

FUNCTIONAL ANALYSIS OF FOUR DIFFERENT RECOMBINANT CYSTATINS FROM COCOA (*THEOBROMA CACAOL.*)

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Phytocystatins are cysteine-proteinase inhibitors from plants implicated in the endogenous regulation of protein turnover, programmed cell death, and defense mechanisms against pathogens. We identified four cystatins ORFs (CysTc1, CysTc2, CysTc3 and CysTc4) using the data from two cDNA libraries corresponding to resistant and susceptible interactions between *T. cacao* and *Moniliophthora perniciosa*. The cDNA genes encode 209, 127, 124 and 205 amino acid residues, with a deduced molecular weight of 24,3; 14,1; 14,3 and 22,8 kDa, respectively. They contain conserved reactive site motif Gln-Val-Val-(ALA/Ser)-Gly of cysteine protease inhibitors, and another consensus LARFAV sequence for phytocystatin. The CysTc1 and CysTc3 contain signal sequence in N-terminus and are 97% identical in amino-acid sequence. The CysTc2 and CysTc4 are 88% identical in amino acid sequence. The sequence identity between CysTc1/CysTc3 and CysTc2/CysTc4 is lower 50%. These ORFs were sub cloned, and His-Tag fused proteins expressed in *E.coli* using pET28a vector. Recombinant proteins were obtained by affinity chromatography in a single step of purification. Polyclonal antibodies against the recombinant cystatins CysTc1 and CysTc2 were raised, allowing the immunodetection of the endogenous proteins in the plant tissues. Moreover, activity against papain was identified on gelatin/SDS-PAGE. Investigation of its antifungal activity clearly revealed a toxic effect on the mycelium growth of *Moniliophthora perniciosa*, causal agent of witches' broom in cocoa.

Keywords: *Theobroma cacao*, plant-pathogen interaction, cysteine-protease Inhibitor, *Moniliophthora perniciosa*.