S U M M A R Y

The biochemical changes during germination and growth of mung bean (*Vigna radiata*) was studied with special reference to different hydrolases. The finding of the study presented in this dissertation can be summarised as :

1] The germination and growth of seedling is accompanied with a number of biochemical changes in axis and cotyledon. Total and soluble protein content of the axis increased gradually from early hours of germination and reached it maximum after 72 h following imbibition. This increase in axis protein is associated with concomitant decrease in protein content of the cotyledon. It appears that during growth, the reserves in the cotyledon are being gradually catabolised to produce the precursor for the anabolic reactions in the growing axis part. The enzymes which play the key role in this process of utilisation of reserve foods present in the cotyledon for the growth of axis are the hydrolases. Therefore, properties of different hydrolases during growth of the seedling were studied in details.

2] The hydrolases studied are acid phosphatase, β-galactosidase, ATPase, amylase and proteinase. Except for amylase, which is absent in axis, all of these hydrolases are present in both cotyledon and axis. As expected the hydrolase activities present in cotyledon are much higher than that present in the axis. The amylase present in the cotyledon is of α-type. The specific activities of all these hydrolases present in the cotyledon increase gradually upto 120 h following imbibition and thereafter the activities of most hydrolases decline. The increase in acid phosphatase, β--galactosidase and ATPase are more pronounced in the early phase of growth whereas increase in α-amylase and proteinase are prominent in the later phase of growth. In axis part, except β-galactosidase, which remain unaltered, all the hydrolases tested show a slow and gradual increase in specific activity upto 144 h following imbibition.

3] To examine the role of axis on the development of hydrolases,

time course experiments of all hydrolases were carried out with half seeds e.g. cotyledon with axis and cotyledon without axis. In both halves, the specific activities of all hydrolases increase upto 120 h, but the specific activities of all hydrolases in axis attached half was always significantly higher than the corresponding activities in other half. This observation is a definite indication that appearance of a part of hydrolases are controlled by the growth of the axis. So, it may be suggested that axis may act as a sink.

4] Zymogram pattern reveals the presence of multiple forms of amylase in cotyledon and that of acid phosphatase in both organs. The patterns of both enzymes also change with the period of germination. Removal of axis from the cotyledon cause qualitative change in zymogram pattern of acid phosphatase in addition to quantitative decrease in enzyme activity.

5] Exogenous agents like indoleacetic acid (IAA), colchicine and para-coumaric acid (PCA) which influence the growth by different mechanisms, were tested to examine their effect on the development of hydrolases. PCA inhibits all the cotyledon hydrolases tested. Colchicine only inhibits acid phosphatase, ATPase and α-amylase of cotyledon. At higher dose, IAA inhibits acid phosphatase and α-amylase of cotyledon, but have no significant effect on axis hydrolases at the same dose.

6] Transcription inhibitor, actinomycin D (Act-D) and protein synthesis inhibitors chloramphenicol (CHL) and cycloheximide (CHX) inhibit growth of axis as well as development of hydrolases in both organs. Among the antibiotics, CHX was the most potent, it inhibits drastically all the hydrolases of both organs. Act-D inhibits hydrolase activities of cotyledon, but no significant effect on axis hydrolases. However, CHL is less potent.

7] Two forms of acid phosphatase (AP-I and AP-II) were separated and partially purified from axis by alcohol precipitation and DEAE

cellulose column chromatography. 50 and 70 fold of purification of AP-I and AP-II respectively was achieved over the crude extract with a total recovery of 57%.

8] Acid phosphatase from cotyledon have been also purified by employing $(NH_4)_2SO_4$ fractionation, DEAE and CMcellulose column chromatographies. At the level of DEAE cellulose chromatography, the activity splitted into 4 sets of fractions. Upon CMcellulose, only 3rd and 4th set of fractions splitted into 2 and 3 component peaks of activity respectively. Therefore, seven forms were obtained. They were designated as AP-I, AP-II, AP-IIIa, AP-IIIb, AP-IVa, AP-IVb and AP-IVc respectively. These forms reached 10-167 folds of purification over the crude extract with a total recovery of 43%.

9] Axis enzymes are more stable to heat and freezing compared to the cotyledon enzyme. Temperature and pH optima for enzyme activities of both organs are comparable.

10] Each of the forms isolated from axis and cotyledon shows single activity band upon PAGE (pH 4.6), although trace of contaminating band are seen in some of them. SDS-PAGE (at pH 8.3) shows single band for some of the fractions.

11] Molecular weights determined by gel filtration on Sephadex G-200 were estimated to be 101,000 and 118,000 daltons for AP-I and AP-II of axis while molecular weight of seven forms of cotyledon were in the range of 50,000-130,000 daltons.

12] Substrate specificity, metal ion requirement for all different forms of acid phosphatase were determined. None of them requires metal ions for activity. pNPP is the best substrate for axis enzymes whereas different forms of cotyledon enzyme differ in substrate specificity.

13] Effect of inhibitors on different forms of acid phosphatase were

also tested. Among them, molybdate and tungstate were the most potent whereas NaF, AsO_4^{-3}, PO_4^{-3} were less potent.

14] Activities of all different forms were enhanced significantly by Triton X-100 whereas all of them were inhibited by another detergent, SDS.

15] Urea has a interesting effect on the forms of axis. In presence of 8 M urea. AP-I is inactivated unlike AP-II.

16] Activation energies of all forms have been calculated to be in the range of 3.69-8.48 KCal/mole. Among the forms, AP-IVc and AP-IVa possess the lowest and highest values respectively.

17] Besides acid phosphatases, 4 forms of β-galactosidase have been isolated and purified from cotyledons of 4 day germinated mung bean seeds by employing $(NH_4)_2SO_4$ fractionation, DEAE and CM-cellulose column chromatographies. The four forms were designated as Gal-I, Gal-II, Gal-III and Gal-IV respectively. Gal-I achieved the highest fold of purification (224 fold) and it showed single band on SDS-PAGE (pH 8.3).

18] pH optima and temperature optima lie in the range of 3.5-4.0 and 55-60^{o}C respectively.

19] Molecular weights were estimated to be in range of 44,000-96,000 daltons.

20] Effect of metal ions and inhibitors on them were tested.

21] K_m values of different forms reveal that pNPG is better substrate for Gal-I and Gal-III whereas oNPG is better substrate for the others.

22] Activation energies of the four forms were in the range of 7.1-10.45 KCal $mole^{-1}$.

23] All the forms are rather stable towards heat. The activities of them do not decay considerably by preincubation of the enzymes at $55^{o}C$ for 30 min. But at $60^{o}C$, most of the activities were lost within 30 min.