

Cysteine Proteinases (CysP) Are Present in Legume Seed Coats
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Programed Cell Death (PCD) is a process that occurs in pluricelular organisms causing the suicide of a cell. The final dehydration stage of developing seeds, followed by the subsequent re-hydration necessary for seed germination, implies on a previsible process of PCD in seed coat cells. The present work aims at the diagnosis of such phenomenon, through biochemical analysis, using quiescent seed coats of *Albizia sp.*, *Cicer arietinum* and *Lens culinaris*. The detection and quantification of molecules potentially involved in PCD, such metacaspase-like CysP, are the primary targets of this work. Seed coats were ground and pigments removed with methanol 80% (1:5 w/v). Proteins were extracted by 100 mM sodium phosphate buffer, pH 7.5, 1% PVPP (1:10 w/v) at 4°C for 2h and centrifuged for 20min at 10.000 xg. All samples were dialysed against water (48h) and lyophilized, before protein quantification and proteolytic assay, using azocasein as substrate. Enzymatic assay was only performed with *Albizia sp.* and *C. arietinum* seed coat extracts. Protein profiles and enzymatic activities were visualized by SDS-PAGE and zymography. The *in vitro* assays did not revealed enzymatic activities. Despite these apparently negative data, gelatinolytic activities were observed in all extracts. Activity bands of ~ 30 kDa for *Albizia sp.*, ~33 kDa for *C. arietinum*, and of ~19, 14, 12, 10 and 8 kDa for *L. culinaris* were observed. After boiling the extracts for 2 min, bands from *Albizia sp.* and those of 19 and 14 kDa from *L. culinaris* remained active. Characterization of these enzymes as metacaspases shall employ substrates such as Ac-YVAD-pNA, Ac-DEVD-pNa, Ac-VEID-pNA, Ac-VAD-AFC, as well as caspase inhibitors such as Ac-YVAD-CHO and Ac-DEVD-CHO.

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