Resumos do 59º Congresso Brasileiro de Genética • 16 a 19 de setembro de 2013
Hotel Monte Real Resort • Águas de Lindóia • SP • Brasil
www.sbg.org.br - ISBN 978-85-89109-06-2

The pathogenesis-related protein PR-4 from *Theobroma cacao* is involved in the defense responses of cacao against *Moniliophthora perniciosa*

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Keywords: gene expression, antifungal activity, witches' broom disease, *Theobroma cacao,* TcPR-4

The pathogenesis-related proteins class 4 (PR-4) is known to be involved in plant defense response. The objective of this study was to evaluate the temporal expression of the TcPR-4 gene after inoculation with *Moniliophthora perniciosa*. Plantlets of *Theobroma cacao* L. varieties Catongo (susceptible to *M. perniciosa*) and TSH1188 (resistant to *M. perniciosa*) were inoculated by the droplet method with a basidiospore suspension (2.10^5 basidiósporo.ml^{-1}) of *M. perniciosa*. After inoculation, the plantlets were kept for 24h at 25±2ºC and 100% humidity. Apical meristems were harvested in triplicates at 24, 48 and 72 hours after inoculation (hai), and 8, 15, 30, 45, 60 and 90 days after inoculation (dai). Non-inoculated plants (controls) were kept and harvested in the same conditions at 24 hai, 72 hai, 30 dai, 60 dai and 90 dai. Total RNA was extracted from macerated samples using the RNAqueous Kit® (Ambion). The synthesis of the first cDNA strand was done using the Revertaid Fisrt Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas). Real-time quantitative analysis of TcPR-4b expression was performed using standard settings of the ABI PRISM 7500 and Sequence Detection System (SDS) software, version 1.6.3 (Applied Biosystems). The expression level of *Tc-PR4b* was analyzed on triplicates with the comparative Ct method ($2^{-\Delta\Delta Ct}$) using malate dehydrogenase and actin as endogenous reference genes, and non-inoculated plants (control) were used as a calibrator. In the early stages of infection, an increase of *TcPR-4b* expression at was observed 48 hai in both varieties TSH1188 and Catongo. After the initial stage, the expression of *TcPR-4b* was observed in all times in the resistant variety, while in the susceptible one the *TcPR-4b* expression was concentrated in the final stages of infection. In parallel, The recombinant protein TcPR-4b, overexpressed in the pET28a plasmid,was used in antifungal tests against *M. perniciosa* (pseudo-colony method). The recombinant protein used at different concentrations shows antifungal activity against *M. perniciosa*. The data generated in this study may help to elucidate mechanisms of the TcPR-4b action during the interaction cacao-*M. perniciosa*. Financial Support: CNPq, BNB, FINEP/RENORBIO, CAPES, Cirad.