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Oral Communication Abstract – 7.01

CYTOKININ PATHWAYS REGULATING *MEDICAGO TRUNCATULA* SYMBIOTIC NODULE DEVELOPMENT

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Cytokinin signalling, nitrogen fixing nodules, lateral roots, hormonal crosstalk, Medicago truncatula

Legumes can develop two types of root lateral organs depending on environmental conditions: lateral roots and nitrogen-fixing symbiotic nodules. Genetic data indicate that their development involves common regulatory pathways, including phytohormonal controls.

Cytokinin signalling mediated by the receptor MtCRE1 was indeed shown to be crucial for both organogeneses (1). A combination of mutant and cytokinin responsive gene analyses revealed their role in *M. truncatula* early nodule organogenesis in dividing cortical cells as well as in mature nodules in relation with the cell division and differentiation balance (2). We additionally characterized interaction of this pathway with auxin and ethylene. The CRE1 pathway is notably required to modulate polarized auxin transport and PIN proteins expression and accumulation.

Recently, we searched for genes directly regulated by the MtCRE1 cytokinin signalling pathway by a combination of biochemical, transcriptomic and bioinformatic approaches. Among the new cytokinin response genes able to act in legume roots, some could be directly linked to cytokinin metabolism, or to other cues required for nodule organogenesis. The relevance of this potential direct regulation by the cytokinin pathway in root and nodule development is currently analyzed.

Overall, we could pinpoint specificities of cytokinin signalling pathways acting in legume root and nodule development, and characterize new potential cytokinin primary response genes.

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Oral Communication Abstract – 7.02

PLANT CELL WALL IN PATHOGENESIS: HOW ITS STRUCTURE CAN INFLUENCE PLANT-PATHOGEN INTERACTIONS

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Fungal and bacterial pathogens, plant resistance, pectin, pectin methylesterase

The cell wall represents the first physical barrier between the pathogen and the internal content of the plant cell. The ability of bacterial and fungal pathogens to produce cell wall degrading enzymes (CWDEs) is often related to a successful initiation of the infective process. In general, most virulence-associated CWDEs are involved in pectin digestion. Due to its cohesive and interacting properties pectin is critical for tissue integrity and accessibility to CWDEs. Enzymatic depolymerisation of pectin weakens the cell wall and exposes other polymers to degrading cellulases and hemicellulases. Pectin is synthesized in a highly methylesterified form and de-esterified in muro by pectin methylesterase (PME). Genetic and structural evidences indicate a critical role of pectin esterification in plant defense consistently with the concept that pectin structure may influence the outcome of host-pathogen interactions. It has been shown that PMEs play a role as susceptibility factor in plant response to fungal and bacterial pathogens, nematodes as well as in the systemic spread of tobacco mosaic virus. PME activity is regulated by specific proteinaceous inhibitors (PMEIs). The recent findings about the role of PMEIs in improving plant resistance to fungal and bacterial pathogens open new perspectives to reduce plant susceptibility to pathogens.

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Oral Communication Abstract – 7.03

INVOLVEMENT OF RNA SILENCING IN PLANT RESPONSE TO INFECTIOUS NON-CODING RNAs

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Pathogenesis, PLMVd, siRNAs, miRNAs, peach

Viroids are minimal non-protein-coding RNA replicons (246-401 nt) infecting plants in which they may incite severe diseases. RNA silencing, a regulatory network that mediates host gene expression and antiviral defense, is also involved in plant-viroid interactions. Small viroid-derived RNAs (vd-sRNAs) similar to the host microRNAs (miRNAs) and small interfering RNAs (siRNAs), the hallmarks of RNA silencing, accumulate in plants infected by both nuclear- and chloroplast-replicating viroids, but their biological roles, including their potential contribution to pathogenesis, are still controversial.

Peach calico (PC) is an extreme chlorosis (albinism) induced in peach by atypical sequence variants of the chloroplast-replicating *Peach latent mosaic viroid* (PLMVd*)*, which differ from the latent- and mosaic-inducing PLMVd variants in having a characteristic insertion of 12-13 nt in a defined position of the genomic RNA. The PC pathogenic determinant has been mapped at this specific insertion, which adopts a hairpin conformation with a UUUU capping tetraloop strictly needed for preserving its pathogenic properties. In the present work, we show that not only the UUUU hairpin tetraloop but also the stem, and in particular its nucleotide composition, plays a major role in eliciting PC, thus indicating that the primary structure of the inserted hairpin could also be involved.

High-throughput sequencing (by Illumina-Solexa technology) of small RNAs libraries from healthy and PC-affected leaves allowed identifying vd-RNAs, peach miRNAs and siRNAs, and their potential precursors and targets. Taking into account the differential accumulation of these small RNAs in symptomatic and non-symtpomatic peach tissues, the possible involvement of RNA silencing in PLMVd pathogenicity appears a feasible alternative. Altogether, these data support the view that RNA silencing machinery plays a role in modulating host gene expression as a response to viroid infections.

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Oral Communication Abstract – 7.04

PEA POWDERY MILDEW *ER1* RESISTANCE IS ASSOCIATED TO LOSS-OF-FUNCTION MUTATIONS AT A *MLO* HOMOLOGOUS LOCUS

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Experimental mutagenesis, er1 resistance, mlo resistance, marker assisted selection

The powdery mildew disease affects several crops and is also one of the major threats for pea (*Pisum sativum* L.) cultivation all over the world. The powdery mildew resistance gene *er1*, first described over sixty years ago, is widely used in pea breeding and still maintains its efficiency in the field. Genetic and phytopathological features of *er1* resistance are similar to those of barley, Arabidopsis and tomato *mlo* powdery mildew resistance, which is caused by the loss of function of specific isoforms of the MLO protein family. Here, we describe the obtainment of a novel *er1* resistant line by experimental mutagenesis with the alkylating agent diethyl sulfate. This line was found to carry a single nucleotide polymorphism in the *PsMLO1* gene sequence, predicted to result in premature termination of translation and a non-functional protein. A cleaved amplified polymorphic sequence (CAPS) marker was developed on the mutation site and shown to be fully co-segregating with resistance in F_2 individuals. Sequencing of *PsMLO1* from commercial *er1* resistant cultivars also revealed an important mutation, expected to be associated to a non-functional allele. Taken together, results point to the identity between *er1* and *mlo* resistances and are expected to be of great breeding importance for the development of cultivars via marker-assisted selection. Furthermore, our study strongly suggests that reverse genetics approaches targeting *MLO* homologs are likely to lead to broad-spectrum powdery mildew resistance across crop species.

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Oral Communication Abstract – 7.05

CALCIUM SPIKING IN ARBUSCULAR MYCORRHIZAS: THE WHO AND WHERE OF PRESYMBIOTIC SIGNALING

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Arbuscular mycorrhiza, plant-microbe interactions, Medicago truncatula, Daucus carota

Arbuscular mycorrhizas (AM) are symbiotic associations between 90% of land plants and obligate fungal symbionts belonging to Glomeromycota. AM fungi improve plant nutrient uptake and resistance against pathogens by colonizing the root through intra/intercellular hyphal development and the formation of arbuscules, the highly branched structures that mediate nutrient exchange. Early recognition of the symbiont by the host plant is a crucial step in the interaction, required for setting up a range of local and systemic responses. Certain of these plant responses depend on the so-called 'common SYM' signal transduction pathway which, in legumes is partly shared with the nitrogen-fixing symbiosis involving rhizobia. In this case, calcium is known to play a key role as a second messenger. Since the bacterial symbiosis evolved more recently than the AM association, it is thought that rhizobia have exploited the ancient AM signaling pathway by mimicking symbiotic fungal signals. Therefore, by analogy with bacterial Nod factors, it has been proposed that AM fungi release signal molecules termed Myc factors, which should activate the SYM pathway and its calcium signals to induce AM-specific responses. Furthermore, due to the wide host range of AM fungi, such signals should not be limited to legumes, but extend to all AM host plants.

The aim of this study was to investigate Ca^{2+} responses to AM fungi in the host root epidermis following fungal contact or diffusible signal perception in both legumes and nonlegumes.

We report that sustained nuclear Ca^{2+} spiking can be detected in hyphopodium-contacted epidermal cells of both *M. truncatula* and carrot roots, thus demonstrating that fungal-activated Ca^{2+} spiking occurs in the cell type targeted by AM hyphopodia, and is most probably part of an ancient plant signalling pathway predating the divergence between the rosid and asteroid clades. In addition, we have been able to show that exudates of germinated AM spores (but not purified rhizobial NFs) are able to trigger nuclear Ca2+ spiking in the outer root tissues of ROCs, and that this response is limited to the AM-responsive root zone. Ca2+ spiking responses to both hyphopodia formation and AM exudate application are dependent on genes of the common SYM pathway in *M. truncatula*, but independent of the NFP gene encoding the LysM receptor-like kinase which mediates NF perception.

Together, these findings provide additional evidence that Ca^{2+} spiking is a key component of a highly conserved AM-activated signalling pathway required for intracellular fungal infection, and furthermore suggest that cameleon-expressing ROCs can provide valuable AM-specific bio-tests for the future characterization of fungal symbiotic signals.

AM specificity in the SYM pathway-mediated response is a point of major interest in legumes, which must discriminate between symbiotic fungi and bacteria. This point will also be discussed in the light of our recent studies on the specificity of calcium signatures in either case.

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Oral Communication Abstract – 7.06

OPHIOBOLIN A ACTIVATES DIFFERENT DEFENCE RESPONSES DEPENDING ON THE APPLIED DOSE IN TBY-2 CELLS

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Programmed cell death, cell cycle regulation, cellular redox state

Ophiobolins are a class of secondary metabolites produced by some fungi (*Bipolaris spp., Aspergillus*) pathogens of various plants of agronomic interest, such as rice, sorghum and maize. These compounds are reported to produce different effects in plants, such as inhibition of roots and coleoptiles growth, reduction of seed germination, changes in cell membrane permeability, but their mode of action is still unknown. Ophiobolin A has been mostly studied for its effect on calcium metabolism, being a natural irreversible calmodulin inhibitor.

Here we report a study concerning the effect of ophiobolin A on Tobacco Bright Yellow-2 (TBY-2) cells. We found that ophiobolin A triggers different responses depending on the applied concentration. At concentrations equal or higher than 10 μM, ophiobolin A induces programmed cell death (PCD) in tobacco cells, showing different PCD markers such as DNA laddering, cellular shrinkage, micronuclei formation.

Intriguingly, the ophiobolin A- triggered PCD appears not to be mediated by an overproduction of reactive oxygen species (ROS). Indeed, in ophiobolin A- treated cells an increase in hydrogen peroxide production occurs only lately after PCD induction and ROS scavenging treatment reduces ROS level without preventing PCD. This behaviour differs from other kinds of PCD well characterized in the same cell culture (Vacca et al. 2004; Locato et al. 2008).

Ophiobolin A concentrations that do not affect cell viability arrest cell cycle in a reversible manner. In particular, 5 μM ophiobolin A treatment induces cell block in S/G2 phase. Concomitantly, ophiobolin A freezes the activity of the poly ADP- ribose polymerases (PARPs), nuclear enzymes involved in DNA metabolic transitions, which normally increases during the exponential growth phase in plant cells (Pellny et al. 2009).

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Poster Communication Abstract – 7.07

STUDY OF *FUSARIUM*-INDUCED BIOSYNTHETIC PATHWAY SHIFTS IN *ZEA MAYS* L.

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Zea mays L., Fusarium verticillioides, artificial inoculation, microarray analysis, bio-informatics

During the summer of 2009, 36 maize inbred lines together with 6 reference lines were tested for their tolerance towards Fusarium infection, by means of a *Kernel Inoculation Assay*. Primary open pollinated cobs were infected 15 days after silk emersion with a spore suspension (10^6 spores/ml) obtained from a mix of two *Fusarium verticillioides* strains isolated in Northern Italy. Inoculation with sterile water was used as a negative control. Subsequently, the number of infected kernels near the inoculation site was counted using the following scale and expressing the surface area of the cob covered with mycelium: 1 = 0% (no infection); 2 = 1-3%; 3 = 4-10%; 4 = 11-25%; 5 = 26-50%, 6 = 51-75%; 7 = 76-100%.

Based on these results, two genotypes were selected with diversified responses to Fusarium infection: Lo186, exhibiting a clear infection pattern with abundant mycelium growth and Lo435 with a far more resistant phenotype. Both accessions were subsequently inoculated as described previously, either with spore suspension or with sterile water, and kernel material was collected around the infection sites. Material was collected at two time points after inoculation (1 day and 5 days) from 5 independent cobs from each of the two genotypes. Total RNA was then isolated from each of the collected samples. Total RNA was, moreover, extracted at the two time points from non-inoculated cobs.

Three independently isolated RNA samples obtained from Lo186 after a 5 day infection period with Fusarium, three RNA samples isolated from identical material but obtained through inoculation with water, and three RNA samples again obtained from identical material, but collected from non-inoculated cobs, were used to prepare hybridization probes, which were subsequently used to hybridize an Affymetrix maize array. The comparison of the expression profiles obtained with non-inoculated, water-inoculated, and Fusarium-inoculated kernel material allowed identifying numerous genes exhibiting differential expression patterns across the series assayed. The genes identified, their putative functions with respect to fungal infection, and the biosynthetic pathways involved will be discussed.

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Poster Communication Abstract – 7.08

STUDY OF *MtN5* TRANSCRIPTIONAL CONTROL AND OF ITS INVOLVEMENT IN *MEDICAGO TRUNCATULA* NODULATION PATHWAY

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Medicago truncatula, early nodulin, MtN5, Rhizobia, symbiosis

The symbiosis between legumes and rhizobia starts with an exchange of molecular signals between the two partners. In response to the plant-derived flavonoids, bacteria synthesize Nod Factors (NFs), which are able to induce a series of events, such as ion fluxes, root hair deformation and the expression of the early nodulin genes, that eventually lead to the formation of root nodules. We previously demonstrated that *MtN5* is an early nodulin, required for the establishment of the symbiosis and also present in mature nodules. In order to investigate the role of *MtN5* in root nodules induction pathway, its expression profile during the early stages of infection was studied. *MtN5promoter::GUS* fusion showed that the promoter was active in epidermis and root hairs a few hours after inoculation, whilst in mature nodules, GUS was observed in the distal zone. The *in silico* analysis of the promoter sequence revealed the presence of two consensus regions (AAAGAT and CTCTT), also found in the promoter region of genes activated in rhizobia-colonized cells within nodules. Other motifs identified are putatively responsible for the hormonal control of gene expression. On the basis of these observation, the responsiveness of *MtN5* gene toward bacteria-derived molecules (*i.e.* NFs, EPS, Chitin Oligomers) and plant hormones (*i.e.* NAA, BAP, Ethylen precursor ACC) was tested. In a time course nodulation experiment, *MtN5* showed to be co-expressed with early markers of rhizobia infection, such as *RIP1, NIN* and *ENOD11*, and resulted to be more precocious than *ENOD20* and *MtN6*. In transgenic adventitious root silenced for *MtN5* expression (*MtN5hp* roots), we observed that upon rhizobia infection the nodulin *MtNIN* was not induced, whilst *ENOD11* was strongly upregulated with respect to control roots. Furthermore, in *MtN5hp* roots the expression of *FLOT4*, a nodulin gene known to be involved in the infection thread growth, was unaffected by the inoculation with symbiotic bacteria, in contrast with what observed in control roots. With the aim of gaining a further insight on the role of *MtN5* in the establishment of symbiosis, we carried out microscopic observation of infected *MtN5hp* roots by means of rhizobia carrying a reporter gene. *MtN5hp* roots displayed a reduced colonization of nodule primordia as compared to control. All these observations suggest that the activity of *MtN5* is required for the penetration of bacteria within the nodules.

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Poster Communication Abstract – 7.09

UNDERSTANDING THE ROLE OF AN EXTRACELLULAR ENDO-1,4-β-GLUCANASE IN THE *PYRENOCHAETA LYCOPERSICI*-TOMATO PATHOSYSTEM

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Pyrenochaeta lycopersici, cell wall degrading enzymes, endo-1,4-β-glucanase, pathogenicity

Many fungal plant pathogens secrete an array of cell wall degrading enzymes mainly involved in the pathogenesis. An extracellular endo-1,4-β-glucanase (named PlEGL1) from the causal agent of the Corky Root Rot (CRR) of tomato, *Pyrenochaeta lycopersici*, was isolated and characterized. *Plegl1* gene is strongly induced during the disease. We are currently investigating its putative role in the pathogenesis and its mechanism of action. We have sequenced *Plegl1* from 18 *P. lycopersici* isolates from different geographic areas of Italy and other world countries. Surprisingly we found that *Plegl1* coding sequence was identical in all the tested isolates, maybe suggesting a key role of this enzyme in the host-pathogen interaction.

In order to obtain preliminary data about the potential of the fungal cellulolytic activity in the virulence on its host, we set up a leaf infiltration assay using *P. lycopersici* culture filtrates with and without cellulolytic activity. The area of the leaf treated with the fungal filtrates becomes chlorotic in 2-5 days which evolves to necrotic lesions, in about 10 days, only with the filtrate having cellulolytic activity. The result shows that both fungal filtrates contain phytotoxins but the cellulase-containing filtrate develops necrosis faster than the other one, suggesting that the cellulolytic activity can be implicate in the pathogen virulence. We investigated the potential hydrolytic activity of *P. lycopersici* cellulolytic filtrate on the bacteria and fungal cell walls by an antibiogram assay for the bacteria and by a spore germination inhibition assay for the fungi. The results were none activity, in the tested conditions, toward the assayed bacteria and fungi, showing that the fungal cellulolytic ability is plant cell wall specific. These data are confirmed by a polysaccharide affinity precipitation assay carried out with the recombinant PlEGL1: chitosan and two types of cellulose, water-soluble and insoluble, were used as substrates and the results showed that the enzyme recognizes only the cellulose-based substrates.

In silico studies showed that PlEGL1 belongs to glycoside hydrolase family 61 (GH61): this enzymes are known to have a poor cellulolytic power but it was shown that they can dramatically enhance the activity of other cellulases in synergism assays. These data are in accordance to the results of the recombinant PlEGL1 acivity *in vitro*, that shows a moderate cellulolytic ability on carboxymethylcellulose substrate. For these reasons the only way to understand the real role of PlEGL1 in the cellulolytic arsenal of *P. lycopersici* is by silencing *Plegl1* expression. We are setting up a gene replacement protocol mediated by *Agrobacterium tumefaciens* transformation. The flanking genomic sequences of *Plegl1* available coding region were obtained by TAIL (Thermal Asymmetric InterLaced) PCR and they will be used for the gene replacement vector construction.

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Poster Communication Abstract – 7.10

ANALYSIS OF PECTIC ENZYME ACTIVITIES PRODUCED BY THREE PHYTOPATHOGENIC FUNGI GROWN ON PLANT CELL WALLS

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Pectic enzymes, plant cell wall, phytopatogenic fungi

The plant cell wall, the main structural element that a pathogen needs to overcome in order to penetrate and colonize the plant tissue, is organized as a complex network of polysaccharides such as cellulose, hemicellulose and pectin. Pectin is a family of complex mixture of highly heterogeneous and branched polysaccharides rich in D-galacturonic acids present in all plant primary cell walls. Phytopathogenic fungi produce a variety of pectin degrading enzymes either to facilitate the invasion of the plant tissue and to release nutrients to be used as carbon source. Most of the degradative enzymes are glycoside hydrolases, which degrade the pectate matrices by the addition of water to break the glycosidic bonds. The pectate network is also degraded by polysaccharide lyases, which cleave the glycosidic bonds via a β-elimination mechanism. Three species of phytopatogenic fungi (*Rhizoctonia solani, Sclerotium rolfsii and Fomitopsis pinicola*) were compared regarding production of pectic enzymes on basal liquid colture medium containing 1% of a crude extract of monocot and dicot plant cell walls (*Zea mays* and *Brassica rapa*). Polygalacturonase, pectin methyl esterase, pectate and pectin lyase activities were quantified in a time course of 4, 9, 13 and 16 days. The results showed that the three fungi have significantly different abilities and timing to produce pectic enzymes, which may be associated to their pathogenic habit.

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Poster Communication Abstract – 7.11

CERATO-PLATANIN AND CERATO-POPULIN INDUCE DIFFERENTIAL GENE EXPRESSION IN *PLATANUS ACERIFOLIA*

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Platanus acerifolia, fungal proteins, defence genes, cDNA microarray

Cerato-platanin (CP) and cerato-populin (Pop1) are small non-catalytic proteins produced by the ascomycetes *Ceratocystis platani* and *C. populicola. C. platani* is responsible for the canker stain disease of plane trees, and *C. populicola* for the black canker of poplar trees. CP and Pop1 are PAMPs (pathogen-associated molecular patterns) inducing typical defense responses in various host and non-host plants. CP and Pop1 have an identity of about 63% and the conservative substitution of approximately 12% of amino acids, and both belong to the "cerato-platanin family" (Pfam PF07249).

The aim of the present research was to analyse the gene expression induced in *Platanus acerifolia* leaves after treatments with CP and Pop1 using cDNA microarrays containing sequences isolated from suppressive subtractive libraries (SSH) from *P. acerifolia* after treatment with CP, and from *Populus* after cold or ozone treatment.

There are many reports on cross-tolerance induction by abiotic stresses against other biotic stresses and vice versa and on the existence of a network of regulatory signalling occurring in plants during the interaction with biotic and abiotic stresses.

PCR amplified sequences of the clones isolated from the cDNA libraries were spotted on microarray glass slides by CRIBI (Padoa, Italy). For the cDNA microarray analysis, mRNA was reverse transcribed in the presence of Cy3-dUTP or Cy5-dUTP. RNAs were extracted from leaves treated with sterile distilled water droplets containing CP or Pop1, or sterile distilled water as a control.

Out of the 318 genes, 131 and 50 genes resulted to be modulated in CP- and Pop1-treated leaves, respectively. Moreover, several transcripts were differentially regulated. In both treatments the up-regulated genes were more than the down-regulated ones. Inducible expression of some candidate genes selected from the microarray results was confirmed by using semi-quantitative RT-PCRs.

These results show that several differentially regulated genes induced by the PAMPs CP and Pop1 in *P. acerifolia* are in common with those induced in response to ozone and cold stresses in *Populus*, underlying the existence of a conserved network of genes activated by different stresses during plant defence responses.

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Poster Communication Abstract – 7.12

DE NOVO SEQUENCING OF *ANEMONE CORONARIA* TRANSCRIPTOME TO DISCOVER PUTATIVE GENES INVOLVED IN *TRANZECHELIA DISCOLOUR* INFECTION RESPONCE

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454 sequencing, Gene Ontology classes, KEGG pathways, Prunus/Anemone rust, R-genes

The genus *Anemone* (*Ranunculaceae*) includes many species that are cultivated as ornamentals, either as garden or as cut-flowers plants. The poppy anemone *A. coronaria* is the most valuable winter-flowering species (Yonash *et al*., 2004) and is the progenitor of most varieties currently grown for cut-flower production.

The *Prunus*/*Anemone* rust, caused by the fungus *Tranzschelia discolor*, has become aggressive in *A. coronaria* cultivation in recent years. Teliospores, formed on *Prunus* leaves, infects *A. coronaria* seedling during the growth cycle required to produced rhizomes; infected plants remain asymptomatic. The disease appears in the next vegetative cycle, on plants cultivated to crop flowers. *T. discolour* infection dramatically reduces flower production and quality. Aeciospores released from *A. coronaria* leaves only infect *Prunus*.

Over the few last years, next-generation sequencing (NGS) technologies have led to revolution in genomics and genetics and provided cheaper and faster delivery of sequencing information (Morozova et al., 2008; Mardis 2008). To date, 454 pyrosequencing technology is widely used for *de novo* sequencing and analysis of trascriptomes.

Total RNA was isolated from leaves of *A. coronaria* either healthy or infected by *T. discolor*. Two 3'-fragment cDNA libraries (labeled S_1S and S_2I) were prepared for pyrosequencing with the GS FLX 454 Titanium system (by Eurofins MWG-Operon; Ebersberg, Germany).

454 reads from the two libraries were independently assembled using the MIRA Assembler. The 304.786 and 306.439 HQ reads obtained with an half-plate run from the S_1S and S_2I libraries were assembled in 154.039 and 150.421 unigenes (contigs plus singletons), respectively. The unigene sequences were annotated by BLASTx versus the UniProtKB database as well as the *Puccinia graminis tritici* and *Puccinia triticina* database (http://www.broadinstitute.org/annotation/genome/puccinia group/multiDownload.html). Cross-library comparison were performed with BLASTn to detect library-specific transcripts. A functional classification of the unigenes according to Gene Ontology (GO) was performed with a custom tool and statistical analyses were performed to highlight differentially expressed GO classes within a plant-specific GO-Slim. UniProt-ID retrieved by BLAST were used to relate transcripts to know

molecular pathways available at KEGG. Comparison of unigenes to a plant resistance gene database (PRGdb; http://www.prgdb.org), was also performed with Blast2GO (www.blast2go.org).

The overall analyses allowed the individuation of ontology classes displaying statistically significant differences in expression levels between the two libraries. In addition, 611 and 524 sequences from 1S and 2I library, respectively, presented homology with R-genes collected in PRGdb.

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Poster Communication Abstract – 7.13

OIDIUM NEOLYCOPERSICI INFECTION INDUCES DIFFERENT RESPONSES ON RESISTANT AND SUSCEPTIBLE TOMATO PLANTS

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Antioxidants, Oidium neolycopersici, reactive oxygen species, tomato plants

Oidium neolycopersici is a highly widespread fungus which infects tomatoes, causing powdery white lesions on leaves. Severe infections lead to leaf chlorosis and premature senescence that severely affect plant yield. Resistance to infection by *O. neolycopersici* has been found in *Lycoperiscon esculentum* var. *cerasiforme* (R-28) and appears to be due to a single recessive *ol-2* gene (Ciccarese et al., 1998). The recessive *ol-2* gene, confers race-non-specific resistance via papilla formation, a non HR-based mechanism (Bai et al. 2005). Very little information is available regarding the involvement of biochemical responses of R-28 plants to *O. neolycopersici* . Therefore, in this work different defence mechanisms have been studied during the infection of *O. neolycopersici* on the *s*usceptible (Super Marmande -SM) and R-28 tomato plants. The analyses have been conducted on R28 and SM plants at different times after inoculation (4-24-48-72 hpi). Data obtained show different trends in various biochemical parameters. In particular in SM plants a decrease in ascorbate (ASC) content, ascorbate peroxidase (APX) and total peroxidase (POD) activities occurs underlying that a general decrease of plant defence in response to fungus penetration could be responsible of the symptoms of the disease. On the other hand, R28 plants show a peak in hydrogen peroxide production that is parallel to the arrest of fungus penetration (4-24 hpi). At the same time an increase in ASC, APX and POD occurs. Moreover, after 48-72 hpi a higher content of total antioxidant power and phenolic compounds is also evident.

Changes in redox metabolites and enzymes, in relation to the resistance response of R-28 tomato plants, will be discussed.

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Poster Communication Abstract – 7.14

IDENTIFICATION OF EARLY TRANSDUCTION ELEMENTS INVOLVED IN OLIGOGALACTURONIDE SIGNALLING

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Plant defence, Arabidopsis thaliana, plant cell wall, oligogalacturonides

Alpha 1-4-linked oligogalacturonides (OGs) derived from plant cell walls function as damage-associated molecular patterns (DAMPs) and activate the plant immune response. OGs also regulate developmental responses, likely due to their ability to antagonize auxin. So far, little is known about the intracellular elements involved in the early events triggered by OGs. One of the objectives of our work is to identify Arabidopsis Mitogen-Activated Protein kinase kinase kinases (MAPKKKs) involved in the signal transduction cascade activated by OG. Here we describe a MAPKKK mutant defective in both early and late responses to OGs. Notably, responses to microbe-associated molecular patterns (MAMPs), such as elf18 and chitin, are not affected in these mutants. These results suggest the presence of two different and convergent signalling pathways in response to DAMPs and MAMPs in Arabidopsis.

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Poster Communication Abstract – 7.15

HAPLOTYPE ANALYSIS OF THE *RDG2A* LOCUS IN DIFFERENT BARLEY VARIETIES

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Resistance gene, barley, leaf stripe, haplotype analysis

Leaf stripe disease on barley is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea*. Race-specific resistance to leaf stripe is controlled by two known *Rdg* (*R*esistance to *Drechslera graminea*) genes: the *H. spontaneum*-derived *Rdg1a*, mapped to chromosome 2HL and *Rdg2a*, identified in *H. vulgare*, mapped on chromosome 7HS and cloned in the resistant cultivar (cv.) Thibaut. The *Rdg2a* locus contains a gene cluster of three sequence-related Coiled-Coil, Nucleotide-Binding site, and Leucine-Rich Repeat (CC-NB-LRR) encoding genes. However, only one gene conferred resistance to isolate *Dg2*, against which *Rdg2a* is effective, when the susceptible cv. Golden Promise was transformed with the *Rdg2a*-candidates. The high level of sequence similarity between the three genes most likely contributed to significant rearrangements during evolution, probably derived from un-equal crossing-overs resulting in sequence exchange between paralogs and in the generation of recombinant genes, as well as in expansion/contraction of gene copy number. To examine haplotype variation at the *Rdg2a* locus, the sequencing of the allelic *Mrdg2a* (Morex *rdg2a*) locus of the leaf stripe susceptible barley cv. Morex was carried out and revealed large rearrangements including two deletions that generated an *Rdg2a*-homolog gene. This gene most likely derived from an un-equal crossing-over between the *Rdg2a* ancestor and its paralog *Nbs2-Rdg2a*. PCR analyses performed with informative markers at five loci within the *Rdg2a* locus identified four different haplotypes. The Thibaut haplotype was observed to be largely conserved in *Dg2*-resistant barley cultivars. The re-sequencing of the *Rdg2a* gene in barley genotypes showing the same Thibaut haplotype or the same resistant phenotype revealed high sequence similarity to Thibaut *Rdg2a*, demonstrating the widespread conservation of the gene. Nonetheless, some sequence variation were identified in at least two barley genotypes that were verified for possible differences, with respect to *Rdg2a*, in the range of resistance specificities towards different leaf stripe isolates.

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Poster Communication Abstract – 7.16

IDENTIFICATION AND MAPPING OF A NEW LEAF RUST RESISTANCE GENE DERIVED FROM *TRITICUM TURGIDUM* VAR. *DICOCCUM*

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Leaf rust, resistance gene, Triticum turgidum, QTL analysis

Leaf rust, caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*) is one of the most important diseases for wheat, causing significant yield losses annualy in many wheat growing regions of the world. The utilization of resistance genes is the most viable and economical strategy to minimize the yield losses.

Nevertheless the source of resistance gene is rather limited in durum wheat genetic background, a situation that requires the search of new resistance genes in related wheats.

In order to investigate the genetic basis of leaf rust resistance, we are currently developing a genetic linkage map on a RILs population (122 F9 lines) derived from a cross between the susceptible durum wheat cultivar Latino and the resistant accession MG5323 of *T. turgidum* var. *dicoccum*.

The phenotypic characterization (Infection Type and Relative Disease Severity) of the RIL population by means of artificial inoculation of two *P. triticina* (Jerez 05 and 16081-1) isolates was performed in greenhouse experiment carried out in Italy and Spain.

More than 400 microsatellite markers with known position and well distributed on the whole genome were tested on parents of the segregant population, and 79% of the markers analysed were found polymorphic.

A genetic linkage map for QTL analysis was developed using of 300 SSR markers distributed within 14 linkage group and spanned greater than 2000 cM.

The QTL analysis carried out using the disease response data (IT and RDS scoring) of all the RILs allowed us uncover one major QTL localized in a genomic region where no previously identified leaf rust resistance genes (Lr genes) have been positioned. This major QTL was mapped on the short arm of 1B chromosomes and SSR markers strictly associated were identified. This result therefore suggests the identification of a new resistance gene to leaf rust in the *durum* wheat genetic background.

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Poster Communication Abstract – 7.17

MICROARRAY ANALYSIS OF GENE EXPRESSION VARIATION IN EGGPLANT ROOTS SUBJECTED TO INOCULATION WITH *FUSARIUM OXYSPORUM* AND *VERTICILLIUM DAHLIAE*

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Microarray analysis, Fusarium oxysporum, eggplant, fungal inoculation

The two fungal *Fusarium oxysporum* and *Verticillium dahliae* are the causal agents of root rot and wilt diseases in several plant species, including eggplant. Perception of these fungal diseases by model plants follows the concept of the elicitor-induced immune response, which in turn activates several defence signalling pathways. Our purpose is to compare the expression profiles of genes involved in defence response mechanisms in roots samples of an *Fusarium* resistant eggplant line after different fungal inoculations and different timings (0, 4 and 8 hours after dipping in fungal suspension). Transcriptional changes in eggplant roots induced by *Fusarium*, *Verticillium*, and mixed (*Fusarium* + *Verticillium*) inoculations were undertaken by microarray analysis, using mock-inoculated samples as control. The analysis was performed in a new CombiMatrix platform, with a 4x2K customized chip and containing 2000x4 eggplant probe sets, resulting in the hybridization of four independent samples on a single slide. The probes were selected and designed from a collection of three subtractive cDNA libraries of eggplant genes putatively involved in the plant-pathogen interaction. Changes in gene expression were examined and compared between the three different timings (T0,T4,T8), considering the different fungal inoculations and the control. Stringent control measures were applied for all steps of the experiments and for the following data analysis; thus all results obtained were within the windows of resolution of the microarray hybridization method. About 160 genes were found to be modulated in at least one condition or timing. Results show that the number of up-regulated genes was slightly higher in T8 versus T0 than in T4 versus T0. The number of down-regulated modulated genes remain comparable between T8/T0 and T4/T0. The up- and down-regulated genes were functionally assigned according to the principal GO categories (molecular function, biological process and cellular localization), and preliminary data analysis showed that most of modulated genes belong to defence response and stress induced categories. The validation of microarray data by RT-qPCR is in progress.

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Poster Communication Abstract – 7.18

PRODUCTION OF TRANSGENIC WHEAT PLANTS EXPRESSING THE PROTEIN INHIBITORS AcPMEI AND PvPGIP2 TO ENHANCE RESISTANCE TO FUNGAL DISEASES

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Defence genes, PMEI, PGIP, transgenic plants, wheat

Many pathogens produce a wide range of cell wall degrading enzymes (CWDE) and among them polygalacturonases (PGs) are one of the first enzymes produced during the infection process. Plants possess cell wall inhibitors that inhibit PG activity (PGIPs). Wheat plants expressing a bean PvPGIP2 showed a reduced disease symptom caused by *Bipolaris sorokiniana* and *Fusarium graminearum*. PG activity is also limited by the level of methyl esterification of the cell wall pectin. Pectin methyl esterification is controlled by the activity of the pectin methyl esterase (PME) and its inhibitor (PMEI). Wheat plants expressing a kiwi AcPMEI showed a reduced disease symptom caused by *Bipolaris sorokiniana* and *Fusarium graminearum*. In order to test the possibility to enhance further wheat resistance against fungal pathogens by limiting the degradation of cell wall pectin, we used traditional breeding to cross the transgenic wheat plants expressing PvPGIP2 and AcPMEI. PCR assays and inhibition analyses of F1 and F2 progenies demonstrated the possibility to combine the expression of both transgenes. Parental lines and F1 and F2 hybrid plants expressing both inhibitors were tested against *B. sorokiniana* and the evaluation of the symptoms showed similar level of protective effect of hybrid plants and AcPMEI and PvPGIP2 parental lines. Additional tests with *B. sorokiniana* and *F. graminearum* on AcPMEI/PvPGIP2 homozygous plants are under evaluation.

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Poster Communication Abstract – 7.19

ISOLATION AND CHARACTERIZATION OF XYLANASE INHIBITORS FROM CHROMOSOME GROUP 5 OF WHEAT

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Xylanase inhibitor protein, xylanases, wheat, transgenic plants, defence response

Cereals contain xylanase inhibitor proteins (XIs) which inhibit microbial xylanases from glycoside hydrolase families 10 and 11. In wheat, three types of XIs have been identified: *Triticum aestivum* XI (TAXI), xylanase inhibitor protein (XIP) and thaumatin-like XI (TLXI), and each of them is represented by a multiple number of genes. Several observations suggest that these inhibitors are important plant defence components. For example, XIs are effective against xylanases of microbial origin and not against plant xylanases. XIs share a significant sequence similarity with pathogenesis-related proteins (PR), are localized in the apoplastic region and are induced by wounding, jasmonic acid and pathogen infection. Moreover, the importance of xylaneses during pathogenesis has been demonstrated for the fungal pathogens *Botrytis cinerea* and *Septoria tritici*. Here we report the isolation and localization on chromosome group 5 of some XI genes. Moreover, to verify *in planta* the role of these XIs in wheat defence, we produced a number of transgenic plants over-expressing XipI, XipIII and TaxiIII. We are also attempting to silence TaxiIII and XipIII and a number of regenerated wheat plants have been obtained. The over-expression of XIs under control of the maize *Ubi-1* promoter endows wheat with the capacity to produce these inhibitor constitutively and through *in vitro* inhibition assays using total protein extract from transgenic tissue we demonstrated the capacity of the transgenic XI to retain the inhibition properties against fungal xylanases. The transgenic wheat plants are under investigation to verify their response to the fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*.

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Poster Communication Abstract – 7.20

THE EXPRESSION OF A FUNGAL POLYGALACTURONASE CAUSES CELL WALL PECTIN MODIFICATION AND ALTERS PLANT GROWTH IN WHEAT

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Cell wall, homogalacturonan, Polygalacturonase, wheat development

Pectin is a major constituent of the primary cell wall of dicotyledonous plants and is composed mainly of homogalacturonan (HGA). This pectic polysaccharide consists of a linear homopolymer of 1,4-linked α-D-galacturonic acid (GalA) with a degree of polymerization of about 100 residues. Pectin is synthesized in the Golgi apparatus, secreted into the cell wall in a highly methyl esterified form and here de-esterified to varying degree in a spatial regulated manner by the activity of pectin methyl esterase (PME) under control of its inhibitor (PMEI). Differently from dicotyledonous plants, grass species contain a low level of pectin in their cell wall, however, recent evidences and the presence of a large number of genes encoding PME and PMEI in the model species *Oryza sativa* and *Brachypodium distachyon* suggests that pectin structure could play a relevant role in plant development and defense also in grass species.

In order to gain information on the role of pectin, and in particular of HGA, in wheat grow and development, we have specifically modified HGA in wheat transgenic plants by expressing the endopolygalacturonase II of *Aspergillus niger* (AnPGII). We obtained a limited number of transgenic wheat plants (*Triticum aestivum* cv Bobwhite and *T. durum* cv Svevo) and some of them showed a dwarf phenotype that was associated to AnPGII activity. The pollen grains of these plants showed an altered morphology and a reduced vitality. Immunodot analysis on cell wall pectin of the transgenic and control plants using monoclonal antibodies that recognize pectin epitopes with different methyl ester distribution revealed a lower level of PAM1-binding epitope on transgenic lines compared to WT, indicating a reduced level of long stretches of de-esterified pectin in the transgenic lines. A reduced level of LM19 binding epitopes was also detected on transgenic lines compared to the WT, indicating a low level of short contiguous unesterified GalA residues in the transgenic lines. These observations indicates that the de-esterified HGA is a crytical component in wheat growth.

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Poster Communication Abstract – 7.21

THE LACK OF RECOGNITION OF THE POLYGALACTURONASES SECRETED BY *CLAVICEPS PURPUREA* BY PVPGIP2 IS RESPONSIBLE FOR SUSCEPTIBILITY IN WHEAT TRANSGENIC PLANTS

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Claviceps purpurea, Polygalacturonase, PGIP, wheat

Claviceps purpurea is a biotrophic fungal pathogen of grasses and cereals, causing the ergot disease. The infection process of *C. purpurea* spore mimics a pollen grain growing into the ovary during fertilization and the subsequent pathogenic development is strictly limited to the ovary and the connected vascular tissue. The infection process of *C. purpurea* on rye (*Secale cereale*) flowers is accompanied by pectin degradation and PG activity represents a pathogenicity factor. Because of the importance of PG in the infection process of rye flower, we tested whether in the interaction system *C. purpurea*/wheat the presence of PvPGIP2 can affect pathogen infection and ergot disease development. We first verified the expression of PvPGIP2 in the ovary of transgenic wheat line MJ23a that showed a reduced symptom disease caused by *Bipolaris sorokiniana* and *Fusarium graminearum*. Subsequently, we evaluated the ergot disease symptoms both by assessing the honeydew production on a scale from 1 to 4 and by measuring the sclerotia weight. Both methods produced slight, though statistically significant, differences in mean value between transgenic and control plants. These data suggest that PvPGIP2 affects pathogen growth but its contribution does not produce a noticeable phenotypic effect on ergot symptoms. To verify the possible reason of these lack of improved resistance in MJ23a transgenic plants, we isolated and expressed in *Pichia pastoris* both *pg* genes contained in the *C. purpurea* genome. In vitro assays using the heterologous expressed proteins and purified PvPGIP2 showed that both PGs are poorly affected by this inhibitor. These data suggests that the lack of increased resistance in MJ23a line is due to the lack of inhibition of the PGs of *C. purpurea* by PvPGIP2, thus reinforcing the notion of the effectiveness of PGIP as defence response when recognition occurs.

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Poster Communication Abstract – 7.22

CO-TRANSFORMATION OF PECTINASE AND XYLANASE INHIBITORS (PGIP, PMEI AND XI) TO ENHANCE WHEAT RESISTANCE TO FUNGAL DISEASE

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Plant disease, cellwall reinforcement, Polygalacturonase inhibitor protein, Pectin methyl esterase inhibitor, xylanases inhibitor

The reinforcement of plant cell wall compartment can efficiently protect plants against those pathogens that need to overcome this barrier to colonize the host tissue. Plant cell wall contains Polygalacturonase inhibitor protein (PGIP) that inhibits the endopolygalacturonase (PG) secreted by pathogens during the initial phase of infection. Moreover, plants cell walls with higher level of methyl esterified pectin are less susceptible to the hydrolysis by pectin enzymes such as fungal PG. The degree of cell wall pectin methyl esterification can be controlled by increasing the level of the protein inhibitor PMEI that inhibit the activity of pectin methyl esterase (PME), that is the enzyme responsible for removing the methyl ester group from the newly synthesized pectin. Cereals contain also xylanase inhibitors (XIs) which inhibit microbial xylanases from glycoside hydrolase families 10 and 11. Endo-β-1,4-xylanases are key enzymes in the degradation of arabinoxylans (AXs), the main non-starch polysaccharides from cereal cell walls.

Transgenic plants overexpressing the bean PvPGIP2 or the kiwi AcPMEI showed increased resistance to fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*, whereas the effect of XI in the defence response has not been yet reported. Transformation experiments using single components makes possible to define the contribution of the specific component. However, the feasibility to test several components at the same time offer the possibility to verify their combined contribution and to reduce the necessary to analyze more components. Moreover, depending on linkage relationships between the transgenes used, transgenic plants with single components can be also obtained. The aim of this study was the determination of the co-transformation frequency of four trangenes, *Pvpgip2, Acpmei, TaxiIII* and *Bar* genes, and their mode of inheritance. The four constructs were co-bombarded into immature embryos of durum wheat cv Svevo. Sixteen transgenic lines were obtained in two separate bombardment experiments. PCR analysis of T0 plants revealed a co-trasformation frequency of all four transgene of about 50%. The segregation frequency in those lines containing the four transgenes were analyzed in the T1 and T2 progenies. Three of these lines showing the three transgenes of interest linked were subjected to infection experiments with *B. sorokiniana*. The analysis of the data showed a significant reduction in symptom severity of about 60% compared with non transgenic plants. Further characterization of the transgenic lines will be also presented.

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Poster Communication Abstract – 7.23

EXPRESSION PROFILING OF THE WRKY MONOCOT-SPECIFIC CLADE IN RICE

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WRKY genes, rice, monocotyledons, Magnaporthe oryzae

WRKY proteins constitute a large family of plant transcriptional factors which have been shown to be involved in a range of biological processes. They are usually classified in three main groups. In this study, we showed the existence of a monocot-specific (MCS) clade within group 3 in rice WRKY family, composed of 19 genes. Fourteen out of these 19 MCS *WRKYs* fell into three segmental duplicated blocks on chromosomes 1, 11 and 12. Several residues and/or motifs are conserved within these 19 MCS proteins.

Expression analysis indicated that some MCS OsWRKY genes are developmentally regulated in rice while others are not. In addition they are differentially regulated in response to several abiotic stress conditions, pathogens or parasites. In particular, the MCS *OsWRKY* genes were transcriptionally regulated upon rice blast fungus infection. Duplicated MCS OsWRKY genes have divergent expression profiles, likely reflecting a diversity of rice responses to environmental constraints. The MCS *WRKYs* are also poorly co-regulated among them as showed by co-regulation analysis. To conclude, we defined the existence of a monocot-specific *OsWRKY* clade with diverse transcriptional profiles supporting their role in rice specific regulatory pathways.

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Poster Communication Abstract – 7.24

ISOLATING RESISTANCE GENES AGAINST *FUSARIUM* EAR ROT IN MAIZE

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Fusarium ear rot, disease resistance, Zea mays, molecular markers

We investigated global gene expression in maize ears at several time points after infection with *Fusarium verticillioides*. In kernels at 48 h post infection with a fumonisin-producing strain, about 800 differentially expressed sequences were identified and nearly 10% assigned to the category cell rescue, defence and virulence. The expression analysis was extended to early (12, 24 h) and late (72, 96 h) phases after infection with a fumonisin-nonproducing strain. The mutant strain was able to activate host defence genes later than the wild type strain. When resistant and susceptible maize genotypes were compared, in the resistant lines the expression of defence genes were induced upon infection, indicative of a basal defence response against the fungus. In the susceptible genotypes defence genes were induced specifically after pathogen infection. The basal defence response was also active against several fungal species invading maize kernels. The differentially expressed genes were selected as candidate genes for mapping. SNP markers were developed for resistant and susceptible maize lines. SSR markers were selected according to their chromosomal positions on the maize reference map with the aim to test the polymorphisms in the two parental lines and in a segregating F_2 population. The molecular markers associated to resistance will be localized on a high density molecular map and exploited to detect quantitative trait loci (QTLs).

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Poster Communication Abstract – 7.25

TOWARDS THE MODELING OF TOMATO-FORL INTERACTION

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FORL, cytochrome P450, ethylene, glucosinolate

In recent years models have been widely used to study pathogenicity mechanisms thanks to the availability of high throughput sequence data and computational resources. Gene expression approaches constitute a starting point from which to determine the best strategy for building a biological model of a plant –pathogen system. A common strategy in gene expression analysis is to identify a set of genes of interest following their expression profile in different hosts and/or treated tissues. *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) is one of the most destructive pathogen of tomato plants. This pathogen is responsible of the crow and roots rot until to determinate the plant's death. Large-scale microarray analysis has been performed on infected and non-infected root samples of resistance (Momor) and susceptible (Monalbo) tomato genotypes. The differential expression of cytochrome gene family in compatible and incompatible reaction helped us to generate hypotheses about its behavior in susceptible and for resistance response to FORL. The results showed that in the incompatible reaction there is an activation of the metabolic pathway of tryptophan whilst in the compatible reaction was detected the activation of the ethylene synthesis. Previous studies showed that the production of glucosinolate compounds are involved in the defense plant and that ethylene suppresses the action of the cytochrome P450. Although still fragmented genes expression network helped us to formulate a hypothesis that provides a overview of the tomato-FORL interaction process. Proteomic techniques as well as data mining and functional genomics tools available nowadays will help us to refine the current model.

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Poster Communication Abstract – 7.26

IDENTIFICATION OF BACTERIA LIVING IN THE CHRYSOLINA HERBACEA GUT BY CULTURE-BASED APPROACHES

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Chrysolina herbacea, Mentha aquatica, monoterpenoids, gut microbial flora, VOCs analysis

Chrysolina herbacea (the mint beetle), is a phytophagous beetle that establishes specific relationship with the plant *Mentha aquatica* (the watermint). In particular, the ability of *C. herbacea* to use volatiles emitted by undamaged *M. aquatica* plants as attractants and the plant's response to herbivory involving the production of deterrent molecules, have been recently described (1). Moreover, there is evidence that some *Chrysolina* species produce deterrents to natural enemies from plant-derived compounds (2-4).

The objective of the present study was to examine the gut microbial flora by using culture-based approach. A total of 10 single insect pools (male or from female *C.herbacea*) were placed in a sterile physiological solution (0,9% NaCl). Each insect pool suspension was decanted and rinsed 3x10ml of sterile 0,9% NaCl solution. Then, the rinsed insect pool was treated with 3% H_2O_2 for 20s, and finally rinsed with 70% ethanol and rapidly flamed. The throat of each insect was cut with a sterile scalpel and the head was removed. Pressing on the paunch of the cutted insects the total intestine was collect in 5ml LB broth containing 2-3g of sterile glass beads (ϕ 0,5mm) , heavily vortexed for 4-5min and left to elute overnight at 4°C. To remove gut debris, samples were centrifuged at 2000 g for 1min. Then appropriate dilutions of the supernatant were transferred on the surface of NA, LB and YEPD solid media, respectively, and incubated at 30°C. The bacterial titers by plating on the upper different solid media were: on NA plates 10^7cell/ml ; LB plates 10^7 cell/ml ; YEPD plates 10^5 cell/ml . After 24 h incubation at 30°C , a number of colonies, from each agar plate media, were picked up randomly and streaked onto fresh plates. Only plates with colony numbers ranging from 50 to 200 were used for isolation of pure cultures. Pure cultures were checked by microscopy, and were stored either in LB agar slants or in LB broth plus 60% glycerol at -20°C.

By using culture-based approach we have analyzed more than 200 bacterial isolates from the digestive tract of *C. herbacea*. Most bacterial isolates belong to three species of the genera *Serratia*, *Pseudomonas* and *Sphyngomonas* with marked quantitative differences between male and female

individuals. Furthermore, Head-Space analysis by GCxGC qMS revealed the differential emission of hydroxylated cineole derivatives between male and female faeces (frass) Volatile Organic Compounds (VOCs). The hypotheses that these bacteria may be involved in metabolism of plant-derived compounds and that biotransformed metabolites may be used as recruitment/sex pheromones by female insects will be discussed. Because *C. herbacea* is quite diffuse in mint fields and herbivore feeding alters the aromatic profile of essential oil-producing plants like *M. aquatica*, the issue is not only ecologically but also economically relevant.

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Poster Communication Abstract – 7.27

CHARACTERIZATION AND DIVERSITY OF BACTERIAL ENDOPHYTES OF *VITIS VINIFERA*

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Vitis vinifera ("Prosecco"), bacterial endophytes, ARDRA

Plant-growth-promoting-bacteria (PGPB) are associated with many plant species and are commonly present in many different environments. Recent studies have evidenced that some of these micro-organisms can also enter the root interior, move within the plant and establish endophytic populations. Endophytes can be defined as micro-organisms that colonize the internal tissues of the plant showing no external sign of infection or negative effect on their host and that could be isolated from surface-sterilized plant tissues. It has been demonstrated that they can accelerate seedling emergence, promote plant growth and yield; they also can act as biocontrol agents to manage plant pathogens: disease development is prevented through endophyte-mediated synthesis of novel metabolites which are effective on different plant enemies. In addition they may help to remove contaminants, solubilize phosphate or contribute assimilable nitrogen to plants.. This work is part of a F.S.E. research-project focusing on the biodiversity of bacterial endophytes, living in stems and roots of *V. vinifera* (Prosecco) that grow in Conegliano (TV) area. In order to evaluate and isolate culturable and non-culturable bacterial endophytes leaves, roots and shoot were sampled. Grapevine tissues were sterilized by treating with ethanol 70% for 1 min, sodium hypochlorite 1,5% for 3 min and washing with sterile water 5 times. Sterilized tissues were aseptically ground in a mortar; loopful of the ground tissue suspension was utilized for plating; overflow tissues were stored at -80°C for later culture independent molecular analyses. The diversity of culturable bacterial endophytes of grapevine was examined using plate-dependent cultivation methods in NA (Nutrient Broth Agarized Medium), and incubated. Many isolates were selected; they have already been grouped on the basis of phenotypic characteristics such as colour, form, surface, opacity, texture, cell morphology, streaked onto individual NA plates, checked for purity and stored at -80°C. These isolates have also been screened for nitrogenase activity, production of indole acetic acid (IAA) and siderophore, and phosphate solubilization. Prokariotic DNA has been extracted from the endophyte candidates using standard procedures and has been analyzed by ARDRA (Amplified Ribosomial DNA Restriction Analysis) technique. Bacteria identification will be performed by 16S rDNA amplification and sequencing; their screening for plant growth promotion and biocontrol will result in good candidates for practical application.

Obtained results suggest that certain isolates may be exploited for developing a potential endophyte application in improving grapevine productivity and disease biocontrol.

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Poster Communication Abstract – 7.28

TOMATO, ARBUSCULAR MYCORRHIZAL FUNGI AND GEMINIVIRUSES: PLANT HEALTH IN A TRIPARTITE INTERACTION

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Tomato, biotic stress tolerance, symbiosis, TYLCSV, AM fungi

Arbuscular mycorrhizal (AM) symbiosis is one of the most widespread mutualistic associations, established between soil fungi belonging to the Glomeromycota phylum and the majority of land plants. This symbiotic association is considered a natural instrument that improves the productivity and health of host plants. Several investigations have in fact demonstrated that mycorrhizal plants often show higher tolerance to abiotic and biotic stresses, i.e. attack by bacterial and fungal pathogens. However, the impact of the AM symbiosis on the infection by viral agents is still largely unknown.

The aim of this project is to study the effects, at a phenotypic and a molecular level, of the AM symbiosis on the infection by a virus. The experimental system is: the model plant *Solanum lycopersicum* (tomato), colonized by the fungal symbiont *Glomus mosseae*, an AM fungus widespread in agricultural and natural systems, and infected by Tomato yellow leaf curl Sardinia virus (TYLCSV), an economically important DNA virus of the Geminiviridae family.

To reach our goal four biological conditions were set up: control plants (C), TYLCSV-infected plants (V), mycorrhizal plants (M) and mycorrhizal plants infected by TYLCSV (MV). Tomato seedlings were initially inoculated with *G. mosseae*. After one month, when mycorrhization was well established, plants were inoculated with TYLCSV. One month after virus inoculation a tissue print assay was performed to assess virus infection, and plants were examined for biomass, mycorrhization level, viral symptoms, and sampled for DNA and RNA extraction.

Shoot and root biomass was not significantly different in any group of plants, but V plants presented a high variability. The level of mycorrhization was comparable in M and MV plants. Viral symptom severity was lower in MV plants than in V plants.

Molecular analyses are in progress to quantify viral DNA and to monitor the expression level of important genes involved in the complex tripartite interactions.

This study will allow to extend the present knowledge on the molecular bases of plant/virus/mycorrhizal fungus interactions, and to identify key genes in the host response, candidate to represent important molecular determinants of plant health.

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Poster Communication Abstract – 7.29

CTV-INDUCED MODULATION OF THE *CITRUS* BARK PROTEOME –AND PHOSPHOPROTEOME – DURING COMPATIBLE AND INCOMPATIBLE INTERACTIONS

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Citrus, CTV, proteomics, 2DE

Citrus tristeza virus (CTV) is the causal agent of various diseases with dramatic effects on citrus crops (*tristeza, stem pitting* and *seedling-yellow* syndromes). It causes the collapse and necrosis of sieve tubes and companion cells leading to the production of an excessive amount of non functional phloem, and the reduction of the root system. The tristeza disease occurs with wilt symptoms and leads to a complete decline of the susceptible plant in a few weeks. The propagation of *Citrus* spp. on tristeza-tolerant rootstocks is the only viable option to lower the incidence of this disease on citrus crops. Although CTV structure, genome organization and expression have been well characterized, little is known about the molecular mechanisms of this host-pathogen interaction, and the actual role of scion-rootstock interactions in the modulation of the response to CTV infection is still unknown. Global analysis of bark proteome and phosphoproteome has been carried out by means of 2DE in Tarocco Scirè sweet orange grafted either on sour orange or Carrizo citrange rootstocks (respectively susceptible and resistant to CTV), in infected and uninfected conditions. Three biological and technical replicates were performed for each experimental condition producing 36 2DE maps that were analyzed by the Progenesis Samespots software (Nonlinear Dynamics, UK). 2DE analysis led to the identification of 125 protein spots differentially accumulated and 97 spots modulated by phosphorylation in the two scion-rootstocks combinations, in infected or uninfected conditions. Differentially accumulated spots were validated by statistical analysis and manually picked up from gels for trypsin digestion and LC/MS/MS analysis, leading to the identification of the protein patterns putatively associated to the resistance/susceptibility of citrus plants to the tristeza disease. The analysis of changes in protein phosphorylation patterns will highlight the possible role of this post-translational modification in the plant response to CTV infection, also providing further insight about scion-rootstock interaction in citrus.

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Poster Communication Abstract – 7.30

A NEW ARCELIN VARIANT RESPONSIBLE FOR RESISTANCE OF COMMON BEAN SEEDS TO BEAN WEEVILS?

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Phaseolus vulgaris, bean weevils, defence proteins, bean resistance, microsatellite markers

In common bean (*Phaseolus vulgaris*) the most abundant seed proteins are the storage protein phaseolin and a family of closely related proteins (lectin related APA proteins, ie Arcelin, Phytohemagglutinin, α-Amylase inhibitor), which are considered to play a role in plant defence. It has been shown that seeds from a restricted number of *P. vulgaris* wild varieties (Arcelin 1 to 7 genotypes) differ in their APA protein composition and the presence of Arcelin has been associated with resistance against bean weevils (the Mexican bean weevil, *Zabrotes subfasciatus* and the bean weevil, *Acanthoscelides obtectus*). So far Arcelin has only been found in wild common bean seeds originating from Mexico. Besides many efforts, a successful breeding of the genetic trait linked to the weevils' resistance into cultivated genotypes has not yet been achieved. This can be explained by the complexity of the APA locus and the lack of knowledge of which and how many APA proteins are involved in the mechanism of resistance.

In the present work, 34 wild bean populations collected in Mexico were used to study the performance of the bean weevil, *Acanthoscelides obtectus* and the Mexican bean weevil, *Zabrotes subfasciatus* and the hymenopteran wasp, *Dinarmus basalis,* an ectoparasitoid of *A. obtectus*. Bean population significantly influenced bean weevil and parasitoid development. From some populations fewer, less heavy beetles and smaller parasitoids emerged and developmental time was significantly longer. Seeds of these populations were subjected to SDS-PAGE and Western blot analyses using APA protein specific antibodies. One of the two most resistant bean populations (QUES) showed a new APA pattern. Biochemical (2D-PAGE coupled to LC-MS/MS analyses) and molecular (cDNA sequence and microsatellite analyses) data confirmed that the QUES genotype contains a new Arcelin variant.

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Poster Communication Abstract – 7.31

IDIAM –PROJECT: IDENTIFICATION OF GENETIC VARIABILITY AND GENES FOR SELECTION OF GENOTYPES TOLERANT TO ROOTWORM DAMAGE IN MAIZE

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Diabrotica virgifera virgifera, Zea mays, larval development, root damage

Recently, *Diabrotica virgifera virgifera*, commonly referred to as the Western corn rootworm (WCR), spread in the Italian areas devoted to corn cultivation. The major damage to maize plants is caused by larval feeding on the roots, which disrupts water and nutrient uptake. In addition, the adult stage can cause silk clipping causing low fertility of the ear with a consequent reduced production.

One of the most promising strategies to deal with infestations of pests is given by the cultivation of resistant varieties. The establishment of plants that can produce their own insecticide is proposed as an effective strategy, safe from an ecological point of view to control the spread of insects. Plants resistant to insects lead to a reduction in production losses, a decrease of the costs of insecticide treatments and improved food safety for animal feed and human.

The most important methods to control this pest are crop rotation, insecticide application, use of resistant maize varieties developed by classical plant breeding methods, or use of transgenic approaches. In particular maize expressing *Bacillus thuringiensis* (bt) toxins or the *caryophyllene synthase* gene which is responsible for (E)- β-Caryophyllene production in maize, have been used as protection from the pests. Recently the use of RNAi might be exploited to control insect pests via *in planta* expression of a dsRNA.

Studies have also revealed a wide range of varieties and genetic variability in inbred lines showing a complex quantitative genetic basis, making difficult and expensive the selection of tolerant genotypes. It is therefore clear that the identification of genes and molecules underlying the defensive response of the plant against the corn rootworm products are of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae.

Our research in the IDIAM project is focused on the identification of genetic variability and genes for the selection of genotypes tolerant to rootworm damage in maize. The main topics involve: i) analysis of genetic variability and identification of hybrids with low-radical damage; ii) identification of genes underlying the plant response to damage inflicted by corn rootworm larvae; iii) validation of candidate genes and polymorphisms mapping.

In our laboratory are in progress experiments to set up an artificial *Diabrotica virgifera virgifera* eggs inoculation method on B73x MO17 genotype, in order to obtain root samples for differential gene expression analysis in comparison with control samples.

In addition, the artificial inoculation method could be useful for a preliminary analysis of genetic variability for rootworm damage tolerance in maize.

Research developed within the project: “IDIAM-Interventions to counterattack the spread and damage from rootworm in maize Italian crop”, funded by the Italian Ministry of Agricultural Food and Forestry Policies".

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Poster Communication Abstract – 7.32

INTERACTIONS BETWEEN BUGS' FEEDING AND WHEAT QUALITY

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Sunn pest, Triticum aestivum, bread-making quality, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, western blot

Several species belonging to the genera *Aelia* (Heteroptera: Pentatomidae) and *Eurygaster* Heteroptera: Scutelleridae), known also as sunn pests or cereal bugs, have long been recognized as detrimental to wheat, *Triticum aestivum* L. In particular, *E. maura* (L.) and *E. austriaca* (Schrank) are considered the most noxious pests in western Europe. Sunn pests feed on wheat, piercing stems, leaves, and ears: with early attacks during crop life cycle, they can cause serious economic losses related to a decrease in grain yield, mainly due to losses in kernel weight, while later attacks during the grain filling period can cause a reduction in baking quality, the maximum damage occurring with sunn pest feeding at the late milk-ripe stage. In the United States also the green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is responsible of reductions in germination, kernel weight, and kernel texture even when milk stage kernels are infested with few individuals. In the present work, six different species of bugs collected in wheat fields in Piedmont were tested for their capacity to induce damage to wheat. In addition, the possibility to develop a rapid diagnostic method to detect residual bug salivary proteins in wheat kernels using biochemical markers was investigated. The study was conducted according to a completely randomized experimental design with six treatments [*E. maura*, *E. austriaca*, *Ae. acuminata* (L.), *N. viridula*, *Carpocoris* sp., and control without insects], two wheat cultivars (Bologna and Aubusson) and five replications, in two growing seasons (2008-09 and 2009-10). At maturity, the following analyses were performed on the harvested material: thousand kernel weight, damaged kernels (i.e., the percentage of seeds showing, at visual inspection, the typical discoloured area around the point of bug stylet penetration), protein content, hardness, sodium dodecyl sulfate sedimentation volume, specific sedimentation volume. For the biochemical analyses, single seeds damaged by each bug were crushed and incubated with extraction buffer (66 mM Tris HCl pH 6.8, 2.2% SDS, 10% glycerol, 5% 2-mercaptoethanol) for 1h at room temperature and 5 min at 95°C. The extract was separated by SDS-PAGE (12.5%) and then blotted on PVDF transfer membrane. After blocking in skim milk (diluted in PBS/Tween 0.2%), the membrane was incubated with primary antiserum against *E. maura* salivary glands diluted 1:1000 in skim milk and incubated with secondary antiserum diluted 1:5000 for 2.5h. The bug proteins were detected with diaminobenzidine tablets. In both growing seasons, the heaviest quality depletion was caused by *E. maura* whereas no detrimental effects were observed for *N. viridula*. The western blotting analysis was effective in the detection of damage from *E. maura* and can be the basis for the development of a rapid diagnostic method to identify damaged wheat stocks.

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Poster Communication Abstract – 7.33

CHITOSAN-INDUCED CELL DEATH IN SYCAMORE CULTURED CELLS

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Chitosan, programmed cell death, oxidative stress, Acer pseudoplatanus

Programmed cell death (PCD) plays a pivotal role in many developmental plant processes and in defense mechanisms against biotic and abiotic stresses. At least three different forms of PCD have been reported in plants: a "nuclear" (apoptotic-like) form, a "chloroplastic" form and a "vacuolar" form[1]. In plant cell cultures different stress conditions induce cell death that only in a fraction of the dead cells presents the typical morphological hallmarks of apoptosis, i.e. cell shrinkage, chromatin condensation, DNA fragmentation[2,3]. Recently, our attention has been focused on chitosan (CHT). CHT is a natural, non-toxic and inexpensive compound obtained by partial alkaline deacetylation of chitin, the main component of the exoskeleton of crustaceans and other arthropods as well as of the cell wall of many fungi[4]. Although the exact mode of action of CHT is still unknown, in agriculture it has been shown to be a versatile compound that controls numerous pre and postharvest diseases of various horticultural commodities[5]. In sycamore (*Acer pseudoplatanus* L.) cultured cells CHT rapidly induces a set of defense/stress responses that include accumulation of dead cells and of cells with fragmented DNA accompanied by release of cytochrome *c* from the mitochondrion[6]. In this work we further investigated the cell death process induced by CHT. In particular, we tested the capability of CHT to induce oxidative stress (superoxide anion and malondialdehyde production) and to increase the activity of caspase3-like proteases.

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Poster Communication Abstract – 7.34

ROLE OF MAP KINASES IN THE ACTIVATION OF *ARABIDOPSIS* DEFENSE RESPONSES TRIGGERED BY ELICITORS

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Elicitors, MAP kinases, defense responses, Botrytis cinerea, Arabidopsis thaliana

Mitogen-activated protein kinases (MAPKs) are fundamental components of the plant innate immune system. MPK3 and MPK6 are Arabidopsis (*Arabidopsis thaliana*) MAPKs activated by pathogens and elicitors such as oligogalacturonides (OGs), which function as damage-associated molecular patterns (DAMPs), and flg22, a well-known microbe-associated molecular pattern (MAMP). However, the specific contribution of different MAPKs to the regulation of elicitor-induced defense responses is not completely defined. We have investigated the roles played by MPK3 and MPK6 in elicitor-induced resistance against the fungal pathogen *Botrytis cinerea*. Analysis of single *mapk* mutants revealed that lack of MPK3 increases basal susceptibility to the fungus but does not significantly affect elicitor-induced resistance. Instead, lack of MPK6 has no effect on basal resistance but completely suppresses elicitor-induced resistance to *B. cinerea*. Interestingly, MPK3 and MPK6 are not required for the oxidative burst induced by elicitors, indicating that this response is not regulated by MAPKs. Overexpression of AP2C1, a MAPK phosphatase, leads to impaired elicitor-induced phosphorylation of both MPK3 and MPK6, and a to a phenotype that recapitulates that of the single *mapk* mutants. These data indicate that OG- and flg22-induced defense responses effective against *B. cinerea* are mainly dependent on MAPKs, with a greater contribution of MPK6.

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Poster Communication Abstract – 7.35

A MUTATION IN THE FZL GENE OF *ARABIDOPSIS* CAUSES A LESION MIMIC PHENOTYPE

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Lesion Mimic Mutant (LMM), Hypersensitive Response (HR), chloroplast, Reactive Oxygen Species (ROS), signalling pathway

We report on the characterization of an Arabidopsis mutant showing reduction in size, compared with the wild type, and presence of chlorotic lesions on rosette leaves. These traits are characteristic of a group of mutants displaying spontaneous HR cell death, the lesion mimic mutants (LMM). They are useful tools to unravel the regulatory mechanisms of this form of PCD (Programmed Cell Death), in which reactive oxygen species (ROS), nitric oxide (NO) salicylic acid (SA), jasmonic acid (JA) and ethylene are known to play a central role as signalling molecules. Histochemical and expression analysis showed that lesions formation on mutant rosette leaves correlates with the presence of specific biochemical and molecular markers usually associated with the HR cell death program.

Analysis of double mutants, obtained by crossing our mutant with mutants affected in HR signalling pathways, showed that the mutant phenotype was dependent on SA but independent from JA and ethylene signalling.

Positional cloning and sequence analysis indicated that the mutation was in the FZL gene, encoding for a membrane-remodelling GTPase with a unique role in the determination of thylakoid and chloroplast morphology, and histological analysis confirmed the presence in mutant leaves of chloroplasts with altered shape, size and number in comparison with the wild type.

Chloroplasts are the major ROS source in plant cells and even if the signalling role played by ROS in HR progression has long been recognised many aspect of ROS function remain obscure, thus the characterisation of this LM mutant will help in understanding the role played by chloroplast-derived ROS in HR signalling pathway.

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Poster Communication Abstract – 7.36

AN INTERACTION NETWORK MEDIATED BY THE OLIGOGALACTURONIDE RECEPTOR WAK1 REGULATES ARABIDOPSIS LOCAL RESPONSE TO WOUNDING

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Damage-associated molecular patterns (DAMPs), wall associated kinase 1, glycine rich-protein 3, kinase-associated protein phosphatase, *immunity*

An efficient sensing of danger and a rapid activation of the immune system are crucial for the survival of plants. Conserved pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and endogenous molecular patterns, which are present only when the tissue is infected or damaged (damage-associated molecular patterns or DAMPs), can act as danger signals and activate the plant immune response. These molecules are recognized by surface receptors that are indicated as pattern recognition receptors (PRRs). Oligogalacturonides (OGs), released from the plant cell wall, are well-known DAMPs that have long been considered as signals in the wound response. Since they are negatively charged and have a limited mobility, their activity as a wound signal is likely to be restricted to the areas that are close to the damaged or wounded tissue. Recently, through a chimeric receptor approach, we have demonstrated that the Arabidopsis Wall-Associated Kinase 1 (WAK1) is a receptor of OGs. WAK1 has been described to form a complex with an apoplastic glycine-rich protein (GRP3) and a cytoplasmatic kinase-associated protein phosphatase (KAPP). Using Arabidopsis plants overexpressing WAK1 and *grp3* and *kapp* null insertional mutants, we have investigated the role of the three proteins in the perception/transduction of the OG signal and in the regulation of the wound response.

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Poster Communication Abstract – 7.37

A PROTEOMIC APPROACH TO STUDY THE AUTOREGULATION OF NODULATION IN *MEDICAGO TRUNCATULA*

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Autoregulation of nodulation, Sinorhizobium meliloti, two-dimensional gel electrophoresis, mass spectrometry

Plant belonging to *Leguminosae* family can establish a symbiotic relationship with nitrogen-fixing bacteria, termed rhizobia, that lead to the formation of a new organ, the root nodule. This organogenetic pathway starts as a consequence of a molecular cross-talk between plants and bacteria. Plants release flavonoids in the rhizosphere, which induce the rhizobia to produce Nod factors (NFs). NFs perceived by the host plant trigger at least three signalling pathway that control nodule formation and nodule number. Two of these pathways are root-specific whilst the third is systemic. The systemic pathway, also termed Autoregulation Of Nodulation (AON), is a mechanism by which a plant controls the number of nodules inhibiting further nodule formation after the first few nodules have been formed, as a consequence of a molecular communication between root and shoot. Several researches on long-distance signaling in nodulation have identified a number of genes involved in autoregulation; however this process is still under investigation. With the aim of gaining a deeper insight in the signalling cascade of AON, we compared the root and shoot proteomes of *Medicago truncatula* plants inoculated with the symbiotic rhizobium, *Sinorhizobium meliloti,* with those of uninoculated plants. We utilized the technique of two-dimensional polyacrylamide gel electrophoresis for the identification of differentially expressed proteins. Twenty plants per biological sample were used, and 5 technical replicates were performed for each biological sample. We detected 19 differentially expressed spots comparing root samples from rhizobia-infected and uninfected plants and 16 differentially expressed proteins for the aerial parts. The differentially accumulated proteins were isolated and subsequently identified by mass spectrometry.

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Poster Communication Abstract – 7.38

ANALYSIS OF S-NITROSYLATED PROTEINS *IN ARABIDOPSIS THALIANA* LEAVES SUBJECTED TO OXIDATIVE STRESS OR WOUNDING

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Arabidopsis thaliana, hypersensitive response, nitric oxide, S-nitrosylation

Oxidative burst, is defined as a rapid, transient production of high levels of reactive oxygen species (ROS) in response to different external stimuli. Plants produce ROS in response to biotic and abiotic stress, in chloroplasts, mitochondria and peroxisomes. Recent data indicates that oxidative stress occurs when ROS are produced more than they are metabolised in the cell, a condition common in plants exposed to various kinds of stress (heat, water, light, ozone, heavy metals, pathogens or elicitors). ROS not only cause oxidative damage, but are also involved in signaling. Controlling the concentration of ROS is important for the survival of the plant itself, and Nitric oxide (NO) seems to have a pivotal role in protecting cells from oxidative damage. NO is involved both in the control of the redox status of the cell, regulating the concentration of ROS, as well as in response signaling, to modulate cell physiological and pathophysiological processes. NO signaling involves direct post-translational modifications (PTM) of protein targets, which results in the formation of nitrosothiol adducts from the thiol group of target cysteines. In this study the effect on protein S-nitrosylation pattern in *Arabidopsis* leaves treated with Paraquat (methyl viologen) or subjected to mechanical injury (wounding) has been investigated. Paraquat is a redox-reactive compound that generates superoxide anions in chloroplast and is widely used to mimic oxidative stress in plants, whereas mechanical injury is a component of biotic stress induced by insect attack. The involvement of protein nitrosylation in the plant response to wounding is poorly investigated. Proteins have been extracted from control and treated leaves, subjected to biotin switch, separated by two-dimensional gel electrophoresis (2-DE), blotted and decorated with an anti-biotin probe, and identified by mass spectrometry.

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Poster Communication Abstract – 7.39

METABOLIC AND MOLECULAR RESPONSES OF ITALIAN RICE CULTIVARS TO BTH TREATMENT

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Italian rice cultivars, BTH, Reactive Oxygen Species, Transcription factors, metabolomics

Benzothiadiazole (BTH), a functional analog of Salicylic Acid (SA), is one of the so-called plant activators that protect various plants from infectious diseases. BTH treatments have been used by several authors to study disease plant response, since it is a potent inducer of both SAR and PR genes. It has been proposed that in rice the SA/BTH-signalling pathway branches in at least 2 subpathways downstream of SA (OsWRKY45-dependent and OsNPR1-dependent). Both OsWRKY45 and OsNPR1 play an essential role in BTH-induced blast resistance. Most of the current Italian varieties have intermediate to low resistance to blast in field conditions and the development of new rice varieties with a higher resistance to blast disease is of great interest, but precise molecular information on resistance genes present in Italian commercial varieties is still missing.

In this work, we provide a characterization of 10 Italian rice cultivars, selected on the basis of their resistance or susceptibility to blast disease, for metabolic content and gene expression after BTH treatment.

We performed a metabolic profiling of mock and BTH-treated plants through ^{1}H-NMR. Interestingly, statistical analysis of the obtained spectra showed a distribution of resistant cultivars separated from the susceptible ones. Resistant varieties were characterized by a higher content of sucrose, whereas susceptible ones presented a higher content of fructose, suggesting that a difference in metabolic response occurs between resistant and susceptible cultivars.

A qRT-PCR analysis showed that, after 24 hours from BTH treatment, *OsWRKY45* was induced in all the cultivars, whereas *OsNPR1* was induced only in three blast resistant cultivars. Besides, the transcription level of two *myb* and one *WRKY* genes increased in most of the resistant cultivars and decreased or did not change in the susceptible ones, thus suggesting a positive correlation between their expression level and the resistance phenotype of the rice cultivars. Moreover, we analysed some genes involved in the ROS signalling pathway (SOD and CAT encoding genes), belonging to the OsNPR1 pathway. Whereas the *CAT* gene was not induced in any of the analysed cultivars, the *SOD* gene expression was up-regulated after BTH treatment in two of the resistant cultivars that showed an induction of *OsNPR1*, suggesting the occurrence of different levels of H_2O_2 among cultivars.

These preliminary data suggest that in Italian cultivars the resistance to blast could be due to the activation of different pathways. Further analysis of the expression of TF genes and of other genes involved in ROS scavenging and signalling are needed to understand the role of these branches of the SA/BTH-signalling pathway in Italian cultivars.

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Poster Communication Abstract – 7.40

THE SPECIFICITY OF TYROSINE NITRATION QUESTIONS THE REDUNDANCY OF AtMKK4 AND AtMKK5 DURING PLANT DEFENSE RESPONSES

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Hypersensitive response, peroxynitrite, MAPK, tyrosine nitration, Arabidopsis

Peroxynitrite is a reactive nitrogen species formed from the reaction between NO and O_2^-, both produced concomitantly during the hypersensitive response (HR) in plants. Accordingly, using the specific dye HKGreen-2 in a photometric assay, we recently demonstrated that infection of *Arabidopsis thaliana* plants with an avirulent strain of *Pseudomonas syringae* (Pst AvrB) induces peroxynitrite accumulation[1] with a timing that correlates with an increase in tyrosine-nitrated proteins[2].

In plants, peroxynitrite is not involved in NO-mediated cell death and its physiological function is poorly understood. However, it is emerging as a potential signaling molecule during the induction of defense responses against pathogens and this could be mediated by the selective nitration of tyrosine residues in a small number of proteins.

In an attempt to identify specific targets of Tyr-nitration displaying signaling functions during the HR, we focused our interest on MAPK cascades, a complex network of phosphorylation events involved in plant defense responses and known to be regulated by Tyr-nitration in animals. In this context, we demonstrated that AtMKK4 is specifically nitrated on two Tyr residues, namely Y76 and Y94, leading to an inhibition of its activity. According to the location of both Tyr close and inside the ATP-binding site, AtMKK4 nitration prevents the binding of ATP to the protein. *In vivo* peroxynitrite-treatment strongly delays the hypersensitive cell death induced in tobacco plants by the active AtMKK4. Interestingly, despite 78% of sequence homology with AtMKK4, AtMKK5 is not nitrated by peroxynitrite and its activity is not modulated by such a treatment. This raises the question of AtMKK4 and AtMKK5 redundancy in mediating defense signal in plants and highlights the specificity of Tyr-nitration for signaling modulation.

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Poster Communication Abstract – 7.41

cGMP SIGNALING IN PLANT-PATHOGEN INTERACTIONS

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cGMP, plant signaling, nitric oxide, biotic stress, Arabidopsis thaliana

Cyclic GMP (3', 5'-cyclic guanyl monophosphate) is known as a key second messenger which regulates a wide variety of cellular responses in several living organisms such as bacteria, fungi, and animals. Its presence and role in plants has been long debated but in the last decade a growing number of reports have described both the occurrence and function of cGMP in higher plants. These reports, mainly based on pharmacological approaches, clearly highlighted its involvement in mediating nitric oxide and other hormones plant signaling in several important physiological processes like biotic and a-biotic stress responses, but detailed knowledge about the mechanisms is still very limited. We are interested in clarifying the genetic basis of cGMP signaling in plant especially during plant- pathogen interaction.

One of the main limitations in cGMP studies in plants is due to the difficulties related to measurement of its content in plant tissues. All available detection systems are expensive and allow processing only limited number of samples. In this project therefore we started applying a new Alpha-Screen (Perkin Elmer) FRET based immunoassay for high throughput cGMP measurement which was never applied in plants. Reproducibility of the assay as well as error introduced by different cGMP extraction procedures from plant tissues have been carefully evaluated and quantified in order to establish a protocol for measurement of cGMP content in a high number of plant samples using this system. We are currently employing this assay in our lab in order to analyze the dynamic of changes in cGMP content in plant tissues upon nitric oxide or pathogen treatment.

cGMP content in plants is regulated by the activities of the enzymes guanylate cyclase (GCs), that specifically synthesize cGMP from GTP, and phosphodiesterase (PDE), that hydrolyze cGMP to GMP. Unfortunately the specific enzymes operating this turnover in *Arabidopsis thaliana* have not yet been clearly identified limiting our possibility to alter through a classical genetic approach the cGMP content in plant. As an alternative we expressed the heterologous mammalian genes for a soluble guanylate cyclase and a phosphodiesterase in *Arabidopsis thaliana* plants. Transgenic lines constitutively expressing a functional soluble mammalian guanylate cyclase (alpha and beta sub-units) and presenting a cGMP content 10 to 50 time-fold higher than wild type plants have been obtained. Moreover, transgenic *Arabidopsis thaliana* plants expressing either a constitutive or inducible bovine phosphodiesterase were also obtained. We are currently characterizing these plants both morphologically and in terms of response to biotic and a-biotic stresses with the final aim of better clarifying thorough this approach the role of cGMP signaling in plant-pathogen interaction.

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Poster Communication Abstract – 7.42

ROLE OF *KNOX1* TRANSCRIPTION FACTORS OF *MEDICAGO TRUNCATULA* IN ROOT NODULE FORMATION AND HORMONE SIGNAL TRANSDUCTION

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Transcription factors, M. truncatula, cytokinins, symbiotic nodule formation

Legumes represent the second family of crops after cereals in terms of cultivated surface and constitute a crucial source of proteins for food and feed to use in sustainable agriculture. Plant root architecture is determined by the number and distribution of lateral roots, which is adapted to the soil environment. Legumes, additionally, are able to develop symbiotic interactions with bacteria of the Rhizobia family to form another secondary root organ, the nitrogen-fixing nodule. Recently, a crucial role of cytokinin signalling mediated by the MtCRE1 receptor was revealed in *M. truncatula* nodule and lateral root formation. Indeed, the MtCRE1/LHK1 cytokinin receptor is crucial for the initiation of symbiotic nodule organogenesis in *L. japonicus* and *M. truncatula* models.

In seed plants, members of the KNOX1 homeodomain transcription factor family control multiple hormonal pathways involved in the determination of cell fate and organ formation, including cytokinin biosynthesis. In the *M. truncatula* model legume, we identified eight novel *KNOX* genes (*MtKNOX*s). Gene expression studies revealed organ-specificity, possible cytokinin-dependent transcriptional activation of two *MtKNOX*s and expression of seven *MtKNOXs* in roots. Moreover, three *KNOX* genes have been found to be modulated in response to *Rhizobium* infection in transcriptomic studies.

Within the framework of the bilateral Scientific Cooperation between CNR (IT) and CNRS (France), we are investigating the role of *KNOX* genes in rhizobia-induced nodule organogenesis as well as in the control of hormone homeostasis in *M. truncatula* roots.

Real Time qPCR experiments were carried out to analyze *MtKNOXs* early response to different plant hormones related to root and nodule development such as cytokinins, auxin, ethylene, abscissic acid, gibberellins and brassinosteroids. *In situ* hybridizations have been carried out to localize the message RNAs of the MtKNOXs of interest during nodule formation. Constructs for constitutive (i.e. 35S:CaMV) overexpression as well as for RNAi-mediated silencing of MtKNOXs have been obtained. One *mtknox3* knock-out heterozygous line was isolated in collaboration with The Samuel Roberts Noble Foundation (US) and its characterization is in progress to assess the role of this gene in nodulation processes.