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

BOOK OF ABSTRACTS

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

S.P. Menezes[1], E.M.A. Silva[1], K.P. Gramacho[2], C.P. Pirovani[1], A.S. Gesteira[3] and F. Micheli[1,4].
[1] Universidade Estadual de Santa Cruz, Centro de Biotecnologia e Genética, Rodovia Ilhéus-Itabuna, Km 16, 45662-000, Ilhéus-BA, Brasil.
[2] CEPLAC/CEPEC, 45600-970, Itabuna-BA, Brasil.
[3] Embrapa Mandioca e Fruticultura, Rua Embrapa, s/nº, 44380-000, Cruz das Almas-BA, Brasil.
[4] CIRAD, UMAR AGAP, Avenue Agropolis, Montpellier, France.
Email: menezes_sp@yahoo.com.br

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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