THE USE OF CHAGASIN, A *Trypanosoma cruzi* INHIBITOR OF CYSTEINE PROTEINASES TO PRODUCE INSECT-RESISTANT TRANSGENIC PLANTS.

Monteiro ACS, Paes NS, Scharfstein J, Abrahamson M, Aragão FL and Grossi de Sá M F. Embrapa Recursos Genéticos e Biotecnologia. norma@cenargen.embrapa.br

A cysteine proteinase inhibitor of *T. cruzi* was found by screening of a lgt11 cDNA expression library. Several full-length clones encoding the inhibitor, named chagasin, were identified and the non-coding sequence up to the *T. cruzi* mini exon sequence was determined by RT-PCR. The amino acid sequence is composed of 110 residues (Mr 12.039), lacks cysteine residues and is devoid of the QXVXG motif characteristic for cysteine proteinase inhibitors belonging to the cystatin super family. A recombinant form of chagasin was produced in high yields in a periplasmic *E. coli* expression system, isolated and characterized. Similarly, to observations made with members of the cystatin super family, chagasin displays broad target specificities, being active against cysteine proteinases from different species of insects, including bruchids. Besides that, artificial seeds of the common bean, *Phaseolus vulgaris*, containing chagasin showed an inhibitory effect on the development in feeding trials using artificial beans made with flour of the susceptible bean (cv. Jalo) containing 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 1 and 1.5% (w/w) of lyophilized protein. Since chagasin can act as a plant defense protein, genetically engineered legume plants that express this inhibitor in their seeds should be resistant to these bruchid larvae. The coding region of chagasin was amplified by PCR from its cDNA using a oligonucleotide primer SPINH, corresponding to the signal peptide sequence of a-AI2 (amylase inhibitor from *Phaseolus vulgaris* seeds) and to the 5' end of the coding region of chagasin, and a second primer CHACT, corresponding to the 3' end of the coding region of chagasin. The PCR product was cloned in frame with phytohemagglutinin promoter and 3' UTR region. This construction was then cloned into Puc bar followed by transformation of bean embryo. Órgão Financiador : Embrapa e CNPq