demonstrating that this expression causes an increase in plant resistance to various pathogens. This study investigates the involvement of chitinase against disease "fusarium wilt" developing a transgenic plant, overexpressing the gene of interest in cultivar Santa Clara. Seeds cv Santa Clara were disinfected and after 10 days of germination the cotyledons were cut into transverse segments for inoculation with the co-culture of *Agrobacterium,* strain EHA 105, under the control of the duplicated CaMV 35S promoter overexpressing this gene. Then, the explants were transferred to a growth room to wait regeneration. For the molecular analysis of transgenic plants that survive the selection of transformation, using the antibiotic Kanamycin (50 mg/L) and Timetin (250mg/L), the genomic DNA of regenerated plants will be extracted for analysis by PCR and Southern blot and positive plants are then challenged against the pathogen.
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S03P03
Identification and cloning of soybean Hub proteins for plant-nematode interaction studies.
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Phytopathogenic nematodes cause serious losses to agriculture and the use of pesticides for the control of these pests pose risks to the environment and people's health. Understanding the plant defense mechanisms is very important to prevent pest damages. *Meloidogyne incognita* is a root-knot nematode (RKN), sedentary and obligate parasite to important cultures, including are soybean and rice. There are few studies on the interaction mechanisms that cause the nematodes virulence in plants, which are usually performed with model plants. In this way, we are conducting the investigation of the molecules responsible for the nematodes virulence and their interactions with plant proteins. It is known that some plant-host proteins, called Hubs, interact with other proteins of the plant cell and can be targeted by pathogens. In this work we show partial results of a project that aims to identify virulence effector proteins of *M. incognita* that interact with target rice and soybean Hubs. Initially, we searched 15 soybean candidate proteins orthologs to the Hubs from *Arabidopsis thaliana* that are targeted by effectors from two different pathogens. We then proceeded to the steps towards cloning the gene sequences corresponding to these candidates. The bioinformatics sites Phytozome, Plaza and Expressolog Tree were used to the get protein orthologous, and the choice of candidate proteins to soybean Hubs was based on similarity of sequences, phylogeny and gene expression. Primers were designed to specifically amplify the soybean Hubs obtained from cDNAs of different tissues (root, leaves, pods and apical meristem) collected in 9 specimens of the Williams82 cultivar. We obtained five positive PCR amplifications out of the 8 sets of specific primers we tested. PCR products of the expected sizes were identified in 1% agarose gel, and the corresponding band excised and DNA purified from the gel. The isolated DNA sequences were ligated to the pGEMT-Easy vector and transformed into *Escherichia coli* XL1Blue. The DNA samples of the selected colonies were sequenced and analyzed with Blastn and Blastx for verification of homology of sequences with the data of the Genebank showed that the cloned soybean fragments correspond to the Hubs sequences chosen *in silico*. The results indicate that five soybean Hubs were successfully cloned and will inserted into Yeast-two-hybrid (Y2H) vectors for hybridization essays with RKN effectors candidates.
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S03P05
Genetic transformation of 3336 clone of *Eucalyptus urograndis* via *Agrobacterium tumefaciens*
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Abiotic stresses can cause several physiological damages in plants, leading to economical losses that can reach up to 100%. Global warming has been intensified in the last two centuries, and plants are submitted to extreme stressing conditions. Functional genomic studies have revealed several genes encoding proteins *related to abiotic stress response in plants. These genes can further be used in genetic*

transformation, in order to obtain transgenic plants tolerant to abiotic stresses. In this work we developed a genetic transformation protocol for 3336 clone of *Eucalyptus urograndis*, aiming further transformation with genes related to abiotic stresses. Leaf explants were collected from *in vitro* grown plants maintained on semi solid MS medium containing BAP and ANA. The leaves were cut and placed into a Petri dish containing MS liquid medium with the *Agrobacterium tumefaciens* EHA105 harboring the pCAMBIA2301 vector with the *nptII* and *uidA* genes. After 20 minutes in this liquid co-culture, explants were placed in regeneration semi solid medium containing WPM salts, TDZ and ANA and maintained for 3, 4 or 5 days at 23 ºC and 16 h photoperiod. Afterwards, the explants were washed three times to eliminate the *Agrobacterium* and transferred to fresh regeneration medium supplemented with 12.5 mg L^{-1} kanamycin and 250 mg L^{-1} Augmentin. To induce shoots, the explants were transferred to a WPM medium supplemented with BAP and ANA after one month. Kanamycin dose was enhanced every 15 days, when the explants were transferred to fresh medium. In a second experiment, we tested the effect of 100 µM acetossiringone in the liquid co-culture (for four days) and compared to a control, without acetossiringone. The experiments were repeated twice. Forty five and 90 days after the beginning, we carried out a gus histochemical assay to observe the blue staining at the calluses and shoots formed, respectively. Blue spots were observed on calluses in all treatments in both experiments, suggesting that transient expression did not depend on time of co-culture or acetosseringone. However, 90 days later, the shoots tested proved to be escapes, and did not stained. We conclude that the transformation is effective, although the selection step still needs to be improved, and new kanamycin concentrations must be tested in order to regenerate transgenic shoots.
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S03P06

Genetic transformation of tobacco plants with a cacao pathogenesis-related protein 4 for tolerance to water stress study

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Drought is an important environmental factor limiting the productivity of various crops worldwide. The development of crop cultivars with improved adaptation to drought is a major goal in many crop breeding programs. In addition to classical breeding approaches, genetic transformation to introduce candidate genes into plants for better tolerance to water deficit has been successfully developed. Pathogenesis-related proteins (PR proteins) are defined as plant proteins induced in response to pathogen attack. However, it is known that these proteins may also be involved in response to abiotic stresses. The objective of this study was to transform tobacco plants (as plant model for subsequent analysis of cultivated plant such as citrus) with a PR-4b protein from *Theobroma cacao* (TcPR-4b) and to select and test the tolerance of such transformed plants to water/osmotic stress. First, an *in silico* analysis of the TcPR-4b using the BLAST, Pfam,InterProScan, ORF-Finder programs, as well as a search on Cocoa GenDB databank were performed. The TcPR-4b belongs to a small family of PR-4 proteins whose members were mainly located on the chromosomes 5 (five genes) and 10 (one gene). The complete *TcPR-4b* sequence is 802 bp in length and contains two exons (171 and 258 bp), and one intron (82 bp); the corresponding protein is 142 amino acids in length. For plant transformation experiment, the *TcPR-4b* cDNA (from cacao-*M. perniciosa* interaction library) was cloned into the pGem-T Easy vector then subcloned on the pCambia binary vector 1390. Then, *Agrobacterium tumefaciens* strain EHA 105 was transformed with the*35S::TcPR-4b::pCambia* construction, and the transformed *A. tumefaciens* used for *Nicotiana tabacum* cv.Havana transformation by co-cultivation of leaf segments in selection medium. Transformed shoots from three transformation events are under selection for subsequent *in vitro* water/osmotic stress, using, among others, mannitol and NaCl.
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