Serine Proteinases in Developing, Quiescent and Germinating *Vigna unguiculata* Seeds

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Plant serine proteases have been somehow neglected and their physiological roles in seeds are not yet well established. A considerable variation in the levels of serine proteinase activity from *Vigna unguiculata* seeds has been found during development and germination. The purpose of this research is to isolate and characterize serine proteases activities from embryonic tissues along these life stages as well as to identify their potential endogenous substrates. Dry mature seeds were cultivated in the field and developed pods were collected at the stages of 12, 14, 16, 18, 20, 22 and 24 days after pollination (DAP). Dry mature seeds were also germinated in an incubation chamber and hypocotyls and radicles were collected at 2, 4, 6, 12, 24, 36, 48 and 72 hours after imbibition (HAI). Seed or seedling tissues were dissected and separately ground to a fine powder, which was submitted to albumins' and globulins' extraction. Extracted proteins were quantified by Bradford. Proteolytic activities from albumin fractions were measured using BApNA as substrate. Band profiles were visualized by SDS-PAGE followed by zymography. During maturation, a ~70kDa protein was present at all stages, being more intense at 12 DAP. A ~45 kDa band was found exclusively at 12 and 14 DAP. During germination, a similar ~70kDa was present from 12 HAI until 72 HAI. A major ~34.7 kDa protein appears after 12 HAI, being then conserved until 72 HAI. Serine proteinases from quiescent seeds were isolated by aprotinin-agarose affinity column and visualized as five gelatinolytic bands. We are establishing the best activity parameters for the isolated proteases, which shall be useful to the identification of the potential endogenous proteinases substrates.

Keywords: serine proteases, Vigna unguiculata, seeds, germination, development.