ISOLATION OF A Vigna vexillata CYSTATIN AND ITS ANTIFUNGAL ACTIVITY AGAINST Fusarium solani

Da Cunha, P.C.., Pinto, M.S.T., Gomes, V.M, Oliveira, A.E.A., Fernandes, K.V.S.
UENF; CBB; LQFPP

Phytocystatins have been correlated to several physiological roles, as control of endogenous proteolysis and defense against exogenous proteases from plant predators. A suggestion of a singular attribute for them has been proposed involving its participation in cellular signal transduction and intertissue interaction processes. We isolated a *V. vexillata* cystatin by using an affinity Sepharose 4B CM-Papain column. The extraction was performed using Tris-HCl 0.1M, NaCl 0.5M pH 7.2 (300g of flour/L), overnight. The suspension was centrifuged (5,000xg, 30 minutes /4ºC) and supernatant (10 mL) was percolated through the Sepharose 4B CM-Papain column. The equilibration buffer was sodium phosphate 0,1M, NaCl 0,5M pH 7,0 and retained fractions were eluted by the same buffer at pH 11.5. The isolated protein had a Mr of 13 kDa and cross-reacted with an anti-cowpea cystatin antibody by Western blotting. Antifungal activity microplate assays showed that the isolated protein completely inhibited the growth of *F. solani* at a concentration of 200µg. As the *V. vexillata* cystatin has previously been shown by us to be located at extracellular space of seed cotyledons, a defense role against predators is suggested. However, binding of this cystatin to cell wall and cell membrane components are being tested by ELISA, to investigate its potential participation in the above mentioned role of involvement in signal transduction and intertissue interaction processes.

Supported by: CNPQ/PIBIC, FAPERJ, UENF.