incidence or whole treatment average ELISA values nor did it result in higher squash fruit yields compared with the non-treated control in spring or fall trials.

Identification of plant defense signaling components induced in response to fungal elicitor EIX

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Plant microbe interactions involve a large number of global regulatory systems, which are essential for plants to protect themselves against pathogen attack. An ethylene-inducing xylanase of Trichoderma viride (tvEIX) is a potent elicitor of plant defense responses, like hypersensitive response (HR) in specific cultivars of tobacco and tomato. The molecular genetic analysis of the signal transduction pathway that modulates HR is an important step in understanding the plant defense responses. In this study, we attempted to identify signaling components involved in tvEIX and its cognate *R* gene of tomato, *LeEix2*, in inducing HR in *Nicotiana benthamiana*. We used virus-induced gene silencing (VIGS)-based fast-forward genetics approach to screen about 3,300 cDNA clones from a mixed elicitor treated and normalized *N. benthamiana* cDNA library and documented the responses of these plants to co-expression of tvEIX and LeEix2. *Agrobacterium*-mediated transient co-expression of LeEix2 and tvEIX in the leaves of *N. benthamiana* plants showed HR whereas several gene-silenced plants showed differential HR symptoms like delayed HR, early HR, and no HR. To identify EIX specific responses, we simultaneously observed HR mediated by two other gene-for-gene interactions Pto-avrPto and Cf9-avr9 as positive controls. Based on this screening approach we identified about 10 candidate genes that when silenced clearly compromised LeEix2-tvEIX mediated HR. In order to achieve uniform HR which might facilitate better screening and characterization of identified signaling genes, we also developed *N. benthamiana* transgenic plants stably expressing *LeEix2* gene.

Identification of genes involved in nonhost disease resistance in *Nicotiana benthamiana* and *Arabidopsis thaliana*

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Plants have evolved different defense mechanisms, both active and passive, to combat pathogen attack. The well studied *R*-gene mediated defense is often very specific to a particular plant genotype or cultivar and a particular race of a pathogen. In contrast, nonhost resistance can act against all races of a particular pathogen and can occur in all cultivars of a host plant species. In this study we attempted to dissect the signaling pathway during nonhost pathogen and plant interaction. We used virus-induced gene silencing (VIGS) based fast-forward genetics approach and screened a cDNA library to identify the candidate genes possibly involved in imparting nonhost resistance in *N. benthamiana*. GFPuv labeled nonhost pathogenic bacterial growth patterns was studied in these silenced plants. We documented the differences in nonhost bacterial growth between the mock and the gene silenced plants. We have identified several candidate genes that when silenced compromises nonhost resistance in *N. benthamiana*. We are now characterizing the function of these genes and their relevance to plant defense using gene overexpression, RNAi and Arabidopsis mutants.

Differences in constitutive and induced expression of two phenolic compounds in coast live oaks susceptible and resistant to infection by *Phytophthora ramorum*

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Phytophtora ramorum is the causal agent of sudden oak death (SOD), a devastating disease of tanoaks and oaks in California and Oregon. Apparent resistance to infection by *P. ramorum* in coast live oak (CLO) has been observed in natural populations and in laboratory inoculation trials. No practical controls for this disease are available, therefore characterization of natural resistance is highly desirable. In a preliminary test, we used HPLC analysis to evaluate branch phloem phenolic profiles for CLO's previously identified as relatively susceptible (S) (N = 4) or resistant (R) (N = 5) to *P. ramorum*. Separate sets of branches from the same trees were wound-inoculated with *P. ramorum*. Two compounds, identified as tyrosol and catechin, were present in constitutively higher amounts in R than in S trees, but due to the low replication the differences were not significant. However, a significant overall negative correlation was found between lesion length and tyrosol concentration ($r = -0.667$, $N = 9$, $P = 0.026$). These preliminary findings may be important in establishment of chemical biomarkers, which has great significance in applications such as screening of oak germplasm for resistance to SOD. Follow-up studies in different seasons and with trees exhibiting prolonged resistance in the field under high disease pressure are planned.

Development of real-time PCR for the detection of exotic potyviruses infecting imported plant germplasm

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Several viruses can infect and move with imported germplasm causing serious threats to American agriculture. We developed fast, sensitive and reliable real-time PCR primers and probes for the detection of four grass-infecting viruses and six sweet potato-infecting viruses. These assays are an improvement over the conventional PCR assays for grass and sweet potato-infecting potyviruses that we developed last year. Sequences of grass-infecting potyviruses [*Cocksfoot streak virus* (CSV), *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), and *Sugarcane mosaic virus* (SCMV)] and sweet potato-infecting viruses [*Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), *Sweet potato virus Y* (SPVY), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), and *Sweet potato mild speckling virus* (SPMSV)] were used to design TaqMan probes and primers. Partial viral sequences (viral mini-genes) were synthesized and used to evaluate, optimize and standardize quantitative real-time PCR (qPCR) assays. All the qPCR assays developed showed a high sensitivity, with detection levels as low as 30 copies of the synthetic targets. Our results demonstrated a viable approach of using synthetic target DNA (mini-genes) of restricted and unavailable pathogens for the development of reliable diagnostic tools. The real-time PCR primers and probes developed in this study promise to be useful tools for screening foreign plant germplasm.

Development of real-time PCR for the detection and identification of potato cyst nematode

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Potato cyst nematode (PCN), *Globodera pallida*, a major potato pest and a quarantine pest in the USA, was detected in Idaho for the first time in 2006. PCN detection and identification is based on morphological features followed by conventional PCR based protocols. In this study, we developed TaqMan primers and probes for PCN detection and identification using real-time PCR. The primer set (PITS PAL3mf/PITSp4) with the TaqMan probe Gp were used to successfully detect and identify *G. pallida*. Another TaqMan probe (Prosto-rtP) was used for the detection of *G. rostochiensis* and *G. tabacum*. Prosto-rtP probe was also used with the primer set (Prosto-rt1f/Prostartr) for the identification of *G. rostochiensis* and used with the primer set (Prosto-r2f/PITST3) for the identification of *G. tabacum*. Multiplexing the real-time PCR assays for the detection and identification of two species at a time were also successful.

Molecular characterization of the PhoP/PhoQ two-component signal transduction system in *Erwinia amylovora*

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The PhoP/PhoQ system is a pleiotropic two-component signal transduction system that controls many pathogenic properties in several animal and plant pathogens, and is a master regulator of virulence genes in *Salmonella*. Three different cues have been proposed to activate the PhoP/PhoQ system: a mild acidic pH; antimicrobial peptides and low Mg^2. The role of the PhoP/PhoQ system in *Erwinia amylovora* pathogenesis is unknown so far. In this study, phoP/phoQ deletion mutants of *E. amylovora* were generated and characterized. Our results showed that, while phoP, phoQ and phoPQ double mutants were still pathogenic on immature pear fruit, these mutants were more resistant to strong acidic conditions than the wild type strain (WT). The growth of the mutants was same as WT at pH 5.5 and 7 in modified basal medium A (MBMA) at low Mg^2 concentration; however, the growth of the mutants at pH 4.5 and 5 was greatly increased. At 24 h, the bacterial number of the mutants was ~100 fold higher than that of the WT at pH 4.5. At pH 5.5 and low Mg^2 concentration, we also found that the survival of the mutants was ~ 2 and 10 times less than that of the WT when treated with antimicrobial peptides, thionin and cecropin A, respectively, suggesting that the PhoP/PhoQ system renders the pathogen more resistant to antimicrobial peptides. Further analysis demonstrated that phoP/phoQ mutants were more sensitive to osmotic stress and iron than the WT strain at acidic pH as well. In addition,