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Variant response of Liao, Aao, and protease during *in vitro* culture of *Argyrolobiumroseum*

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Indole acetic acid oxidase (IAAO), ascorbic acid oxidase (AAO), and protease were analyzed during in vitro tissue culture of *Argyrolobium roseum*. These enzymes are crucial for biochemical regulation for growth and developmental processes. The enzymes were studied during growth from seed; callus induction from different explants; organogenesis period; and acclimatization conditions. It was observed that leaf derived calli presented 23.7% and 29.4% increase in IAAO and protease enzyme, respectively as compared to stem (16.1% and 91%) and root (17.1% and 23%, respectively). The decline level in Ascorbic acid oxidase was observed during in vitro conditions.Calliproduced fromleaf showed 18.47% decline in AAO level, whereas stem showed 12.5% and root 22.4%. Behavior of IAAO, AAO and protease changed during organ genic phase. Root accessible 19.3% decrease in IAAO level as compared to regenerated stem (31.8%) and leaf (29.9%), however protease showed 28.6% decline in regenerated leaf as compared to stem (26.4%) and root (31.7%). Regenerated leaves showed 39.57% increase in AAO enzyme level. Similarhigher pattern was observed in stem (30%) and root (47%). The study highlights the biochemical behavior of IAAO, AAO and protease during in vitro life pattern of *A. roseum*.

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Purification and characterization of a 32kd β-glucosidase (*seafp*$_{32}$) having antifungal activity from the seeds of *Sechium edule* (jacq) Swartz

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Plant diseases have emerged as a major threat to the global food security. Although, plants have no immune system; they possess a variety of defense mechanisms which include synthesis of pathogenesis related proteins in response to fungal/bacterial infection. The paper reports on the purification and characterization of a 32kD protein which has growth inhibitory activity against *Fusarium oxysporum, Trichoderma viride* and *Rhizopus stolonifer var stolonifer* from seeds of *Sechium edule* (Jacq) Swartz. The protein, designated as *SeAFP*$_{32,}$ inhibited mycelial growth of *Fusarium oxysporum, Trichoderma viride* and *Rhizopus stolonifer var stolonifer*, with an IC_{50} of 10±0.14, 20±0.035 and 8±0.05 µg ml^{-1} respectively. SYTOX Green uptake assay indicated that the protein affected the permeability properties of the fungal cell membranes. MALDI-TOF/MS analysis of the tryptic digested 32kD *SeAFP*$_{32}$ revealed 100% homology with β-glucosidase from *Arabidopsis thaliana* (acc. no. BAD94819). The 3D homology model of SeAFP32 developed with Modeller 9.10 with rice β-glucosidase protein (PDB ID: 3GNO) as template, confirmed it the high degree of homology of *SeAFP*$_{32}$ with β-glucosidase family of proteins. *In silico* docking of p-nitrophenyl β-D-glucopyranoside (pNPG) on 3D model of *SeAFP*$_{32}$ confirmed the presence and stearic position of the conserved Glu_{105}, Ser_{75} and Glu_{161} on the docking domain. Glutamic acid at P'_{105} has been identified as the active site nucleophile required for the enzymatic hydrolysis of the glycosidic bond.

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