AIM AND OBJECTIVE OF THE WORK

The detailed mechanism underlying the transformation of an embryo in the dry seed to a full grown plant or a mighty tree is greatly obscure and is a subject of much interest to the biologists long since. Dry seed is the dorment state of plant and it possesses remarkably low rate of respiration as well as metabolism. But, as soon as it comes in contact with water, enhancement of both processes become apparent. This phenomenon involves series of sequential but complex array of various biophysical and biochemical events. From the foregoing chapters, it is apparent that one of the most obvious biochemical changes associated with the transformation of a seed into its seedling, is an enhancement of hydrolytic activities within the reserve organ. This signifies that the hydrolases play important role during germination and growth of seedling. In fact, they are involved in the mobilisation of stored food from the reserve organ. So that the mobilised products are transported from cotyledons to the axis for utilisation and the subsequent growth of this organ. There are also several other factors that exert control on the metabolism in germinating seeds by influencing the mobilisation process. Axis also possesses some reserves, though much less in quantity compared to the major reserve organ. But this reserve material in the axis is to permit autonomous supply of nutrients in axis during its early germination stage. It is therefore important to learn about the occurrence and nature of hydrolytic activities in the axis organs in order to get a fuller understanding of the biochemical events occurring in the growing axis.

Considerable studies have been done to explore the mechanism and control of reserve mobilisation in the cotyledons and also the role of different endogenous and exogenous factors influencing them. But whether and to what extent the axis organ acts as a supplier of hormonal stimulant or as a sink for the drainage of the mobilised molecules from the cotyledon, thereby facilitating the hydrolase enzymes in the cotyledon thus favouring the reserve mobilisation there; is still matter of debate.

Many enzymes appear to exist in nature as a mixture of multiple forms or isozymes. They are often found to be tissue specific in origin

and function; sometimes they seem to play crucial roles in the metabolism (through differential gene expression) for determining metabolic diversity or as a survival mechanism under environmental stress.

Several hydrolytic enzymes such as acid phosphatase, amylase, glycosidases etc. have been reported to occur in multiple forms in a number of plant tissues (In this area too, most studies have been done on the cotyledon portion of the seeds). However several pertinent questions arise at this point regarding the hydrolytic enzymes in germinating seeds : What mobilising enzymes are involved in the axis ? When do they appear ? Are they newly synthesised or activated through some modification of pre-existing form ? What controls their development ? If they contain multiple forms, in what ways these forms are related (or are different) ?

To answer these questions attempts were made to investigate into the nature and components of some hydrolytic enzymes in germinating mung beans (*Vigna radiata*).

For this, detailed work was undertaken to study different biochemical aspects of some hydrolytic enzymes, such as their changing profiles as well as isolation, purification and characterisation of the individual forms from cotyledons and axis organs.

However, the first phase of work comprises of time course of several hydrolases of germinating seeds as well as effect of various exogenous factors on them. In this phase, studies were also carried out in details on the changing pattern of multiple forms of hydrolases. But these studies were not sufficient to provide knowledge about the properties of individual forms of hydrolases. So, in the second phase attempts have been taken to separate the multiple forms of hydrolases from each other and characterise. For this purpose, acid phosphatase from the axis of germinating mung bean seed has got the prior importance because reports on the detailed studies on the hydrolytic enzymes of axis organs are scanty, particularly in legume seeds. However, along with the axis, cotyledon of this germinating seed has also been

taken for similar study. This parallel side by side purification from two different organ of the same germinating seeds makes us enable to understand their different biological functions and properties. Lastly another important hydrolytic enzyme, β-galactosidase has been considered for purification and separation of multiple forms, because still now attempts have been taken occasionally to purify this enzyme from plant source. Moreover, very little is known about the physiological function of this enzyme during seed germination. So attempt of purification and separation of individual forms of β-galactosidase from the cotyledon of germinating seeds is very rationale in this scheme of work. In this connection it should be mentioned that recently Dey has isolated only single form of β-galactosidase from mature mung bean seeds [188]. Obviously, the isolation and purification of multiple forms of β-galactosidase from the same source during germination has got extra importance.

For these studies, mung beans were chosen because this legume seed is widely available, many aspects of its biochemistry are well known and can be germinated and grown into seedling easily in the laboratory condition.

The entire results of this study as presented in the following pages of this thesis, is conveniently divided into five chapters as follow :

CHAPTER - I : Hydrolytic enzyme activities in mung beans during germination and seedling growth

CHAPTER - II : Multiple forms of acid phosphatase (and amylase) and their changing pattern in germinating mung beans

CHAPTER - III : Isolation, purification and characterisation of multiple forms of acid phosphatase from mung bean axis

CHAPTER - IV : Isolation, purification and characterisation of multiple forms of acid phosphatase from mung bean cotyledon

CHAPTER - V : Isolation, purification, and characterisation of multiple forms of β-galactosidase from mung bean cotyledon

The results have been discussed in each chapter. Material and methods concerning the experiments and discussion are presented in the respective chapter. There is a general discussion following these five chapters and finally the results are summarised in a small section.