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DEVELOPMENT OF A SOYBEAN GENE EXPRESSION DATABASE TO CROSS COMPARE MICROARRAY EXPERIMENTS

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Microarrays are a revolutionary tool to estimate expression levels of genes within an organism. In a fairly quick and simple experiment, one can monitor expression of tens of thousands of genes. The relative ease of use and availability of array platforms for many commonly researched plants, is generating massive amounts of valuable expression data. To assist with the analysis of soybean expression experiments, we used PERL/CGI, C and an ORACLE database management system to develop a web-accessible, public Soybean Gene Expression Database (SGED) with several useful features. SGED contains searchable annotations from NCBI, TIGR and TAIR for all genes represented on soybean cDNA from the University of Illinois as well as from Affymetrix soybean expression chips. A BLAST server will search and parse top hits for nearest match within soybean ESTs and TCs that are represented on soybean cDNA slides. Data querying and clustering tools can analyze expression data with p-values and fold changes from published projects from the Clough lab (will add outside experiments in future). All output files and tables are down-loadable. SGED is housed at the National Center for Supercomputing Applications at the University of Illinois (http://aragon.ncsa.uiuc.edu/soybean-microbe). We plan to expand the BLAST feature to include sequence databases for other plant expression arrays such as Medicago, Lotus, and *Arabidopsis* to allow identification of best matches across different plant arrays and cross comparison to soybean projects. Examples will be shown cross comparing expression data from soybean treated with *Pseudomonas syringae*, *Sclerotinia sclerotiorum*, *Fusarium solani*, *Bradyrhizobium japonicum*, and herbicide.

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INVESTIGATING THE SYSTEMIC RESPONSE OF BARLEY TO BIOLOGICAL INDUCERS OF SAR: A ROLE FOR DIR1

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Systemic acquired resistance (SAR) occurs following a local interaction with a necrotising pathogen and is associated with systemic PR gene expression and a rapid HR response to previously virulent pathogens. The SAR response is well characterised in several dicot species, but in the cereals evidence remains inconclusive. This project aims to examine systemic transcriptional and resistance responses in barley to biological inducers of SAR, facilitated by the development of a SAR screen for barley comparable to that available for *Arabidopsis*. The identification in *Arabidopsis* of *DIR1* (*Defective in Induced Resistance 1*), a putative non-specific lipid transfer protein which acts specifically in the production or transmission of the mobile SAR signal from biologically induced leaves, is an important insight that makes the further investigation of biologically induced SAR in cereals possible. Bioinformatic analyses have identified candidate *DIR1* homologues in rice sharing 40-50% sequence identity with *DIR1*. *Arabidopsis dir1-1* mutant lines heterologously expressing these genes are being produced and may allow us to determine the functionality of these candidates. In addition, there is evidence to suggest that the expression of SAR in *Arabidopsis* imposes yield costs. This leads us to speculate that in the cereals, SAR specific genes such as *DIR1* may have been selected against through breeding for high yield. To investigate this, transgenic barley lines overexpressing the *Arabidopsis DIR1* gene have been generated by agrobacterium-mediated transformation. Both these transgenic lines and a range of barley landraces will now be screened for any variation in systemic response in comparison to cultivated barley.

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SPANISH SITUATION OF VIRAL DISEASES THAT CAUSED NECROSIS IN TOMATO

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Spain is the first fresh-market tomato exporting country worldwide. The particular meteorological conditions of the country let the producers perform intensive and consecutive growing seasons along the year, both in greenhouse or field-grown. The reiteration of the crop entails the appearance of a high number of different diseases of viral etiology. Frequently, these diseases are caused by more than one viral agent in a mixed infection. The term "necrosis" refers to a high diversity of different necrotic symptoms, caused by viral infections. In Spain, different necrosis have been detected associated to *Potato virus Y* (PVY), *Tomato spotted wilt virus* (TSWV), *Parietaria mottle virus* (PMoV) and *Tomato torrado virus* (ToTV). This study is a compilation of the variability of the necrotic symptoms shown by the plants, the different agents implicated in the symptom, the geographical location and extension of the diseases.

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A PATHOGENESIS RELATED PROTEIN, TCPR10 FROM *THEOBROMA COCOA* WITH ANTIFUNGAL ACTIVITY AGAINST *MONILIOPHTHORA PERNICIOSA*

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In 1989, the basidiomycete *Moniliophthora perniciosa* (*M.p.*), the causal agent of witches' broom disease, was introduced in the South of Bahia (Brazil). Consequently, the cacao bean production drastically decreased, and important social and economical problems occurred in the region. Because of the complex problem due to this pathogen, genomic and post-ge-

nomic studies of the *Theobroma cacao-M.p.* interaction were developed, and expressed genes (ESTs) were identified. A *T. cacao* clone encoding a pathogenesis related-protein (TcPR10) was isolated from cacao-*M.p.* interaction cDNA libraries. This gene was cloned in pET28a, the recombinant protein obtained and purified. The recombinant protein has a molecular mass of 18 kDA. The susceptibility to the recombinant TcPR10 (toxicological evaluation) of the dikaryotic and monokaryotic hyphaea of *M.p.* was tested on solid medium containing glucose and glycerol as carbon source, respectively. Amounts of 0, 0.75, 1.5, 2, 4 and 8 μg of TcPR10 were added to 1 ml of *M.p.* cell suspension. It was observed that the surviving of *M.p.* was inversely proportional to the amount of TcPR10 added; the highest amount of protein allowing 15% of surviving. Ribonuclease activity of TcPR10 was tested by incubating at 28°C 1μg of protein with 500 μg of *M.p.* RNA. Different incubation times were tested and the ribonuclease activity was visualized on 1% agarose gel. TcPR10 presented an activity dose x response x time-dependent; when incubation time increased twice, RNA degradation also increased twice. Degradation of DNA wasn't observed. These results showed that the produced PR10 from *T. cacao* has a ribonuclease activity and presents an antifungal activity against *M.p.*, showing that it may be an important factor for the plant-pathogen interaction.

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TRANSCRIPTION FACTORS ORCHESTRATING THE *ARABIDOPSIS* RESPONSE TO CELL WALL DERIVED PAMPS

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An early response to pathogen attack involves recognition of so-called pathogen-associated molecular patterns (PAMPs), molecules that characterize pathogens or infected tissue. PAMPs include pathogen-encoded molecules such as flagellin and host-derived molecules such as oligogalacturonides (OGs), pectic cell wall fragments released by pathogen-encoded polygalacturonases. In an effort to identify molecular markers for the plant response to OGs, we have carried out genome-wide transcriptional gene analysis using *Arabidopsis* Affymetrix chips. A number of transcription factors belonging to families involved in pathogen-responses have been identified as markers for the OGs response. In particular, WRKY11, WRKY15, WRKY33, WRKY40 and MYB51 were shown to have an early, SA/ET/JA-independent and transient induction upon treatment of plant with OGs. In order to analysis the contribution of these genes in the response to pathogens and PAMPs, T-DNA interrupted lines were identified. After identifying T-DNA homozygous plants for WRKY11, WRKY33, WRKY40 and MYB51, infection experiments were carried out to establish whether they are compromised in responding to pathogen or not. Plants lacking a functional WRKY33 gene are more susceptible to *Botrytis cinerea* than wt plants, whereas plants lacking a functional WRKY11 or MYB51 one are more susceptible to *Erysiphe orontii*. Transcription profiling of these four mutants indicates that there is extensive gene cross-regulation among them, suggesting that a complex net of positive and negative regulators is essential to fine-tune the response to PAMPs.

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CORRELATING GLOBAL GENE EXPRESSION CHANGES WITH THE DEVELOPMENT OF VIRUS SYMPTOMS

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Since the advent of microarray technology, experiments have been conducted in *Arabidopsis*, *Nicotiana*, and rice to study the impact of virus infection on host gene expression. Collectively these studies have identified thousands of genes that respond to virus infection, but understanding how these genes relate to pathogenesis and symptom development remains problematic. Here, we compared the gene expression profiles between viruses that cause distinct symptoms in the same host plant in attempt to correlate gene expression patterns with the display of specific symptoms and cytopathic effects. Microarray expression profiling experiments were performed on *Nicotiana benthamiana* leaves infected with one of three fruit tree viruses; *Plum pox potyvirus*, *Tomato ringspot nepovirus*, and *Prunus necrotic ringspot ilarvirus*. The results suggest that chlorotic phenotypes are associated with global repression of chloroplast associated genes. In addition, increased ribosome number and nucleoprotein synthesis caused by PPV was associated with increased expression of cytosolic ribosomal genes. Necrosis and wilting caused by ToRSV was linked to the induction of cell death and abiotic stress responses. Taken together, these data reveal that comparative expression profiling of distinct disease states can reveal insight into the relationships between host gene expression and symptoms.

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REGULATION OF *LOTUS JAPONICUS* ROOT SYMBIOSIS BY SINA E3 LIGASE PROTEINS.

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Lotus japonicus root nodulation and arbuscular mycorrhiza symbioses require at least 7 common proteins in the signaling pathway (Kistner *et al.*, 2005), one of which is the LRR- receptor-like kinase SYMRK (Stracke *et al.*, 2002, Yoshida *et al.*, 2005). A yeast-2-hybrid screening using the SYMRK kinase domain as bait was performed to identify interacting proteins of SYMRK. Four SINA proteins were shown to interact in yeast, 3 of which showed *in vitro* E3 activity. The fourth SINA protein interacts solely with the active SYMRK kinase and lacks enzymatic activity. SINA proteins act as dimers and have been shown to regulate lateral root formation (Xie *et al.*, 2002) and to be involved in rhizobial infection during *Medicago truncatula* nodulation (Den Herder *et al.*, in preparation). E3 ligase control of membrane proteins has been demonstrated in several pathways (d'Azzo *et al.*, 2005; Wang *et al.*, 2006). Transgenic plants ectopically expressing the *L. japonicus* SINA genes or the dominant negative mutants and several TILLING mutant lines were subjected to functional analysis. Protein localization and split-YFP experiments are performed to analyse *in vivo* complex formation. In combination with biochemical characterisation, this will allow us to unravel SYMRK regulation and provide new insights into SINA symbiosis-specific functions.