin, some carrying and others lacking \textit{Lr19}, were genotyped by NBS profiling. This PCR-based approach efficiently targets resistance (\textit{R}) genes and \textit{R}-gene analogues using degenerate primers targeted to the conserved nucleotide binding sites (NBS) characteristic of the frequent NBS-LRR class of \textit{R} genes. The fingerprints of two near-isogenic recombinant lines (NIRL) were compared. One NIRL carries \textit{Lr19}, and has 23\% of its 7AL replaced by 7AgL; the other lacks \textit{Lr19}, with 22\% of 7AL replaced by 7AgL. This allowed us to isolate \textit{R} gene fragments located within the 1\% 7AgL chromatin containing \textit{Lr19}. Two such polymorphic bands were identified, cloned and sequenced, and new sets of primers were designed and validated to allow the development of a codominant assay for \textit{Lr19}. The 7AgL and 7AL sequences generated by NBS profiling share high homology with known NBS-LRR wheat genes. In order to identify expressed NBS-like sequences induced by leaf rust infection, and encoded by the 1\% of 7AgL containing \textit{Lr19}, a modified NBS profiling protocol was then applied to cDNA obtained from infected seedlings of the two NIRLs. At particular time points post infection, NBS fragments were identified which were specific to plants carrying \textit{Lr19}, and these are currently being characterized. NBS profiling applied to wheat-alien recombinant lines is a promising strategy to describe, at the DNA and RNA levels, the \textit{R}-gene content of introgressed segments. It may also represent a first step for cloning important \textit{R} genes such as \textit{Lr19}. 

THE GENE ENCODING CERATO-ULMIN, AN OPHIOSTOMA-PRODUCED PROTEIN INVOLVED IN THE DUTCH ELM DISEASE, HAS BEEN INTROGRESSED OR HORIZONTALLY TRANSFERRED IN AN UNRELATED SPECIES OF THE GENUS GEOSMITHIA

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Ophiostoma, DED, Geosmithia, hydrophobin, cerato-ulmin

Cerato-ulmin (CU) is a class II hydrophobin protein of about 8000 Da, produced by the Ascomycota Ophiostoma novo-ulmi, O. ulmi and O. himal-ulmi (Whiteford and Spanu 2002, Mol Plant Pathology 3: 391–400). They are responsible of the Dutch elm disease (DED), well known to have destroyed in the 20th century the most part of native European and North-American elms (Ulmus spp.). CU is a component of the mycelial surface and is released in the medium when the fungus is grown in liquid shake culture (scarcely by O. ulmi). Many evidences suggested a key role of CU in the virulence of DED pathogens (Takai 1974, Nature 252: 124-126), but the involvement of CU in DED pathogenesis is still debated (Del Sorbo et al 2002, In "Advances in microbial toxin research and its biotechnological exploitation", Upadhyay R. (ed.), Kluwer Academic/Plenum Publishers, NY, U.S.A: 93-104). Another ophiostomataceae species, O. quercus, non pathogenic towards elm trees, has been shown to possess the CU protein in the fungal cell walls (Scala et al. 1997, Mycol Research 101: 829-834); recently, the gene encoding CU in O. quercus (cu gene) was isolated and characterized (Scala et al 2005, manuscript). Comparison of the cu gene sequences and of the rDNA regions of various Ophiostomas are consistent with the view that the DED pathogens evolved from O. quercus or from an O. quercus-like fungus (Pipe et al 2000, Mycol Research 148: 533-539). Until now, attempts to find the cu gene and/or CU-like protein in other fungal species were unsuccessful.

In this paper we demonstrate that a strain of Geosmithia sp., an unrelated species to the genus Ophiostoma, possesses the cu gene, and a CU-like protein is abundantly excreted in the liquid Takai medium. The strain, named IVV7, was isolated near the Vibo Valentia town (Italy) from elms showing the typical DED symptoms. IVV7 was determined to belong to the genus Geosmithia on the basis of the sequences of the internal transcribed spacers 1 and 2 and the 5.8S ribosomal RNA genes. The IVV7 cu gene has been isolated using primers based on the published cu gene sequence from O. novo-ulmi (Bowden et al 1994, Curr Genetics 25: 323-329); the analysis of the sequence showed that the cu gene of IVV7 is identical to that of O. novo-ulmi (score > 1000; E value = 0.0). The strain IVV7 has been defined on the basis of various morfological, physiological and pathological characters: (i) the morfology of the colony onto Malt Extract Agar (MEA) and of the conidia grown in liquid shake culture in Takai medium; (ii) the radial growth rate on MEA and the temperature growth optimum; (iii) the cerato-ulmin production index, assayed by the turbidimetric method and by ELISA; (iii) the pathogenicity on elm trees (Ulmus glabra Huds.).

The significance of the cu gene presence in a fungal species so distant from the Ophiostomas will be discussed also in order to try to understand if the cu gene has been introgressed in IVV7 by sexual contact and hybridization, or if it has horizontally been transferred via hyphal anastomosis.
GENETIC ENGINEERING FOR WHEAT PROTECTION AGAINST MYCOTOXIGENIC FUNGAL PATHOGENS

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Wheat Head Scab (WHS), genetic transformation, b-32, Triticum aestivum

Wheat Head Scab (WHS) or Fusarium Head Blight (FBH) caused mainly by *Fusarium culmorum* and *F. graminearum* is a destructive disease of wheat. The harvested grain is often contaminated by fungal-secreted mycotoxins which cause serious illness and immunorepression in humans and animals, as well as yield loss *per se*. In this respect, it is important the development of improved wheat genotypes with increased pathogen resistance using traditional breeding biotechnology strategies. A maize endosperm cytosolic albumin, with a molecular weight of 32 kDa, termed b-32, has homology with several previously characterized Ribosome-Inactivating Proteins (RIPs). It was found that b-32 is a functional RIP by the criteria of inhibition of *in vitro* translation in a cell-free rabbit reticulocytes system and specific N-glycosidase activity on 28S rRNA. Additional evidence indicated that transgenic tobacco plants expressing b-32 showed an increased tolerance against infection by the soil-borne fungal pathogen *Rhizoctonia solani*. In order to further explore the antifungal activity of the maize b-32, we have placed the b32.66 cDNA clone under the constitutive promoter 35S CaMV and introduced it into hexaploid wheat (*Triticum aestivum* L.) cv. Veery via particle bombardment. Six lines expressing b-32 were raised and brought to homozygosity through genetic analysis of progeny. Expression of b-32 protein was confirmed through four subsequent generations and throughout the plant life cycle. The six lines at T4 level were challenged for response against *Fusarium culmorum* (WHS).

The six transgenic homozygous progenies western-b32 positive, and cv. Veery, as negative control, were raised to maturity into a containment-greenhouse and used, at the flowering stage, for a detailed analysis of b-32 expression and for pathogenicity tests. A differential b-32 expression in both leaves and immature spikelets of the various progenies, was recorded. Preliminary experiments on cv. Veery, supported the choice of “single floret injection inoculation method” parameters (spore concentration, detection time) useful for a reliable evaluation of the genotypes. Plants at the early flowering stage were inoculated by injection of *F. culmorum* spore suspension (5x10⁸ spore/ml) into a floret in a central spikelet of a spike. For each genotype, controls were non-inoculated and sterile water-inoculated spikelets. Fusarium scab is easily recognized by premature bleaching of spikelets on emerging heads. According to literature, a visual scale, based on percentage of infected spikelets, was chosen to estimate scab disease severity. Visual inspection has been conducted 7 and 14 days after inoculation, counting discoloured spikelets per inoculated spike and recording other visible symptoms of infection such as mycelial growth. The cv. Veery, not expressing b-32, was the most susceptible to *F. culmorum* attack, in comparison to all the transgenic progenies tested. No significative resistance differences between the progenies have been noticed. At maturity, the spikes were manually harvested, glumes were removed and scab-infected kernel symptoms, were evaluated. The percentage of “tombstones” (shriveled, light weight, dull greyish or pinkish in colour of kernels), indicative of scab disease severity, was recorded. Transgenic plants constitutively expressing b32 have a higher level of resistance to scab because the percentage of
tombstones was significantly reduced in comparison to cv. Veery. Data obtained suggest that b-32 protein may provide protection to wheat against mycotoxigenic fungal pathogens, as *F. culmorum*. Experiments using higher spore concentrations applied to WHS inoculation method, are in progress to establish the proper conditions for better discriminating the response to Fusarium infection of the different genotypes.
THE MAIZE RIBOSOME-INACTIVATING PROTEIN (b-32): ROLE IN THE DEFENCE AGAINST FUNGAL PATHOGENS

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Ribosome Inactivating Protein, b-32, plant defence, Fusarium verticillioides, Zea mays L.

The development of improved maize genotypes with increased resistance to fungal pathogens is one of the major objectives of breeding biotechnology strategies. Fusarium species are widespread pathogens in cereals. F. verticillioides attacks maize causing root, stem, and ear rot diseases, and produces mycotoxins (fumonisins) 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 mycotoxicoses in livestock and also in humans. In the 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. Both cDNA and genomic clones encoding b-32 have been isolated. It was shown that the b-32 genes form a small gene family. The b-32 gene, as well as the 22 kDa storage protein zeins, are under the control of the seed-specific transcriptional activator Opaque-2 (O2). In opaque-2 mutants the b-32 protein is expressed at very low levels. Although, the role of b-32 in maize endosperm remains unclear, this protein displays structural and functional homology with other previously characterized Ribosome-Inactivating Proteins (RIPs). It was found that b-32 is a functional RIP by the criteria of inhibition of in vitro translation in a cell-free rabbit reticulocytes system, and specific N-glycosidase activity on 28S rRNA. Additional evidences indicated that transgenic tobacco plants expressing b-32 showed an increased tolerance against infection by the soil-borne fungal pathogen Rhizoctonia solani.

Research is in progress in our laboratories to verify if maize plants expressing b-32 in various organs and tissues display an increased resistance against fungal pathogens in comparison with normal control plants. For these purposes transgenic plants were obtained via genetic transformation using the vector pSC1b32 containing the b-32 coding sequence under the constitutive promoter 35SCaMV and the cassette ubil-bar for L-glufosinate resistance as a selectable marker. A set of six homozygous progenies PCR-b32 and western-b32 positive, and a progeny PCR-b32 positive and western-b32 negative (as 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. Various progenies, characterized by a differential b-32 expression in the leaves, were identified; these ones were used 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. Preliminary experiments supported the choice of bioassay parameters (spore concentration, detection time) useful for a reliable evaluation of genotypes. Leaves of progeny not expressing b-32 protein were surface sterilized and square segments dissected and plated on PDA (Potato Dextrose Agar); they were inoculated with 5µl spore suspensions at four different concentrations; control leaf squares were non-infected and treated with sterile water. Infection progression was daily monitored measuring fungal colony diameter around inoculated leaf squares. A concentration of 10^5 and/or 10^6 spores/ml and 3-7 days following inoculation as detection time, were the parameters adopted for pathogenicity experiments. All progeny, previously
quantified for b-32 expression in leaves, were tested with this method. As a result, the negative control was the most susceptible to *F. verticillioides* attack, whilst in all progenies expressing b-32, a differential resistance was detected.
SELECTION OF BREAD WHEAT LINES CARRYING THE \textit{Pm13} RESISTANCE GENE IN A BACKCROSS PROGRAMME SUPPORTED BY MARKER ASSISTED SELECTION

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\textit{plant disease}, powdery mildew resistance, \textit{Pm13} gene, common wheat

Plant breeding for disease resistance has long been of foremost importance in improving crops yield and quality. Powdery mildew (\textit{Erysiphe graminis} D.C.) is one of major fungal wheat disease.

Resistance genes to powdery mildew in wheat have been found both in the cultivated gene pool and in wild relatives but most of them have already been overcome by new virulent strains. Therefore wild relatives are constantly under study for new resistance sources.

Aim of this research was the introduction of the \textit{Pm13} gene from \textit{Aegilops longissima} in 18 bread wheat cultivars widely cropped in Italy and characterised by high yield and/or good quality. A backcross breeding programme, using these cultivars as recurrent parents and \textit{Pm13}-Chinese Spring as resistance donor, was carried out. Marker assisted selection (MAS) with a closely linked STS marker has been applied giving good and reproducible results; the method allowed to overcome the artificial inoculation, skipping the environment-related problems, and permitting an early-stage selection on 2-3 leaves seedlings.

Agronomic and quality evaluations carried out on sixty selected lines led to the identification of three of them, two derived from a high quality cultivar and one from a high yielding cultivar, possessing adult plant resistance associated to the parent’s peculiar traits.


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*Pyrenophora teres*, *Pyrenophora graminea*, mating type, sexual reproduction, *Hordeum vulgare* L.

The mating-type genes from the heterothallic ascomycetes *Pyrenophora teres* and *P. graminea* are here isolated and described: *P. teres* f. sp. teres: MAT-1: 1190 bp; MAT-2: 1055 bp; and *P. graminea*: MAT-1: 1190 bp; MAT-2: 1055 bp. The predicted protein products of MAT-1 and MAT-2, of 379 and 333 aminoacids, respectively, are similar to those of other fungi belonging to *Pleosporales* and strikingly similar to those of *P. teres*. Moreover, *P. graminea* appears slightly closer to the spot form (SF; four fixed nucleotide differences in the coding region) than to the net form (NF; seven fixed differences in the coding region and one in the intron) of net-blotch disease.

Fragments of the MAT-1 (1158 bp) and MAT-2 (1068 bp) genes have also been sequenced from *P. teres* isolates of both the NF (22 isolates; 12 MAT-1 and 10 MAT-2) and the SF (17 isolates; 10 MAT-1 and 7 MAT-2) collected from Sardinian barley landrace populations and worldwide. The polymorphism within each *forma specialis* was low. When the two forms were pooled, polymorphism increased. More than 80% of the total nucleotide variation was found between *formae specialae* (FST = 0.837 for MAT-1 and FST = 0.879 for MAT-2); the two forms do not share any polymorphisms. Diagnostic nucleotide polymorphisms were also found, in the MAT-1 intron (1) and in the MAT-1 (3) and MAT-2 (2) exons. When the putative peptides were compared, three diagnostic non-synonymous mutations were found, one in MAT-1 and two in MAT-2.

Neutrality tests are consistent with the null-hypothesis of a pure drift mutation process, i.e. no selection effects (positive or purifying) were detected, both in considering the two forms or all of the three taxa (NF, SF and *P. graminea*).

Overall, these data suggest that hybridization between the two forms of *P. teres* is rare or absent under field conditions.
ANTAGONISM TESTS IN VITRO AND IN PLANTA AGAINST DIPLODIA MUTILA ASSOCIATED WITH OAK DECLINE IN SOUTHERN ITALY

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antagonistic fungi, Diplodia mutila, oak decline

During the last decades numerous oak stands have been affected by oak decline in southern Italy. Various fungal microorganisms have been found associated with the syndrome, showing either an endophytic asymptomatic or a pathogenetic behaviour or both in succession. Among the microrganism found Diplodia mutila (Fr.) Mont. was often recorded.

The aim of this research was to assess the antagonistic degree of some fungal isolates belonging to Trichoderma viride Pers.: Fr., Epicoccum nigrum Link., and to Fusarium, Alternaria and Cytospora (teliomorph: Valsa sp.) genera, also occurring in epigeous declining oak tissues by means of antagonistic tests performed in vitro and in planta against an isolate of D. mutila.

In vitro the isolates of T. viride, E. nigrum and Fusarium sp. demonstrated high antagonistic activity against D. mutila, inhibiting growth by 33%, 42% and 59%, respectively. The isolate of Fusarium sp. also partially overgrew the colony of D. mutila after an initial deadlock at a distance by the mycelium of the pathogen. The isolate of T. viride completely overgrew the one of D. mutila, whereas the tests involving both Cytospora sp. and E. nigrum revealed a mutual inhibition between the two species and D. mutila. In planta the most effective results were given by the isolate of Fusarium sp., which was able to reduce the area of oak tissue infected by D. mutila compared to controls.

If such results gain further positive confirmation, biological control against fungal pathogens involved in oak decline by using antagonistic fungi could represent a promising tool for the sustainable management of forest ecosystems against disturbing biological constraints.
PROTECTION OF MICROPROPAGATED PLANTS AGAINST INFECTION BY RHIZOCTONIA SPP BY USING THE PLANT GROWTH-PROMOTING BACTERIUM AZOSPIRILLUM BRASILENSE Sp245

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micropropagation, Azospirillum, Rhizoctonia, BCA, PGPR

An interesting approach for protecting micropropagated plant material from early infections by the pathogens and to reduce chemical inputs is the use of biocontrol measures, during plant propagation and/or at planting. Enhanced micropropagation may be due to direct or indirect actions of beneficial microorganisms (Monler et al 1998) with an improvement of plant performances under stress environments where microorganisms can be employed as plant protecting (Biocontrol Agents, BCA) or plant growth promoting rhizobacteria (PGPR).

PGPR of the genus Azospirillum have been extensively used as inoculum for crops phytostimulation (Dobbeleare et al., 2001; Basaglia et al., 2003; Russo et al., 2005) which entails the nitrogen fixation and the phytohormones production, particularly auxin-related compounds such as indole-3-acetic acid (IAA) (Steenhoudt and Vanderleyden 2000; Dobbeleare et al., 2001). Most informations on the phytostimulation activity of Azospirillum has often been derived from studies regarding cereals. The results reported here show the positive effects of A. brasilense Sp245 inoculum observed during micropropagation of Prunus cerasifera L. clone MrS2/5. In vitro-derived shoots of MrS2/5 clone, propagated on Murashige and Skoog (MS) medium, were inoculated at root level with wild-type A. brasilense strain Sp245 leading to better both in vitro rooting of explants and biomass production with the respect to the non-inoculated explants. Moreover, the Sp245 strain induced apical growth during the post in vitro acclimatation phase. In addition, Sp245 strain was able to protect the MrS2/5 clone during the acclimation phase against Rhizoctonia attacks with a more than 90% of plant survival against the 0% of the control. Rhizoctonia is a phytopathogenic fungus causing the damping-off disease responsible of important economic loss in the plant micropropagation. In vitro studies confirmed the biocontrol activity of the Sp245 strain against Rhizoctonia fungus.

Based on these experiments, it can be concluded that the A. brasilense Sp245 inoculation, by improving both the radication phase (increased % of rooting and biomass production) and the acclimatation phase (phytopatogenic biocontrol and apical activity), may represent a useful tool to enhance micropropagation response of Prunus cerasifera.


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A SELECTED LINE OF MELON (Alban-12) RESISTANT TO FUSARIUM OXYSPORUM F. SP. MELONIS, RACE 1-2

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**melon, resistance, Fusarium oxysporum f. sp. melonis, physiologic race, biodiversity**

Fusarium-wilt by *Fusarium oxysporum* f. sp. *melonis* causes heavy losses in all melon cultivation areas. Use of resistant cultivars is the most effective control method of the disease. At present, four physiologic races of the pathogen signed as 0, 1, 2 and 1-2 and two resistant host genes (*Fom-1* and *Fom-2*) are known. Nowadays melon hybrids F1, characterized by resistance to races 0, 1 and 2, are grown. Resistance factors against race 1-2 of *F. oxysporum* f. sp. *melonis* are not available. In screenings for resistance to Fusarium-wilt carried out on numerous accessions and lines of *Cucumis melo* collected directly from farmers in Albania, one line (Alban-12) showed good resistance characteristics with a low disease severity index (Ciccarese *et al.*, 2002). Progenies obtained by self-fertilized single plants of selected line were tested for resistance toward each race of pathogen. Artificial inoculation was made in glasshouse at 26±2°C with isolates belonging to race 0, 1, 2 and 1-2 of *F. oxysporum* f. sp. *melonis* by dipping, for 2-3 minutes, roots of seedlings in a fungal suspension (4 x 10^6 CFU/ml). Disease severity on each plant was assessed according on empirical scale of values ranging, from 0 to 4 in which 0 = healthy plant and 4 = dead plant or plant with extremely severe symptoms. Severity values were used in order to calculate the index of McKynney. Results pointed out a higher level of resistance towards *F. oxysporum* f. sp. *melonis*, race 1-2, of Alban-12/S1 than parent S0. The level of high resistance to race 1-2 of *F. oxysporum* f. sp. *melonis* was confirmed in test carried out on the progenies obtained by self-fertilization of selected single plants. In particular, disease severity index observed on Alba-12/S2 line was 1.8%. If the polygenic base of this resistance will be confirmed by further studies, Alban-12 line represents a promising resource for Fusarium-wilt control in consideration of high capacity of *F. oxysporum* f. sp. *melonis* in differentiating new physiologic races.
MOLECULAR CHARACTERIZATION OF OL-2 LOCUS CONFERRING POWDERY MILDEW RESISTANCE IN TOMATO

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oidium resistance gene, tomato, molecular characterization

Powdery mildew (Oidium lycopersici) is a common fungal disease of tomato. The pathogen can cause severe damages both on glasshouses-grown tomatoes and on field crops, especially when high relative humidity occurs.

A recessive gene, named ol-2 conferring resistance against the pathogen was found in an accession of Lycopersicon esculentum var. cerasiforme. Previous studies, performed by mean of BSA analysis on resistant (R28 accession) and susceptible parents (Super Marmande) and on F2 segregant population, allowed to identify molecular markers linked to ol-2 and localize it on chromosome 4.

The main objective of the present work was to characterize the chromosome region in which ol-2 lies. For this purpose, based on the knowledge of similarity among resistance gene families, a comparative sequence analysis among resistance genes identified in other species was performed.

Nucleotide sequences of mlo and mlo2 (respectively, oidium resistance genes identified in barley and arabidopsis) were used to identify homologous sequences in Solanaceae database. An EST of tomato was found and, on the basis of its nucleotide sequence, a set of primer were drawn to amplify mRNA and genomic DNA of the two parents used in the bulk segregant analysis.

Molecular characterization of the cDNA and the genomic region in which lies ol-2 was performed using different primer combinations in order to establish molecular diversity between resistant and susceptible parents.
WHEAT TRANSGENIC LINES OVEREXPRESSING PGIP SHOWED INCREASED RESISTANCE TO FUNGAL PATHOGEN


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Triticum aestivum, PGIP, Bipolaris sorokiniana, Fusarium spp, defence gene

A possible strategy to control plant pathogens is the improvement of natural plant defence mechanisms against the processes that pathogens commonly use to penetrate and colonize the host tissue. One of these defence mechanisms is the ability to inhibit the pathogen’s capability to degrade plant cell walls. Polygalacturonase-inhibiting proteins (PGIPs) are plant defence glycoproteins associated with the cell wall of both monocot and dicot species. They interact with fungal endopolygalacturonases (PGs) and modulate their activity favouring the accumulation of oligogalacturonides active as elicitors of plant defence responses.

To assess the effectiveness of these proteins in protecting wheat from the fungal pathogens, we have produced a number of transgenic wheat lines expressing a bean PGIP (PvPGIP2) having a wide spectrum of specificities against fungal PGs. The transgene-encoded protein is correctly secreted in the apoplast, maintains the characteristic recognition specificities and endows the transgenic wheats with new PG recognition capabilities. As a consequence, transgenic wheat tissue showed an increased resistance to fungal PG digestion. Two transgenic lines over expressing PvPGIP2 showed a reduced symptom progression through the leaves or spikes following infection with Bipolaris sorokiniana and infection experiments Fusarium spp. are in progress. Due to the technological importance of wheat kernel proteins we are also evaluating the impact of the over expression of PvPGIP2 on the accumulation of major protein components such as gliadins and glutenins.
DIFFERENTIAL EXPRESSION PROFILING FOR RAPID IDENTIFICATION OF DESEASE RESISTANCE GENES IN TWO TOMATO ISOGENIC LINES

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plant resistance gene, SSH, tomato, isogenic line

Comparing patterns of gene expression by PCR-select technology had important applications in a variety of biological system. We used this approach to investigate a resistance genes hot spot chromosome region difficult to analyze with conventional molecular methods. In particular two tomato near isogenic lines (Momor and Monalbo) were examined for differential gene expression profile. Momor contain two resistance genes (Frl and Tm2a), located on short arm of to chromosome 9, that are absent in Monalbo. The first gene confers resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* and the second to TMV virus. Since both lines have a common genetic background, deriving from Moneymaker cultivar, any difference between them is due to the region that contain these genes. PCR select methodology has been used to investigate mRNAs represented only in resistant variant. A suppressive subtraction hybridization (SSH) cDNA library, using Momor genotype as tester, was prepared and analyzed. A total of 200 cDNA fragments present in the library were selected following the criteria established for differential expression. Several clones coding for major pathogenesis-related proteins searching in public databases by BLAST (basic local alignment search tool) were identified. Interestingly, one clone showed sequence homology with Gpa2 gene that confers resistance to *Globodera pallida* nematode in potato. Molecular characterization of these clones is in progress to validate the their location on tomato genome.
IDENTIFICATION OF RESISTANCE GENE TO *RALSTONIA SOLANACEARUM* IN POTATO


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resistance gene, plant disease, Ralstonia solanacearum, AFLP-TP

Bacterial wilt of potatoes caused by *Ralstonia solanacearum*, which used to be a widespread disease in tropics and subtropics, has become a threat to potato production in temperate regions. It can affect more than 200 plant species among which tobacco, banana, tomato and potato. The diploid wild species *Solanum commersonii* has several desirable characteristics including cold tolerance and resistance to *R. solanacearum*. The bacteria enters plant roots via wounds or where secondary roots emerge, colonizes the root cortex, invades xylem vessels and rapidly spreads throughout the vascular system. During colonization of host plants, *R. solanacearum* produces a variety of extra cellular products that contribute to pathogenesis and cause disease symptoms. The aim of this study is to better understand the biochemical and molecular basis of plant-pathogen interactions in resistant/susceptible response.

Since in the compatible interaction polysaccharides of *R. solanacearum* play an important role for pathogenesis, isolation and partial purification of extracellular polysaccharide (EPS) from lyophilised culture filtrate and of lipopolysaccharide (LPS) from lyophilised cells of bacteria were carried out. The crude polysaccarides (EPS and LPS) and the filtered culture medium were tested to study whether they have a phytotoxic effects on plants. Preliminary analyses provided evidence of their phytotoxic effect. Other tests will be performed to investigate the chemical composition of phytotoxic compounds.

In order to identify differential genes expressed during the interaction between *R. solanacearum* and host plants, the cDNA-AFLP-TP technique was carried out on the resistant *Solanum commersonii* and the susceptible *Solanum tuberosum* cv Blondy. RNA was extracted from both genotypes 6, 24, 48 and 72 hours after inoculation. Up till now, 32 primer/enzyme combinations were tested and around 40 bands were observed for every combination. A clear uniformity of the samples at 6 and 24 hours after inoculation was found, whereas in the successive times, some polymorphisms induced after infection in both resistant and susceptible genotypes or only in one of them, were evidenced. The polymorphic fragments are being extracted from gels and subsequently, sequenced in order to define if they may represent genes involved in the interaction.

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TRANSCRIPTIONAL REGULATION OF A WHEAT PATHOGENESIS-RELATED GENE PROMOTER

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gene expression, plant defence, chemical inducers of systemic acquired resistance, uidA reporter gene

Plant pathogenesis-related (PR) proteins are a family of pathogen-inducible proteins involved in defence. Induction of defence responses is triggered by a complex network of signal transduction processes resulting in the rapid activation of defence gene expression. A number of secondary signal molecules such as salicylic acid (SA), methyl jasmonate (MeJA) and ethylene act to amplify and regulate defence responses as demonstrated by studies on mutants and exogenous treatments with these chemicals. In several plant species, SA is able to trigger systemic acquired resistance (SAR). Transcription of distinct classes of PR genes has been reported to be activated by specific signal molecules. Moreover some PR proteins show differential tissue-specific expression, regulated by developmental cues.

To gain a better understanding of the expression pattern of defence genes we characterized a genomic wheat clone encoding a wPR4 protein in terms of transcriptional activity of its 5’ non coding region which contains several cis-acting domains found in other defence genes. We constructed a plant transformation vector carrying the 1700 bp 5’ untranslated region of wPR4e upstream of the β-glucuronidase (GUS) reporter gene and evaluated the constitutive and inducible transcriptional activity of the putative promoter in transgenic tobacco plants.

To evaluate the involvement of various signal transduction mechanisms several independent tobacco stable transformants were obtained by A. tumefaciens transformation which were tested for GUS expression upon leaf treatments with SA (100 uM) or MeJA (100 uM) and after wounding. GUS assays confirmed that the wPR4e promoter is able to drive constitutive expression of the reporter gene. Besides, all the tested treatments resulted in strong induction of GUS expression.

The above results confirm that activation of at least one wheat PR4 gene follows both SA- and JA-dependent pathways, while Arabidopsis PR4 genes are specifically induced through JA signaling.

To study the spatial distribution of GUS activity driven by the wPR4e promoter at different development stages of growth, GUS activity was determined in tobacco seedlings and full grown plants. A reproducible constitutive transcriptional activation, which appears governed by developmental cues, was observed in stems, roots, flowers and seeds.

The potential role of PR4 genes in resistance to pathogens was also investigated in wheat by determining the wPR4e gene expression in response to F. culmorum infection and treatments with SAR chemical inducers, namely SA and MeJA. We also addressed the question whether the expression of these genes in wheat was inducible after wounding. The involvement of wPR4e in defence responses triggered by
different signal transduction pathways was evaluated by RT-PCR analysis of its expression in different wheat tissues.
EARLY DIAGNOSIS OF DISEASE IN ORANGE FRUITS BY USING REFLECTANCE SPECTROSCOPY

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Phytophthora citrophthora, Penicillium italicum, orange, post-harvest disease, reflectance spectroscopy

Post-harvest decay is the most important factor limiting shelf-life of fruits. About 30% of the economic loss in harvested fruits is caused by pathogens. Among the post-harvest diseases of the orange fruits Phytophthora citrophthora (browning rot fruits) and Penicillium italicum (blue mold fruit) are the most common. These pathogens attack the host by means of pectic enzymes and they are responsible for metabolic changes as well as the alteration of pigments like chlorophylls, carotenoids and anthocyanins. Since the spectral properties of pigments change in function of the physiological state of the fruits, the study of the changes of the spectral properties of these pigments allow to detect the health and the physiological status of the fruits. The reflectance spectroscopy is a useful tool for studying pigment composition and content in plants in vivo. The technique is non-invasive and can be performed in a real-time mode. The reflectance values in the visible range (Vis, from 400-700 nm), where the pigments absorb light, give information on the principal pigment composition and content. Moreover, in Vis range it is possible to identify the pigment and plant tissue degradation and the appearance of necrotic zones as a response to biotic and abiotic stress. The internal structure of epidermal layer can be monitored in the near-infrared region (NIR, 700-2500 nm). All these characteristics allow an early diagnosis already at the first stages of the disease. The aim of this work was to monitor and estimate the development of specific diseases on cultivar of blood orange in post-harvest by using reflectance spectroscopy. The blood orange cultivars Tarocco, Moro, Sanguinello have been inoculated with Penicillium italicum and Phytophthora citrophthora. Reflectance measurements have been performed on fruits after 24, 48 and 72 h from the inoculation. The range of wavelengths used was 400 nm to 1100 nm (Perkin Elmer Lambda 25 spectrophotometer). A Cary E spectrophotometer was used for the reflectance measurements in NIR until 2500 nm. Both instruments are equipped with the integrating sphere and certified reflectance standards. The results evidence the different infection modality between the two pathogens in both time of appearance of symptoms and reflectance spectra. Peaks corresponding to 6,7-dimethoxycoumarin (scoparone) were detected by second derivate of the reflectance spectra. This substance is involved in the host-pathogenic interaction. The investigation on the fruits inoculated after 24 and 72 h by means of gas-chromatography/mass spectrometry has been performed. The results confirm that the degradation of pigments and the cell damages of flavedo in response to infestation can be monitored by using reflectance measurements at the early stages of the disease. The changes in reflectance spectra of the orange peel caused by P. italicum and P. citrophthora, pathogens can be seen already 24 h after inoculation, when the damage is still not visible. The reflectance spectra of fruits infested by P. italicum diverge from that of P. citrophthora. The differences among the various cultivars in response to the pathogens have been related with the areas of the necrosis.
PROTEOMIC TO ANALYSE PROTEOLYTIC CHANGES IN HELICOVERPA ZEA GUTS UPON FEEDING ON PROTEASE INHIBITOR CONTAINING DIET


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insect protease, protease inhibitors, Helicoverpa zea

Pest insects like Helicoverpa species are a polyphagous pest of many important crop plants throughout the world, responsible for heavy economical losses. Chemical control of H. zea insects is often not effective, as they are able to develop the resistance to chemicals like DDT, organophosphates and pyrethroids. Proteolytic activities in the larvae guts have been investigated and shown to be due predominantly to extracellular serine proteinases of trypsin and chymotrypsin type.

One of the plant natural defence mechanisms against insect pest is inhibition of digestive proteinases by proteinase inhibitors (PIs). The resulting deficiency in free amino acids causes developmental delays, and in some cases, mortality of larvae. PIs have been shown to restrict the growth and/or the development of herbivourous insects either when added to artificial diets or when expressed in transgenic plants. However some insects, including Helicoverpa, are able to overcome the PIs in their natural or enginereed diet by up-regulating a set of "insensitive" proteinases.

In this report, analyses on the proteolytic content of H. zea guts after feeding of larvae on control and SKTI (Soybean Kunitz Trypsin Inhibitor) containing diets are reported. Isolation of H. zea proteinases was carried out by affinity chromatography through a sepharose-CNBr column on which the mustard trypsin inhibitor MTI-2 had been immobilised. Isolated proteinases were characterised by activity gels, SDS PAGE and iso-electric focussing and their interaction with substrates. The effect of plant proteinase inhibitors on the isolated proteinases was also tested. Isolated enzymes were partially sequenced by tandem mass spectrometry. Polypeptide sequences, matched with available cDNA sequences, allowed the assignment to specific trypsin and chymotrypsin genes of Helicoverpa species.

Proteinase identified in this study would be good candidates for further interactions studies with PIs to clarify structural reasons of proteinase inhibitor insensitivity.
CHARACTERIZATION OF AN ARABIDOPSIS MUTANT IMPAIRED IN DEFENSE PATHWAYS

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Arabidopsis, Programmed Cell Death (PCD), Hypersensitive Response (HR), Lesion Mimic Mutant (LMM)

Programmed cell death (PCD) is a metabolically active and genetically controlled process leading to cell death.

In plants this process occurs during normal development and senescence, and during interaction with the environment, in biotic and abiotic stress response.

One of the most studied form of PCD in plants is the cell death associated to an avirulent pathogen attack, known as hypersensitive response (HR). In this process, the death of cells challenged by the pathogen can be seen as a first barrier to limit pathogen growth in plant tissues.

We report on the characterization of the Arabidopsis mutant chlorotic lesions1 (cll1).

The two main characteristics of this monogenic recessive mutant are a reduction in size respect to wild type plant and the presence of chlorotic lesions on rosette leaves. This latter trait reminds of the typical phenotype shown by the so called “lesion mimic mutants”, a group of mutants characterized by alterations in the hypersensitive response pathway.

To better understand the nature of the lesions present in cll1 mutants we have checked for the presence, in mutant rosette leaves, of specific markers associated to HR by histochemical analyses. We have also analyzed the expression level of two senescence-associated genes (SAG) and a group of pathogen-related /stress-related genes, constitutively expressed in the lesion mimic mutants. Phenotypic, histochemical and molecular analyses have been performed also on cll1 mutants aseptically grown on MS medium.

The data thus far obtained indicate that the lesions formation in cll1 mutants correlates with the expression of histochemical and molecular markers of plant disease resistance responses.

The cll1 mutant has been isolated in the Exotic collection, based on the Ac/Ds transposon system of maize. Cosegregation analysis showed that the mutant phenotype did not cosegregate with the Ds element, the positional cloning of the cll1 mutation is in progress.
RFLP AND SEQUENCE ANALYSIS OF NOD GENES OF RHIZOBIUM LEGUMINOSARUM BIOVAR VICIAE STRAINS INDUCING ROOT “SUPER-TUBERCLES” DEVELOPMENT IN PISUM SATIVUM L.

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Rhizobium leguminosarum biovar viciae is a bacterium able to fix atmospheric nitrogen in symbiosis with Pisum, Vicia and Lens species of legumes. Symbiosis is visualized by root tubercles that have variable morphology. In particular, some strains are able to induce the development of very large and polylobate tubercles. These “super-nodulating” strains, when inoculated on pea, showed a dry matter ratio tubercles/root significantly higher with respect to “normal” rhizobia strains. For example, dry matter ratio tubercles/root due to “super-nodulating” strains used in this study was 1.04 for Belinda and 0.92 for Madria varieties respectively, with respect to maximum ratio of 0.17 for Belinda and 0.39 for Madria inoculated with “normal” strains. These “super-nodulating” strains do not increase yield of inoculated plants and, in some cases, yield is considerably reduced. For example, one “super-nodulating” strain reduced yield two and half times with respect to the best efficient “normal” strain, when inoculated on Belinda seeds in the field; on Madria, three “super-nodulating” strains reduced significantly the yield with respect to “normal” strains or naturally inoculated plants. These results indicate that these strains are most similar to phytopathogenic bacteria rather than mutualistic symbiont. Rhizobium genes involved in tubercles development (nod genes) were isolated and sequenced. In this work we show results of RFLP analysis and sequence homology of nodABC genes of R. leguminosarum biovar viciae strains able to induce root “super-tubercles” on pea. RFLP analyses performed with BamHI and HindIII on amplified nodABC genes, have permitted to distinguish three out six “super-nodulating” strains with respect to normal strains. Sequence homology analyses of nodABC genes showed differences in sequences of 5-10% between “super-nodulating” strains and “normal” strains.
MDR-LIKE ABC TRANSPORTER AtPGP4 IS INVOLVED IN AUXIN-MEDIATED LATERAL ROOT AND ROOT HAIR DEVELOPMENT

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ABC transporter, lateral roots, root hairs, auxin, ethylene

AtPGP4 belongs to the MDR (multidrug-resistance protein) ABC transporter subfamily in Arabidopsis thaliana. MDRs were first identified in mammalian cells because their overexpression confers a multidrug resistance phenotype. The first plant ABC protein gene to be cloned was AtPGP1. Noh et al. (2001) and Geisler et al. (2003) showed that auxin transport activity was greatly impaired in atmdr1 (pgp19) and atmdr1-atpgp1 double mutant plants. Epinastic cotyledons and reduced apical dominance were phenotypes consistent with disrupted basipetal flow of auxin, suggesting that MDRs may be essential for normal auxin transport and the development of plant form.

In this study two independent T-DNA Salk lines pgp4-1 and pgp4-2 have been screened by PCR for the homozygous genotypes, and the number of T-DNA insertions checked by Southern Blot. pgp4 mRNA was not detectable neither in root nor in shoot of pgp4-1 seedlings, while still faintly visible in pgp4-2 mutant tissues. In wild type plants pgp4 was most strongly expressed in the root, especially in the lateral root and in the root elongation zone, early in the plant development. GC-MS analysis of wild type and pgp4 mutants root extracts at 5 dag (day-after-germination) showed that the mutants accumulated IAA in the root at significantly higher level than wild type, consistent with a possible defect in transport and redistribution of auxin in this part of the plant. Lateral root formation at 5dag was increased in both mutant seedlings when compared to wild type, in agreement with the observation that auxin promotes lateral root formation. However, the relative differences in the number of lateral roots decreased with the age of the plant (7 dag and 9 dag), suggesting that PGP4 might be required for the initial control of lateral root formation, correlating with the fact that the gene is mainly expressed in the first developmental stages of the plant. When the plants germinated in the presence of synthetic auxin IAA, the relative differences in lateral root formation between wild type and the mutants were reduced. We also found that pgp4 mutants were able to produce lateral roots even in the presence of the polar auxin transport inhibitor N-1-napthylphalamic acid (NPA), indicating that the mutation directly altered auxin sensitivity and transport at the root level. Root hairs on the mutants were significantly longer than wild type root hairs and more variable in length. Auxin and ethylene are known to promote root hair elongation in Arabidopsis; indeed, IAA and NPA treatment led to the development of longer root hairs than untreated seedlings both in wt and mutants, but pgp4 mutants were less responsive to the treatment compared to wild type plants, in agreement with the observed lateral root phenotype. When seedlings germinated in the presence of the ethylene biosynthesis inhibitor, aminovinylglycine (AVG), which abolishes root hairs, we observed that the inhibition ratio in pgp4 mutants was significantly reduced compare to wt, meaning that higher intracellular level of auxin in the mutant seedlings might play an important role in promoting the root hair elongation in the absence of ethylene. Seedlings grown in hydroponic culture were loaded with ^3H-IAA in a physiological solution along a time course experiment and the relative amount of radioactivity in 1 cm of root apex was counted. pgp4 seedlings showed less ^3H-IAA content in the root apex, suggesting that PGP4 might be involved in the import of auxin within the cell.
These observations indicate that AtPGP4 is a key regulator in auxin-mediated lateral root and root hair development in *Arabidopsis*.
TRANSFER OF GENETIC RESISTANCE TO IMIDAZOLINONE IN
SUNFLOWER HYBRIDS (HELIANTHUS ANNUUS L.) FOR CONTROL OF
OROBANCHE (OROBANCHE CUMANA WALLR.)

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sunflower, imidazolinone resistance, inheritance

Orobanche cumana Wallr. Is a dangerous parasite of sunflower, even impeding its cultivation in large areas. Diffusion of sunflower genotypes carrying genetic resistance to different physiological races of Orobanche induced radical changes in the pathogenicity of the parasite, which become more difficult to control, because of the continuous appearance of new virulent races. The discovery of a spontaneous mutant of sunflower resistant to imidazolinone has opened a new possibility to Orobanche control since chemicals derived from this molecule, normally used as herbicides, are efficiently controlling the development of this parasite. The paper reports the transfer of this mutant gene for imidazolinone resistance, found originally in USA in a wild sunflower population, in domesticated sunflower lines restoring male fertility and maintaining self sterility for hybrids production. Using genetic conventional techniques it was also possible to study the genetic basis of inheritance of this character. Resistance was monogenic and dominant and therefore its transfer in highly producing lines is rather simplified. EUROGEN Society has already developed highly producing hybrids, resistant to imidazolinone, which can be successfully cultivated also in areas where Orobanche is strongly diffused.
ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA IN VETIVERIA ZIZANIIOIDES (L.) NASH ROOTS

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Vetiver root, essential oil, physiological-biochemical test, rDNA amplification, endophytic bacteria

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

Root tissues contain oil-producing cells, responsible for its characteristic odor. These secretory cells are localized in the first cortical layer outside the endodermis of mature roots. Essential oil can be detected in the inner bark within the cortical layer. In the latter, lysigen lacunae were also observed, which are a true storage for the essential oil of the Vetiver root. Electron microscope analysis of the root cells of Vetiveria zizanioides (L.) Nash revealed the occurrence of bacteria as well as of electrodense crystals of essential oils in the external layers of cortical parenchymatous cell up to those close to the endodermata (Massardo et al. 2004). The close relationship between bacteria and the essential oil stimulated the idea of a direct involvement of those endophytic bacteria in the essential oil metabolism.

The aim of the present work is the isolation and characterization of the microbial community inside the cells of Vetiveria zizanioides (L.) Nash roots. Planting in Campania Region of the species Vetiveria zizanioides (L.) Nash native to Thailand was performed in the spring 2002 using vetiver culms with short roots and leaves of approximately 20 cm. Root samples for isolating endophytic bacteria were collected during April-September 2004. The isolation of the pure bacterial cultures was performed by:

1. Preliminary treatment of the samples: the roots were initially washed five-ten times with distilled sterilized water, then treated four-five times with 70% EtOH. The root surface was checked for the presence of bacteria. 2. Taking the sample from inside of the roots: the root surfaces were cutted with scalpel and samples were taken with bacterial needle suspending it in 1 ml sterile water. 3. Isolation of single bacterial colony: aliquots of suspensions (previous step 2) were plated on solid rich medium and incubated 48h at 30°C.

The control of purity of microbial cultures and bacterial identification were performed according both microscopic observations and standard physiological and biochemical tests. Besides, independent bacterial isolates from the microbial community inside cell roots were molecularly controlled by analyzing the differences in ribosomal 16S DNA. The bona fide endophytic bacteria species of Vetiveria zizanioides (L.) Nash roots included the cultivable microorganisms Pseudomonas putida, Pseudomonas sp. WDL5, Pseudomonas sp. Fa2, Pseudomonas corrugata, Pseudomonas thiiveringens, Serratia grimesii, Duganella violaceusniger and non-cultivable bacterial strains including an alpha-, two beta- and
one delta Proteobacteria. Work is in progress to analyze the capacity of those bacterial isolates to degrade the vetiver essential oil.