ISOLATION AND CHARACTERIZATION OF A POLYPHENOL OXIDASE (PPO) INCREASED BY WOUNDING IN COWPEA (*Vigna unguiculata*) UNIFOLIATES

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Increases in PPO activity represent a widespread plant response caused by wounding. However, in cowpea it was unknown until this work. Our aim was to isolate and characterize a soluble PPO induced by wounding in this crop. Greenhouse-grown plants were submitted to a wounding treatment on a single unifoliate. Plants were kept under constant 100 mM photons/cm²/s and unifoliates were collected at 3, 8, 24, 48 and 72 hours after wounding. After ground in acetate buffer pH 5.3 (1:3 w/v) and submitted to centrifugation, soluble proteins were subjected to 30 % ethanol precipitation and percolated through a DEAE-cellulose. The PPO activity peak (eluted by 0.25 M NaCl) was purified by gel exclusion chromatography. PPO activity assays were performed using cathecol as substrate, either *in solution* or *in gel* and revealed a sudden increase of enzyme activity 8 h after wounding in both wounded and neighbor-to-wounded unifoliates; maximum levels were reached at 48 h. Major PPO activity was seen to be an acidic protein and by SDS-PAGE-cathecol gel was seen as heterogeneous PPO bands with Mrs of 87 and 95 kDa. Isolated PPO activity was greatly increased by SDS. The purified enzyme had an optimum pH at 6.0, a Km of 3.58 mM and Vm of 250 UI/min when using cathecol.

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