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

BOOK OF ABSTRACTS

6 to 8 • NOVEMBER • 2013
Cana Brava Resort • Ilhéus - Bahia - Brazil
www.ciba2013.net
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) 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 ($\Psi_{PD}$) 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.

Work supported by CAPES-COFECUB, Consórcio Pesquisa Café and INCT-Café (CNpq/FAPEMIG).

S03O02
The pathogenesis-related protein PR-4 from Theobroma cacao has antifungal activity and induces ROS in Moniliophthora perniciosa


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 Agrapiles, 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 25°C, and then incubated at 25°C for 30 min with dihydroethidium which selectively stains the mitochondrial superoxide ($O_2^-_\text{H}$). 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.

Work supported by CNPq, FAPESP, FINEP/RENORBIO, CAPES, Cirad.