ment running from conidia germination to coleoptile penetration. We compared two wheat near isogenic lines (NILs) for the *Pch1* gene upon eyespot infection. The expression of three candidate genes, PR-16, putatively involved in the cell wall reinforcement; lipoxygenase (LOX), involved in the oxidative burst; and PR-5, involved in the antifungal reaction, were analysed by real-time PCR, while H_2O_2 production and callose deposition were microscopically observed by using DAB and aniline blue staining respectively. In both NILs, the PR-5 and PR-16 genes were up-regulated, first slightly during appressoria formation, then more strongly from penetration of eyespot in coleoptile. Callose deposition and H_2O_2 production were observed locally under appressoria. The expression of the LOX gene remained stable and no hypersensitive reaction was observed. Thus, up to the penetration of the pathogen in the coleoptile, cell wall reinforcement and antifungal reaction seems to be activated as general defence reactions because no clear difference could be established between the resistant and the susceptible NILs. Further work is needed to determine if these two defence reactions are involved in the resistance response after the penetration of the coleoptile. The validation of these results is in progress. Other candidates genes are also being tested.

PS 14-675

A NOVEL WRKY-FACTOR IS INVOLVED IN INDUCTION OF PR-1A GENE EXPRESSION BY TOBACCO MOSAIC VIRUS INFECTION

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Infection with tobacco mosaic virus (TMV) of tobacco plants with the N gene results in salicylic acid (SA)-mediated expression of the PR-1a gene and other defense-related genes. One-hybrid screens identified a novel tobacco WRKY-transcription factor (NtWRKY12) with specific binding sites at positions -859 (Wl-box) and -564 (Wr-box) in the PR-1a promoter. The binding sequence of this factor (TTTTCCAC) deviated significantly from the consensus WRKY-factor binding site ([T]TGAC[C/T]). The Wr-box is in close proximity to binding sites in the PR-1a promoter for TGA (position -582) and Myb1 (position -520) transcription factors. Expression of the NtWRKY12 gene was strongly enhanced by TMV-infection or application of exogenous SA. PR-1a promoter / GUS fusions with mutations in the promoter sequence were inserted in the genome of tobacco plants. GUS-expression by the wild-type construct was strongly increased by TMV-infection of the plants or application of SA. Mutations in NtWRKY12 binding sites Wl or Wr, or in the TGA binding site each reduced GUS expression mediated by TMV and SA. A double mutation affecting both the Wl and Wr sites further reduced GUS expression, whereas a deletion removing the Wr, TGA and Myb1 sites abolished induction of GUS-expression by TMV or SA. The results indicate that NtWRKY12 acts synergistically with TGA and/or Myb1 in the induction of PR-1a gene expression. Agro-infiltration of tobacco with Agrobacterium tumefaciens elicited expression of the endogenous PR-1a gene and expression of a PR-1a promoter / GUS-fusion in the T-DNA vector. Mutations in the Wl and Wr sites in the PR-1a / GUS fusion revealed that NtWRKY12 is also involved in expression of the PR-1a gene induced by bacterial elicitors.

PS 14-676

TRANSCRIPTOME ANALYSIS OF ARBUSCULAR MYCORRHIZAL ROOTS TARGETED TO THE PRE-PENETRATION APPARATUS

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Limited information on changes in the gene expression profile during early interaction with arbuscular mycorrhizal (AM) fungi is available, mainly due to the fact that infection is not synchronized and plant markers for early stages have not been identified. We recently described the pre-penetration apparatus (PPA), organised in epidermal cells upon appressorium contact and responsible for the assembly of a trans-cellular tunnel to host the fungus (Genre *et al*., 2005). Here we used the PPA as a marker for cell responsiveness to fungal contact to investigate gene expression at this stage minimizing transcript dilution. PPAs were identified by confocal microscopy in root organ cultures of *M. truncatula* expressing GFP:HDEL, colonized by the AM fungus *Gigaspora margarita*. A PPA-targeted suppressive-subtractive cDNA library was built by subtracting cDNAs from root segments producing PPAs with cDNAs from comparable control root segments. The cDNAs obtained were cloned and sequenced, and led to the identification of 107 putative interaction-specific genes. The expression of a subset of 15 genes, selected by reverse Northern dot blot screening of the cDNA fragments expressed in the PPA phase, and five additional genes was analyzed by real-time RT PCR and compared with an infection stage 48 hours after PPA formation. Comparison of the expression profile of wild-type and the mycorrhiza-defective *dmi3-1* mutant of *M. truncatula* after inoculation with *G. margarita* revealed that an expansin-like gene, expressed in wild type (WT) epidermis during PPA development, can be regarded as a marker for early AM interaction, whereas a putative Avr9/Cf9 rapidly elicited gene, is up-regulated in this mutant, suggesting novel regulatory roles for the DMI3 protein in the early AM interaction.

PS 14-677

MODULATION OF GLUTAMINE SYNTHETASE, ASPARAGINE SYNTHETASE AND SUCROSE SYNTHASE EXPRESSION AND ACTIVITY BY NO IN *MEDICAGO TRUNCATULA – SINORHIZOBIUM MELILOTI* FUNCTIONAL NODULES

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In legume-rhizobium symbiosis, the set up of nitrogen fixation processes is characterized by a dramatic increase in energy and carbon metabolism. Sucrose, issued from photosynthesis, is actively transported into fixating nodule cells, metabolized by glycolysis, and directed, for one part, to the respiration and, for another part, to the anaplerotic pathway. Anaplerotic pathway