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Characterization of acid proteinases present in digestive and reproductive systems of *Setaria digitata*

Characteristic properties of acid proteinase of *Setaria digitata* and their tissue localization were reported previously. In this report purification procedure and enzymatic properties of acid proteinases present in digestive and reproductive systems of ***S***. *digitata* will be presented.

Crude extract of both digestive and reproductive system was prepared separately and c3entrifuged. The supernatant were dialysed and applied into a column of DEAE cellulose – 52. The protein was eluted with a liner gradient of 0-1 M NaCl. Fractions of the peaks with proteolytic activity were combined. Average activity of pooled fractions were 0.26 U/ mg for sample injection, and 0.55 & 0.51 U/ mg for elution peaks of reproductive system and that of elution peaks of digestive system were 0.1 and 0.03 U/ mg. The combined fractions were applied into a column of sephacryl S 200, separately. Fractions with proteolytic activity were combined and dialyzed against 0.002M sodium acetate buffer pH 4. 0. It was applied into pepstatin sepharose column.

The protein was eluted with 0.04 M Tris-hal buffer pH 8.0, containing 1M NAcl. The proteolytic activities were 0.94, 0.92 and 0.68 U/ mg respectively for unbound and two bound proteinases of reproductive system, respectively. Procedure of polyacrylamide gel electrophoresis (PAGE) under non – denaturing condition followed by activity staining was developed to separate and analyse the acid proteinases. Three and two bands were stained with crude extracts of reproductive system, and digestive system, respectively.

These results suggest that presence of three and two acid proteinases in reproductive system and digestive system of *S. digitata*. The molecular mass of these proteinases were around 45 KD. The optimum pH of proteinases were 2.6 and 3.0 Further significant activity was not observed above pH 7.5. The highest activity was recorded at 45^{o}C. Further studies will provide necessary information for the future screening of proteinase inhibitors for therapeutic approach.