

BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT SERINE-PROTEASE
INHIBITOR FROM CACAO (*Theobroma cacao* L.)

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Proteinase inhibitors (PIs) are widely expressed in plants, especially in seeds of Cruciferae, Leguminosae, Solanaceae, Graminaceae and Sterculiaceae. Their expression is regulated at the transcriptional level in response to different conditions, including germination and insect attack. Plant PIs are also involved in response to various biotic and abiotic stresses, e.g., pathogen invasion, wounding and environmental stress. PIs are common in plants and could be used to develop transgenic crop variety resistant to pathogens. Recently, molecular and genomic studies of cacao have been developed by different research team around the world and about 180,000 ESTs are now available on public databases, in which we identified a trypsin-like serine-protease inhibitor. The ORF contains 660 bp and encodes a protein of 219 amino acids. The predict protein has an estimated molecular weight of the 22 kDa and a theoretical pI of 5.9, and contain a peptide signal with a predict cleavage site at position 26. The His-Tag fused protein was expressed in *Escherichia coli* and the recombinant protein (named TcTI) accumulated in soluble fraction from bacterial extract. Purified His-tagged recombinant proteins showed inhibitory activity against porcine trypsin with a K_i of 406 nM. TcTI also showed thermostability up to 70°C. Polyclonal antibody raised against the recombinant TcTI detected that the endogenous protein was more abundant in cacao tissues infected by *M. pernicioso* than in healthy ones. Our data shows that recombinant TcTI is functional and presents potential biotechnology applications.

Keywords: Serine-protease, trypsin inhibitor, witches' broom disease, recombinant protein

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