6. CONCLUSION

Myo-inositol is the most widely distributed inositol which occurs in all living organisms. It is the central component of several biochemical pathways and its products are important in several cellular processes. Lack of cellular level of inositol has been identified as the cause of "inositol-less-death" in *Saccharomyces cerevisiae*. Inositol phosphates are essential for signalling in almost all organisms; in plants, inositol hexakisphosphate contributes to phosphate storage. *Myo*-Inositol acts as a precursor for making phosphatidyl inositol, which is essential in all eukaryotes, including pathogenic fungi and protozoa, as well as in a small but very significant group of eubacterial pathogens that includes the mycobacteria. Galactinol synthesized from UDP-galactose and inositol is the basic substrate for the raffinose series of sugars in plants. These sugars have been implicated in stress tolerance and in carbohydrate transport. It is known that *myo*-inositol is a compatible osmolyte, and the molecule aids in maintaining an ideal osmotic state when a cell is placed in a hypertonic environment. Inositol may also be conjugated to auxins, preventing biological activity and allowing long-distance transport within the plant.

The present study elucidates the study of MIPS concerning its occurrence and fundamental biochemical characterization in bryophytes. During the study, appreciable L-*myo*-inositol-1-phosphate synthase activity has been detected in two bryophytes, *Asterella khasiana* and *Sphagnum junghuhnianum* exhibiting maximum titre of activity at reproductive stages. *A. khasiana* showed a four-fold higher enzyme activity and *S. junghuhnianum* showed a two-fold higher activity in its reproductive parts as compared to the vegetative parts. The enzymes isolated from both organisms showed almost equal

molecular wt of about 180kDa suggesting a trimeric structure of the protein as in many other cases of multicellular organisms. D-glucose-6-phosphate was found to be the specific substrate for MIPS from the bryophytes. However, the enzyme showed a little bit of activity in presence of D-galactose-6-phosphate and mannose-6-phosphate. The *A. khasiana* MIPS showed a K_m of 3.56 mM and 0.56 mM for D-glucose-6-phosphate and NAD^+ respectively while the V_{max} were found to be 0.71 mM and 0.68 mM for D-glucose-6-phosphate and NAD^+ respectively. In comparison, in *S. junghuhnianum* MIPS the K_m for D-glucose-6-phosphate and NAD^+ were 1.81mM and 0.25mM respectively while the V_{max} were 1.42 mM and 1.12 mM for D-glucose-6-phosphate and NAD^+ respectively.

The deduction of NH_4Cl and ME reduced MIPS activity to 31.80% and 34.51% respectively in of *A. khasiana* and 40.01% and 33.35% loss of activity respectively in *S. junghuhnianum.* No enzyme activity was found in absence of glucose-6-phosphate (substrate) in either *Asterella khasiana* or *Sphagnum junghuhnianum*. When NAD^+ was deducted from the reaction mixture, the enzyme from *A. khasiana* exhibited approximately 59.17% loss of activity and that from *S. junghuhnianum* exhibited about 68.41% loss of activity. The bryophytic MIPS operated between a pH ranges of 7.0 to 7.5. However, the maximum activity was found at pH 7.0. The MIPS from experimental bryophytes showed catalytic activity at temperatures 20ºC in *A. khasiana* and 10ºC in case of *S. junghuhnianum.* The activity of the enzyme in temperature as low as 10ºC, is a significant information which may be a good subject for future studies. The isolation of MIPS gene and the analysis of its homology *vis-à-vis* other stress tolerant MIPS genes may provide some interesting insight and pave the way for the use of the same in

the improvement of crop plants through the application of biotechnology. However, such studies were beyond the scope of this present research and will be taken up in near future. Whether the tolerance to cold stress in these plants is also associated with the NAD^+ bound to the enzyme as in the case higher temperature tolerance needs also to be found out through further research.

NH_4Cl was a strong stimulator of the enzyme and increased the rate of reaction in a concentration guided manner by 7.5 and 9.3 fold in *A. khasiana* and *S. junghuhnianum* respectively. K^+ showed stimulatory effect on bryophytic L-*myo*-inositol-1-phosphate synthase enhancing the rate of reaction by 1.6 fold in *A. khasiana* and 1.8 fold in *S. junghuhnianum* in a concentration dependent manner. Na^+ acted as a mild inhibitor of this enzyme from bryophytes, Li^+ acted as a strong inhibitor inhibiting the activity of the same by almost 81.9% and 76.1% in *A. khasiana* and *S. junghuhnianum* respectively. However, $MgCl_2$ acted as a stimulator, increasing the enzyme activity up to 1.6 fold and 1.2 fold in *Asterella khasiana* and *S. junghuhnianum* respectively. Among other divalent cations studied, Ca^{2+} was mildly stimulatory, while Mn^{2+}, Cu^{2+} and Cd^{2+} had varying degree of inhibitory effect from mild to medium. Zn^{2+} and Hg^{2+} showed extreme inhibitory property towards the bryophytic MIPS. Thus the bryophytic MIPS showed both the characteristics of Class-II aldolase that require divalent metals and a Class-III aldolase requiring NH_4^+ for its optimal activity. The finding that both plants exhibited stimulation of activity in presence of NH_4^+ as well as Mg^{2+} pointing to the dual character of MIPS from these amphibian plants is quite interesting. It cannot be ruled out that these plants may belong to a completely different category showing both the characters of Class-II and Class-III aldolases.

The enzyme, MIPS was purified up to about 46.34 fold over the homogenate fraction in case of the liverwort, *A. khasiana* and 58.67 fold from *S. junghuhnianum* over its homogenate fraction. In plants, multiple physiological and biochemical characters are controlled by MIPS and in turn plants also possess multiple MIPS genes suggesting that different activities may be controlled by different MIPS genes. Naturally, the presence of multiple forms of MIPS may not be ruled out in bryophytes also.

Further studies should explore both cytosolic and the particulate form of the enzyme and the enzyme expressed in different parts of the plants as well as different cell organelles at different developmental stages. Effort should also be made to purify the enzyme to homogeneity, to isolate and sequence the gene, to determine its homology with MIPS from other plants, to confirm the cold and drought tolerance properties of bryophytic MIPS, if any, and to overexpress the gene in order to find out whether transgenics expresses desirable traits in terms of stress tolerance and productivity.