S01O02

Comparative analysis of roots´s proteome and metabolome of two genotypes *E. grandis* x *E. camaldulensis* tolerant and susceptible under drought stress conditions

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Several authors have reported the potential of selected *Eucalyptus camaldulensis* and its hybrids with *E. grandis* for production in arid regions of Brazil. The adaptation of species to a particular environment is related to genetic features expressed on its proteomic and metabolomic profiles. Proteomics and metabolomics analysis can help to understand biological processes and provide an overview about how plants react to environmental stresses. We performed comparative analysis of the roots´s proteome and secondary metabolites of two *E. grandis x E. camaldulensis* genotypes, one drought tolerant and another drought susceptible, subjected to different water regimes, 100% of field capacity (FC - control) and 30% (drought stress). We monitored the gas exchange parameters of the two genotypes, as photosynthetic rate, intercellular CO_2 concentration, stomatal conductance and transpiration rate. Root proteins and secondary metabolites were extracted and their mass spectra were acquired by mass spectrometry (MS) associated with reverse-phase ultra performance liquid chromatography (UPLC). The tolerant genotype showed lower photosynthetic rate and hydraulic conductance under 100% FC than the susceptible genotype. Under 30% FC both genotypes behaved similarly, presenting much lower photosynthetic rate and hydraulic conductance than under 100% FC. Proteome analysis revealed a maxium of 2339 and 2768 proteins in the susceptible and tolerant genotypes, respectively. In both genotypes, a higher number of proteins were down regulated under drought stress than under well-watering. Comparing both genotypes under drought stress, a higher number of proteins were up regulated in the tolerant genotype. Most of proteins found are related to response to stress and catabolic process. In the metabolomics analysis, we found a total of 355 different secondary metabolites. The susceptible genotype presented 12 secondary metabolites differentially expressed, 4 of them over expressed under drought stress and 8 of them over expressed under well-watering condition. The tolerant genotype presented 9 secondary metabolites differentially expressed, 7 of them over expressed in the drought and 2 of them in well-watering. Comparing both genotypes under drought stress, the tolerant genotype showed one exclusive metabolite and 3 over expressed, while the susceptible presented only one metabolite over expressed. The key to drought tolerance may be in the different expression of proteins and secondary metabolites in the plant roots. This is one of the first research involving proteomics and metabolomics studies of eucalyptus roots under water stress and new researches are welcome to complement the results.

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S04O01

Phenotyping a *Coffea canephora* population, cultivated at high altitude, aiming at a GWS program for coffee

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Considered the most popular and non-alcoholic drink regularly consumed by 40% of the population, coffee occupies a prominent position in the economy being one of the world's most important export products. Their production is subject to regular oscillations due to the biennial cycle of the plant, and also the abiotic factors, such as water stress and high temperatures. Aiming at the establishment of tools to help accelerate the genetic improvement of this species, significant advances in coffee genomics have occurred in recent years. As an example, one can cite the recent completion of the complete genome sequencing of *Coffea canephora,* which will serve as a reference work for use in advanced molecular genetics, applied directly to the genetic improvement of this perennial crop, such as the establishment of genome-wide selection programs (GWS) in coffee. In that sense, the objective of this study was to characterize the phenotype of a population of *C. canephora,* with about 1300

individuals, cultivated in Planaltina-DF (1175m altitude) in the experimental field of Embrapa Cerrados. Evaluations started in 2012, evaluating characteristics such as vigor, secondary branching, leaf-rust susceptibility, precocity and fruit load. Furthermore, for two consecutive years, 2012 and 2013, the production (in liters - L) of each plant was measured. In 2012, a sample of fruits of each plant selected after harvest, were shelled, to perform the classification, sieve and 100-grain weight analysis. The predawn-leaf water potential (Ψ_{PD}) of a sample of 400 plants was also evaluated in the drought season of 2012/2013. The results obtained so far, allowed us to conclude that there is potential for cultivation, under irrigated conditions, of *C. canephora* at high altitudes and that the phenotypic diversity of the studied population seems suitable for genome-wide association studies in coffee.
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S03O02

The pathogenesis-related protein PR-4 from *Theobroma cacao* has antifungal activity and induces ROS in *Moniliophthora perniciosa*

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The pathogenesis-related proteins class 4 (PR-4) are known to be involved in plant defense response and/or related stress situations. The objective of this study was to evaluate the antifungal activity and reactive oxygen species (ROS) production of the TcPR-4b protein in *Moniliophthora perniciosa*. The *TcPR-4b* gene was cloned into pET28a and the resulting in frame fusion plasmid was used to transform *Escherichia coli* Roseta (DE3) for protein expression. The expression of the TcPR-4b recombinant protein was induced by 0.4 mM isopropyl-β-D-thio-galactoside and purified by immobilized metal affinity chromatography with TALON® Metal Affinity Resin. The TcPR-4b protein was used for *in vitro* assays against dikaryotic *M. perniciosa* broken hyphae. Then, 1 ml of the broken hyphae suspension was incubated for 2h with: i) 10 µg of TcPR-4b in phosphate buffer (PB); ii) 20 µg of TcPR-4b in PB; iii) 40 µg of TcPR-4b in PB; iv) PB (control). Then, 1 ml of each treatment was applied on CPD solid medium (2% glucose, 2% peptone, 2% of agar) and incubated for 7 days at 25°C. The inhibition of hyphal growth was examined by counting the number of pseudo-colonies on three experimental replicates. To detect the production of the ROS in living cells of *M. perniciosa*, 1 ml of hyphae suspension was treated with 10 µg of TcPR-4b in PB (or not – control) overnight at 25ºC, and then incubated at 25°C for 30 min with dihydroethidium which selectively stains the mitochondrial superoxide (O_2^-). The hyphae were mounted on slides and observed under fluorescence microscope DMRA2 (Leica). Images were captured under fluorescent filters using the IM50 software (Leica). The reduction of *M. perniciosa* survival was observed in all tested concentrations of TcPR-4b with a decrease of survival correlated to the increase of the protein concentration. The hyphae treated with TcPR-4b presented a bright red fluorescence with specific more intense fluorescence in some foci. The control did not present fluorescence emission comparing to the hyphae treated with TcPR-4b. This study showed the antifungal activity of TcPR-4b and the induction of ROS in *M. perniciosa*.
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