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Biotic and Abiotic Stress Tolerance in Plants: the Challenge for the 21st Century

BOOK OF ABSTRACTS

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among them, 460 were common to both treatments, 144 were present only in the non infected sample, and 158 present only in the infected one. In the "Pera C21" variety, 394 and 328 spots were detected in infected and non infected samples, respectively; among them, 266 were common to both treatments, 128 were present only in the non infected sample, and 62 present only in the infected one. The differences of protein profiles between varieties as well as between infected and non infected sample for the same variety revealed probable differential variety-dependant biochemical responses that may be explore for further development of Citrus Tristeza control strategies.
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S01P11
In silico* characterization of a pathogenesis-related protein PR-1 from *Theobroma grandiflorum
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The cupuassu (*Theobroma grandiflorum* [Wild. Ex Sperg.] K. Schum) is a native species of Brazil with a large industrial potential related to the use of the fruit pulp and seeds. In particular, the cupuassu presents a great economic value for the Pará State, which invested in cupuassu sweet (e.g. ice-cream) and cupulate (chocolate obtained from cupuassu seeds) production and commercialization. *Theobroma grandiflorum* belongs to the same genus than cacao (*Theobroma cacao*), and, unfortunately, both suffer from the attack of the fungus *Moniliophtora perniciosa* responsible for the witches' broom disease. Several molecular studies of the interaction between cacao and *M. perniciosa* were previously developed, while little is still known in regards to cupuassu resistance to witches' broom disease. Among the well known genes involved in plant-pathogen interactions, the pathogenesis-related proteins (PR proteins) could be highlighted. In particular, the PR-1 family proteins presented several functions that vary according to the organism and that may be involved in different ways in defense to pathogen infection. Recently Next Generation Sequencing of cupuassu expressed sequences tags allowed the identification of several PR proteins, and here we developed an *in silico* analysis of a TgPR-1 sequence. The TgPR-1 ORF encoded a 161 amino acid protein that showed homology with the PR-1 proteins belonging to the serine-carboxyl proteinase superfamily. A multiple alignment using the ClustalW program allowed the identification of domains conserved between the TgPR-1 and its homologs from other organisms. The TgPR-1 protein presented a peptide signal (24 amino acids identified by the SignalP 4.1 software), and had an isoelectric point and a molecular weight of about 8.75 and 17.3 kDa, respectively. The protein presented possible post-translational modification sites such as 10 phosphorylation sites (encountered using the NetPhos 2.0 software) – but no glycolsylation sites were found. The systems biology analysis of TgPR-1 was performed using the BLASTO and OMA browser to find a corresponding ortholog. The best score protein was found for the PRB1 from *Arabidopsis thaliana*. The network was set up using the software Cytoscape 2.8.2. The protein showed direct interaction with other proteins involved in responses to biotic stimuli, fungus, bacteria and stress as well as involved in mechanisms of resistance and plant defense. The *in silico* characterization of TgPR-1 constitute the first steps towards understanding the mechanisms of *T. grandiflorum* defense.
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S01P12
Systems biology of proteins expressed during the *Moniliophthora perniciosa* necrotrophic phase
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The fungus *Moniliophthora perniciosa*, the etiologic agent of witches' broom disease of cacao (*Theobroma cacao* L.) has a hemibiotrophic life cycle, with a biotrophic and a necrotrophic phase. The biotrophic phase, initiating the disease, is characterized by a monokaryotic mycelium, while the

necrotrophic phase is characterized by a dikaryotic mycelium leading to plant necrosis. During the culture of *M. perniciosa* on bran-based solid medium, six different developmental phases were observed according to the dikaryotic mycelium color or the organ produced: white, yellow, pink, dark pink, primordium and basidiomata. A proteomic analysis of the different *M. perniciosa* development stages associated to mass spectrometry allowed the identification of about 250 differentially expressed proteins. In this study, using such differentially expressed proteins, we developed a systems biology analysis to identify physical protein-protein interaction (PPPI) networks related to the fungus development focusing on basidiocarp formation. First, orthologous protein sequences of *M. perniciosa* were obtained in *N. crassa* using the BLASTX tool. The data mining screening and PPPI network design associated with fungal development was performed using the Cytoscape software, version 2.5.0. These data were downloaded from the STRING 8.3 database. The interactome networks obtained from this first screening were analyzed with the Molecular Complex Detection software, a Cytoscape plugin, in order to evaluate potential subgraphs that were used further for network expansion. Gene ontology clustering analysis was performed using the Biological Network Gene Ontology software. Moreover, an analysis of centrality was performed using the software Centiscape 1.2.1.; several hub-bottlenecks, hub and bottlenecks proteins, as well as proteins involved in biological processes important for the *M. perniciosa* development were identified. The main biological processes encountered were anatomy and morphology, reproduction, oxidative stress, cell wall biosynthesis, pigmentation, development and cell differentiation. The identification of proteins involved in the formation of basidiomata, as well as the knowledge about their interactions, may contribute to the future development of witches' broom control strategies. To our knowledge, this is the first system biology analysis of proteins involved in the *M. perniciosa* life cycle.

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S01P13

Use of *Neurospora crassa* as fungus model for systems biology of *Moniliophthora perniciosa* hyphae proteins: case study of polygalacturonases

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Moniliophthora perniciosa, the fungus responsible for the witches' broom disease, brought serious problems to cacao (*Theobroma cacao* L.) cultivation in the infected areas such as in Southern Bahia, Brazil. For this reason, several molecular studies have been recently developed from *T. cacao* and/or *M. perniciosa*, and subsequent computational analyses were developed. Among them, the systems biology provides a framework for assembling models of biological systems from systematic measurements obtained by experimental analysis. In the case of *M. perniciosa*, which is still little studied, the first step for protein-protein interaction (PPI) network analysis by systems biology consists in the identification of an adequate organism for ortholog search. *Neurospora crassa* is a well-known filamentous fungus, considered as a model organism that has been used for more than 90 years to study genetics, biochemistry and fungal biology. Moreover, lots of genomics and molecular data are available for *N. crassa*, including those related to architecture and hyphae development, as well as cell wall degradation apparatus. Here, the objective was to evaluate the possible use of *N. crassa* as model for determination of *M. perniciosa* PPI networks, and as an example to test our hypothesis, two polygacturonases from *M. perniciosa* (MpPG1 and MpPG2) were analyzed. First, a reciprocal BLASTp of MpPG1 and MpPG2 was performed on *N. crassa* database using stringent conditions (10^{-10}). The respective *N. crassa* orthologs (NCU06961 and NCU02369) were used to build the PPI network in STRING 9.05. This network was analyzed in the Cytoscape 2.8.2 software with the Molecular Complex Detection, Biological Network Gene Ontology and CentiScaPe plugins. The PPI network contained 892 nodes (proteins), 43.035 connectors, and was organized in 11 modules corresponding to biological processes, such as ribosome biogenesis, regulation of gene expression, processes related to carbohydrate metabolism, among others. Twenty-two proteins interacted directly or indirectly with MpPG2-NCU02369 and were associated to post-translational protein modification or pectin degradation. From the 892 and 22 specific proteins, 544 (61%) and 14 (63.6%) were found by reciprocal BLASTp when we