ISOLATION AND CHARACTERIZATION OF AN ENZYME L-*MYO*-INOSITOL-1-PHOSPHATE SYNTHASE FROM BRYOPSIDA: *Brachymenium bryoides* HOOK. EX SCHWAGR. FOUND IN DARJEELING HILLS

[1]Sachina Yonzone, [2]Dhani Raj Chhetri, [3]Subhas Chandra Roy

[1]Research scholar, [2,3]Associate professor
[1]Plant Physiology and Biochemistry,
Post Graduate Department of Botany,
Darjeeling Government College, 734101, West Bengal, India.

***Abstract*: Brachymenium bryoides is an important genus of moss generally regarded as an indicator plant to understand the effects of climate change. In Darjeeling Himalayan region, like any other biodiversity hotspots, the moss flora is very rich, although neglected. Any biochemical work on these plants, especially the present species is absolutely lacking. The presence of enzyme L-myo-inositol-1-phosphate synthase (MIPS) is essential for the survival of this moss like any other groups of plants since it forms the cell walls as well as the sexual units in plants. The enzyme MIPS from Brachymenium bryoides utilizes D-glucose-6-phosphate as a substrate NAD^+ as a co-factor respectively. It showed a pH optimum at 7.5 while the temperature maximum was at $40S^0C$. The enzyme activity was remarkably stimulated by Ca^{2+}, NH_4^+ and Mg^{2+} and extremely inhibited by Zn^{2+}, Cd^{2+}, Mn^{2+} and Hg^{2+}. The K_{rn} values for D-glucose-6-phosphate and NAD^+ were found to be as 6.72 mM and 0.78 mM respectively while the V_{rnax} values were 1.62 mM and 1.11 mM for D-glucose-6-phosphate and NAD^+ respectively.**

***Keywords*: Brachymenium bryoides. myo-inositol, L-myo-inositol-1-phosphate synthase (MIPS), inositol synthase, UDP-galactose, D-glucose-6-phosphate**

1. Introduction

D-glucose-6-phosphate (G-6-P) irreversibly isomerizes to L-*myo*-inositol-1-phosphate by L-*myo*-inositol-1-phosphate synthase (MIPS). The product of this enzyme generates free *myo*-inositol on dephosphorylation [15]. The enzyme, MIPS has been isolated and characterized from a number of systems including bacteria [25], protozoa [19], lower plants [18,10,9,26], higher plants and animals [16,12,21]

In plants, *myo*-inositol becomes incorporated in a number of metabolic products viz., inositol phosphates, phosphoinositides, cell wall polysaccharides etc. As a free cyclitol, *myo*-Inositol has been proved essential for normal growth and development of plant tissue. Lack of cellular level of inositol has been identified as the cause of loss of viability in *myo*-inositol requiring mutants of *Saccharomyces cerevisiae* [8] and the same causes an inhibition of cell division in other plants under tissue culture conditions and the phenomenon has been termed as "inositol-less-death".

Inositol is associated with the resistance to both abiotic and biotic responses [29] specifically in halophytes under salt stress [30,28] active as an osmolyte. Different derivatives of *myo*-inositol also serve as compatible solutes and signalling molecules in response to salt stress in plants [13], oxidative stress, temperature stress, drought and desiccation stress etc. The phosphorylated derivates of inositol are considered as the important component of signal transduction in plants and animals [2]. The breakdown product of *myo*-inositol, D-glucuronic acid, is utilized for synthesis of cell wall pectic non-cellulosic compounds [14]. Intact plant tissue readily utilizes the glucuronic acid and rapidly converts it to cell wall polysaccharides and other products of glucuronic acid metabolism [17].

The present study is concerned with the study on the occurrences of MIPS (EC: 5.5.1.4) from a moss from Darjeeling hills. Partial purification of MIPS and characterization of its properties from *Brachymenium bryoides* have been reported here.

2. Materials and Methods

Fresh specimens of bryophyte *Brachymenium bryoides* Hook. ex Schwagr (*B.bryoides*) of Darjeeling Hills were collected from the Birch Hill area in Darjeeling hills (circa 2134 m amsl.) in $87^0$59' - $88^0$53' E and $26^0$31' - $27^0$13' N in the Eastern Himalayas of India.

Purification of L-*myo*-inositol-1-phosphate synthase from *B.bryoides*

The enzyme L-*myo*-inositol-1-phosphate synthase was purified from *B.bryoides* following the method outlined below:
All the operations were carried out between 0˚ C to 4˚ C

Preparation of crude extract: About 100g of freshly collected plant material was thoroughly washed with sterile distilled water twice. The sample was then homogenized in a mortar and pestle with 2 vol of 50 mM Tris-acetate buffer (p^H7.5) containing 0.2 mM ME in presence of neutral sand. The slurry was centrifuged at 1,000×g for 5 min in a Plasto Crafts Superspin-R centrifuge. The pellet discarded and the supernatant designated as crude extract (1K).The crude extract was spun again at 11,400×g for 20 min and the supernatant was collected (10K).

Streptomycin sulphate precipitation: To the 10K sample**, s**treptomycin sulphate powder was added slowly with constant stirring to a final concentration of 2% (w/v) to the 10K supernatant in order to remove nucleic acids. After mixing the required amount of streptomycin sulphate, the mixture was kept in an ice-bucket at 0^{o}C for 15 min and then centrifuged at 11,4000×g for 15 min. The pellet was discarded and the supernatant collected (SS).

Ammonium sulphate fractionation: The streptomycin sulphate treated supernatant (SS) was then made 0-70 % saturated with ammonium sulphate by adding the requisite quantity of the solid salt (43.6g/100 ml) slowly with constant stirring. The mixture was kept in an ice-bucket for 15 min and then centrifuged at 11,400×g for 20 min. The supernatant was discarded and the pellet collected. The pellet was dissolved in minimal volume of 50mM tris-acetate buffer (pH7.5) containing 0.2 mM ME, poured into a dialysis sac and dialyzed against 500 volumes of the same buffer. On completion of dialysis, the ammonium sulphate fraction (A_2S) was recovered from the dialysis sac.

Anion exchange chromatography with DEAE cellulose: The dialyzed A_2S fraction was adsorbed in pre-equilibrated DEAE cellulose. After adsorption for two hours, the preparation was loaded in a glass column (1.2×8.0 cm) and the effluent was collected. After this, the column was washed with one bed volume of the same buffer. Lastly, the adsorbed protein was eluted from the column with the linear gradient of 0-0.5 M KCl in 50mM Tris acetate buffer (p^H7.5) containing 0.2 mM ME. Fractions at the rate of 1.1 ml/10 min were collected. Each fraction was assayed for *myo*-inositol synthase activity. The active fractions (DE) was pooled and used for the next purification step.

Molecular sieve chromatography through BioGel A-0.5 m: The enzymatically active fractions from the previous step was pooled together and loaded in a column (0.8 X 10.0 cm) of BioGel A-0.5m pre-equilibrated with 50 mM Tris-acetate buffer (pH 7.5) containing 0.2 mM ME. Proteins were eluted with the same buffer in fractions of 0.75 ml/8 mins. Fractions containing MIPS activity were pooled and dialyzed against 1.5 L of 50 mM Tris-acetate buffer (pH 7.5) containing 0.2 mM ME (BioGel fraction). This preparation was concentrated and used as the enzyme source for further characterization.

Enzyme activity assay: The MIPS activity was assayed by the procedure of Barnett *et al.*, (1970) with slight modifications. The assay mixture contained 50 mM tris-acetate (pH 7.5), 14 mM NH_4Cl, 0.8 mM NAD^+, 5 mM ME, 5 mM G-6-P and an appropriate aliquot (100-200 µg) of enzyme protein in a total volume of 500 µl. The activity of the enzyme was determined by estimating the product-specific release of inorganic phosphate from *myo*-inositol-1-phosphate by MIPS reaction.

Inorganic phosphate determination: Inorganic phosphate was determined by the method of Chen *et al.*, (1956) with slight modifications. A freshly prepared Pi-reagent (2.8 ml.) containing H_2SO_4 (6N), ascorbic acid (10%, w/v), chilled ammonium molybdate (2. 5%, w/v) and H_2O mixed in 1:1: 1: 2 ratio was added to the reaction mixture and incubated at 37 ºC for 1h. The absorbance was measured at 820 nm and the inorganic phosphate released was quantified with a standard curve prepared using K_2HPO_4.

Protein determination: Protein was determined according to the method of Bradford (1976) with BSA as a standard. The protein content in fractions obtained from column chromatography was also determined by measuring absorbance at 280 nm
.

Determination of specific activity of inositol synthase: The specific activity of L-*myo*-inositol-1-phosphate synthase will be calculated by determining the number of moles of inorganic phosphate released (from *myo*-inositol phosphate) per mg of protein. The activity may be defined as nmol L-*myo*-inositol-1-phosphate (I-1-P) produced per hour per mg of protein i.e. nmol I-1-P produced (mg protein)$^{-1}h^{-1}$**.**

3. Result and Discussion

Purification of L-myo-inositol-1-phosphate synthase

The enzyme, L-*myo*- inositol-1-phosphate synthase was isolated and purified from the thallus of *Brachymenium bryoides.* The activity of the enzyme was much more pronounced in the reproductive part bearing thallus than in vegetative thallus (Table-1). This follows the common trend as in other bryophytes [7]. The summary on the purification of MIPS is given in Table 2. The ultimate chromatography on BioGel A 0.5m column resulted in about 22 fold purification of the enzyme with about 22 fold recovery over the crude homogenate fraction in the present study.

Characterization of the purified enzyme

Requirements for B. bryoides MIPS activity: The *B. bryoides* MIPS, when assayed in presence of 50 mM tris-acetate buffer (pH 7.5), 14 mM NH_4Cl, 0.8 mM NAD, 5 mM 2-mercaptoethanol (ME) and 5 mM glucose-6-phosphate (G-6-P) recorded maximal activity (Table-3). When the specific substrate (G-6-P) was not added in the incubation mixture, the enzymatic synthesis of L-*myo*-inositol-1-phosphate could not be detected. About 33% activity was lost when tris buffer was omitted from the reaction

mixture. Similarly, 31% of enzyme activity was lost in absence of tris-buffer from the reaction mixture in case of *Marchantia nepalensis* [6]. Deduction of NH_4Cl or ME resulted in the loss of enzyme activity by about 23% and 25% respectively. In comparison, the absence of NAD^+, NH_4Cl and ME decreased the activity of *Euglena gracilis* MIPS by 70%, 23% and 30% respectively [9]. In hepaticopsid bryophyte, *Lunularia cruciata*, the omission of Glucose-6-phosphate (G-6-P), tris buffer, NH_4Cl and ME causes the loss of activity by 100%, 25%, 40% and 27% respectively [7]. Curiously enough, in the present study, both the NH_4Cl and ME exert almost equal influence to the enzyme activity.

Substrate specificity: The *B. bryoides* MIPS have been found to utilize G-6-P as the exclusive substrate for the production of L-*myo*-inositol-1-phosphate (MI-1-P). Among other hexose phosphates tested, such as D-fructose-6-phosphate, D-fructose-1, 6-bisphosphate and D-glucose-1,6-bisphosphate used in place of G-6-P at identical concentrations (5 mM), all were ineffective. This result is in conformity with MIPS from other sources [26]. Nevertheless, a basal level of approx 3-4% enzyme activity in presence of all these substrate isomers was seen which may be the result due to non *myo*-inositol specific phosphate release in the assay reaction (Table-4)

Stability of the enzyme: An important feature of *B. bryoides* MIPS is the moderate stability of its catalytic activity. Stability varies with the enzyme at different stages of purification. While the low speed supernatant remained active for 7-8 days when stored at −20 ºC, the BioGel purified fractions maintained its activity only up to 3-4 days when stored at identical temperature. However, repeated freezing and thawing resulted in remarkable loss of activity. Addition of enzyme stabilizer, 2-mercaptoethanol (ME) or dithiothritol (DTT) considerably increased the activity of the enzyme.

Enzyme and time linearity: The *B. bryoides* MIPS exhibited enzyme linearity upto 350 µg of protein concentration under optimal assay conditions (Fig-1). In contrast, the *Diplopterygium glaucum* MIPS showed enzyme linearity upto 280 µg and in *Marchantia nepalensis* it was upto 250 µg [6,4]. The rate of enzyme reaction proceeded linearly upto 60 minutes with G-6-P as the substrate (Fig-2). This is quite different from MIPS from that of the *Acer pseudoplatanus* cell culture that shows time linearity upto 150 minutes [18].

pH – activity relationship: The *B. bryoides* MIPS exhibited optimum activity at a pH range of 7.0 – 7.5 when 50 mM tris-acetate buffer at a pH range of 6.0 – 8.5 were employed (Fig-3). This value is a little less in comparison to the pH optima for MIPS from other species like *Spirulina platensis* -7.8, *Euglena gracilis*-8.2 [26] and rice cell culture-8.4 [11]. However, it is in conformity with earlier studies with *Swertia bimaculata* enzyme [5].

Effect of temperature: The effect of temperature was studied in the temperature range of 0 - 60ºC at 10ºC intervals. The activity of the enzyme was negligible at 10ºC and gradually increased after 20ºC reaching the maximum 40ºC (Fig-4). This optimum temperature is relatively high as compared to that of *Spirulina platensis, Euglena gracilis, Oryza sativa and Vigna radiata* [26].

Effect of metal ions: Effect of different metal ions was tested in 5 mM concentrations using chloride salts of metals. Of the monovalent cations tested K^+ and Li^+ had little effect, while NH_4^+ was an appreciable stimulator of the enzyme. NH_4^+stimulation of *B. bryoides* MIPS was to the tune of 1.86 times in contrast to the *Acer pseudoplatanus* MIPS which is stimulated 2.3 times with NH_4^+ [18]. The remarkable feature of *B. bryoides* MIPS was its stimulation by divalent cations. Using the similar concentrations of divalent cations it was revealed that Ca^{2+} caused the stimulation of the enzyme by 2.39 times while Mg^{2+} was slightly stimulatory to the enzyme activity. Of the other divalent cations Cu^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+} and Hg^{2+} strongly inhibitory (34%, 64%, 67%, 68%, and 86% respectively) (Table-5). In general, the effects of monovalent and divalent cations are similar to those obtained from other sources except for the effect of Ca^{2+} which is highly stimulatory to the MIPS activity in this case.

Reaction rate-substrate concentration relationship: Kinetic studies were carried out using G-6-P (substrate) in different concentrations. The reaction rate was found to increase with respect to G-6-P upto a concentration of 10 mM. The K_m value for G-6-P calculated from Michaelis-Menten plot was 6.72 mM and the V_{max} value was calculated as 1.61 mM. The K_m value for G-6-P is quite far away from that of pine pollen (K_m=0.33) [12], but somewhat closed for that from animal sources e.g., 2.7 for bovine testis enzyme [22]; 3.89 for rat testis enzyme [20] and 4.4 for rat brain enzyme [1].

Reaction rate-co-enzyme concentration relationship: Between concentrations of 0-1.0 mM of NAD (co-enzyme) the activity of purified enzyme was found to increase only upto 0.2 mM concentration. With more increase in co-enzyme concentration the activity could not be increased. The K_m value for NAD was 0.78 and the V_{max} value was calculated as 1.11 mM from the Michaelis-Menten equation. The K_m value for NAD+ was entirely different from that obtained from other sources e.g., *Euglena gracilis* (K_m= 0.16-0.20) [26] and *Entamoeba histolytica* (K_m= 0.66) [9].

The present study reports the occurrence and partial purification of MIPS for the first time from *B. bryoides*. Table-2 summarizes the partial purification of this enzyme from the moss. The enzyme from *B. bryoides* was highly specific for G-6-P. Though the enzyme exhibits its optimal activity in presence of co-enzyme NAD^+, still it could maintain about one third of the total activity when NAD^+ was not added externally (data not shown). This indicates the presence of bound NAD+ in the molecular architecture of this enzyme which has also been reported earlier [9]. MIPS is involved in the metabolic utilization of G-6-P and it also generates Ribulose-5-phosphate as a product. The activity of this enzyme also seems to be related to that of Fructose-1,6-bisphosphatase and the various biochemical activities of the phosphate esters of *myo*-inositol [23,31,24]. This indicates an important function played by this enzyme in metabolism and makes this study worthwhile in understanding the basic metabolism in plants [27]. In *B. Bryoides*, the ultimate chromatography on BioGel A-0.5m yielded a 22 fold increase in the enzyme activity with about 11% recovery. This study may lead to further research to look for homogeneous MIPS preparation from *B. bryoides*, sequencing the responsible gene and analysis of sequence homology.

Table 1. L-*myo*-inositol-1-phosphate synthase activity in vegetative and reproductive stages of the moss, *B. bryoides.*

Tissue type	Enzyme source	Specific activity [nmol I-1-P produced $(mg)^{-1}$ protein h^{-1}]
Vegetative	Homogenate	154.00
	Low speed supernatant	198.00
Reproductive	Homogenate	162.52
	Low speed supernatant	814.53

Table 2. Summary of partial purification of L-*myo*-inositol-1-phosphate synthase from *B. bryoides.*

Fraction	Total volume(ml)	Specific activity [nmol I-1-P produced (mg)-1 protein h-1]	Total activity [nmol I-1-P produced $(mg)^{-1}$ protein h^{-1}]	Recovery (%)	Purification (fold)
Homogenate	72	26.67	1920.96	100	1.00
10K- supernatant	70	15.76	1103.20	57.42	0.59
SS-fraction	69	45.43	3134.67	163.18	1.70
A_2S-fraction	3	155.86	467.58	24.34	5.84
DE-fraction	2.2	190.86	419.89	7.15	21.85
Bio Gel A 0.5m fraction	1.5	283.87	425.80	10.67	22.16

Table-3. Effect of composition of incubation medium on L-myo-inositol-1-phosphate synthase from *B. Bryoides*

Conditions	Specific activity [nmol I-1-P produced $(mg)^{-1}$ protein h^{-1}]	Percent activity
Complete set	155.86	100
Without substrate (G-6-P)	0.00	0.00
Without NH4Cl	49.62	77.33
Without ME	48.38	74.98
Without buffer (tris-acetate)	43.50	67.42
Heat killed enzyme	0.00	0.00

Table -4. Effect of some substrate isomers on L-myo-inositol-1-phosphate synthase from *B. bryoides.*

Compound	Concentration (mM)	Specific activity [nmol I-1-P produced $(mg)^{-1}$ protein h^{-1}]	Percent activity
D-glucose-6-phosphate	10.0	155.86	100
D-fructose 1,6 bisphosphate	10.0	2.48	3.84
D-fructose-1-phosphate	10.0	1.98	3.06
D-glucose-1-phosphate	10.0	1.86	2.88

Table-5. Effect of monovalent and divalent cations on L-myo-inositol-1-phosphate synthase activity from *B. bryoides.*

Cation	Concentration	Specific activity [nmol I-1-P produced (mg)-1 protein h-1]	Percent activity
Control	0	216.76	100.00
K^+	5	232.97	107.47
Li^+	5	268.81	124.01
NH_4^+	5	403.22	185.88
Ca^{2+}	5	519.71	239.58
Mg^{2+}	5	286.73	132.18
Cu^{2+}	5	143.36	66.08
Zn^{2+}	5	79.21	36.51
Mn^{2+}	5	71.68	33.04
Cd^{2+}	5	68.81	31.72
Hg^{2+}	5	30.46	14.04

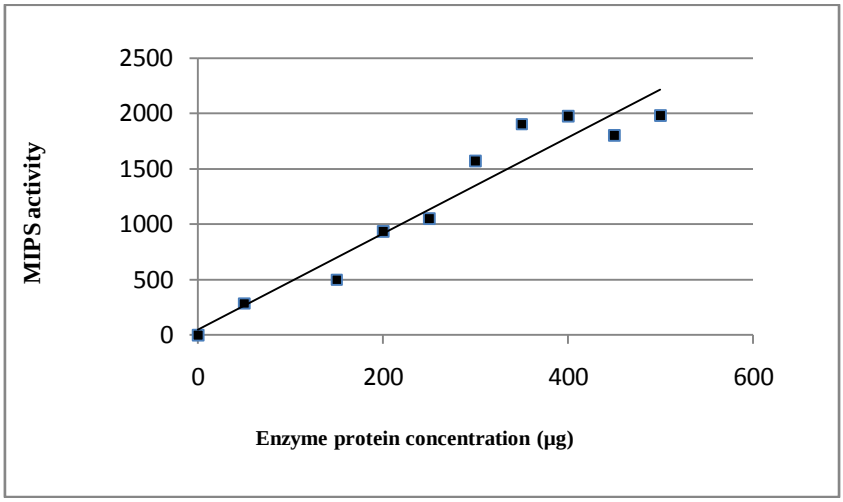

Fig 1. Effect of different enzyme concentration on *B. bryoides* MIPS activity. Enzyme activity is expressed as [nmole of Inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1}]

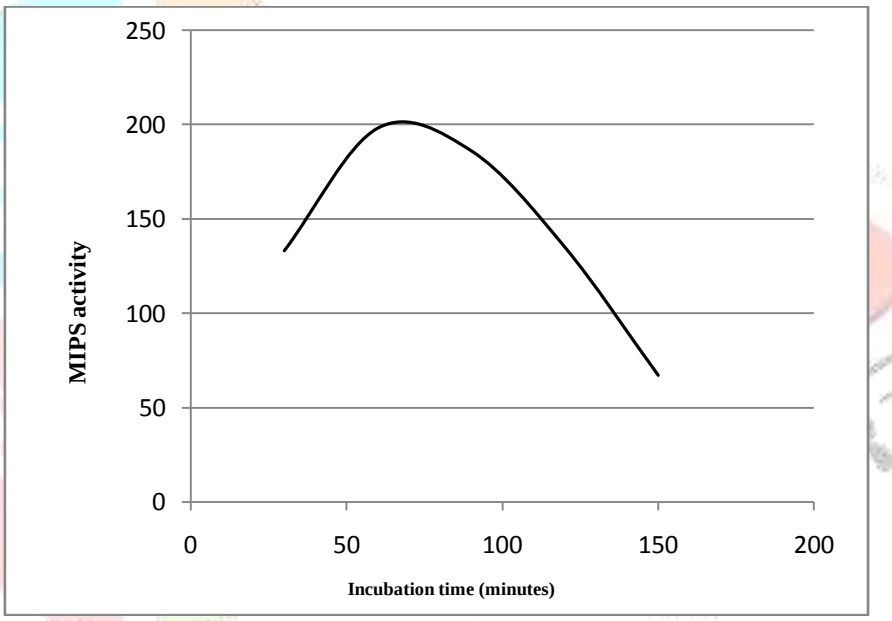

Fig 2. Effect of different incubation time on *B. bryoides* MIPS activity. Enzyme activity is expressed as [nmole of Inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1}]

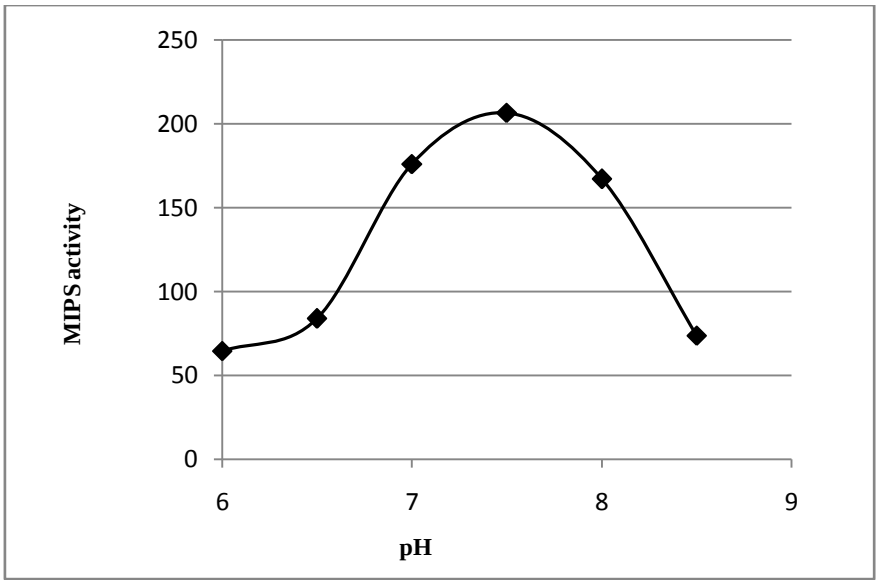

Fig 3. Effect of different pH on MIPS activity in *B. bryoides* MIPS activity. Enzyme activity is expressed as [nmole of Inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1}]

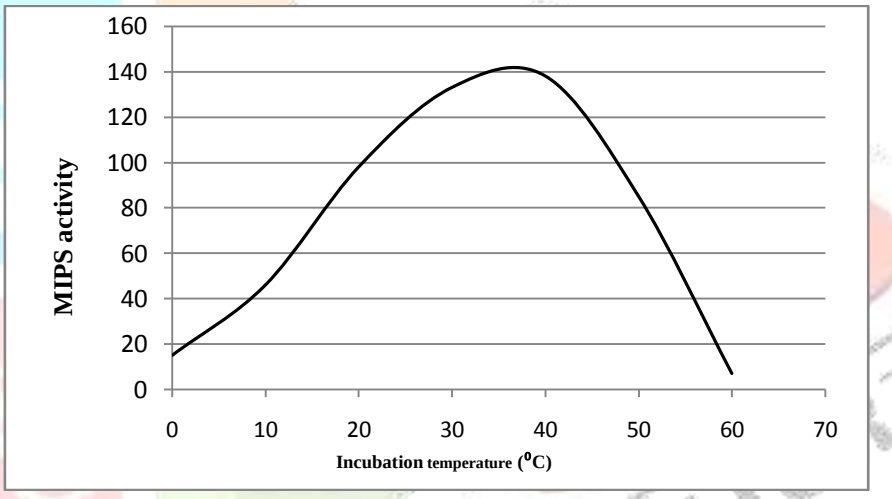

Fig 4. Effect of different reaction temperature on *B. bryoides* MIPS activity. Enzyme activity is expressed as [nmole of Inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1}]

4. Acknowledgement
We are indebted to PG Department of Botany, Darjeeling Government College for allowing laboratory facilities in carrying out these experiments.

References

[1] Adhikar, J. and Majumdar, A.L 1988. L–*Myo*–Inositol–1-phosphate synthase from mammalian brain: partial; purification and characterization of the fetal and adult enzyme. Indian Journal of Biochemistry and Biophysics, 25: 408-412.
[2] Boss, W.F. Davis, A.J. Im, Y.J. Galvao, R.M. and Perera, I.Y. 2006. Phosphoinositide metabolism: towards an understanding of subcellular signaling. Subcellular Biochemistry, 39: 181-205.
[3] Chen, R.S. Toribara, T.Y. and Warner, H. 1956. Microdetermination of phosphorous. Annals of Biochemistry, 28: 1756-1758.
[4] Chhetri, D.R, Mukherjee, A.K. and Adhikari, J. 2006. *Myo*-inositol content in pteridophytes and the isolation and characterization of L-*myo*-inositol-1-phosphate synthase from *Diplopterygium glaucum*. Brazilian Journal of Plant Physiology, 18(2): 291-298.
[5] Chhetri, D.R. Pradhan, B. and Mukherjee, A.K. 2008. Isolation and biochemical characterization of L-*myo*-inositol-1-phosphate synthase from *Swertia bimaculata* Hook.f. &Thomas. Journal of Hill Research, 21(2): 59-66.
[6] Chhetri, D.R. Yonzone, S. Mukherjee, A.K. and Adhikari, J. 2006. L-*myo*-inositol-1- phosphate synthase from *Marchantia nepalensis*: partial purification and properties. General and Applied Plant Physiology, 32(3-4): 153-164.
[7] Chhetri, D.R. Yonzone, S. Tamang, S. and Mukherjee, A.K. 2009. L-*myo*-inositol-1-phosphate synthase from bryophytes: purification and characterization of the enzyme from *Lunularia cruciata* (L.) Dum. Brazilian Society of Plant Physiology, 21(3): 243-250.
[8] Culberston, M.R. and Henry, S.A. 1975. Inositol requiring mutants of *Saccharomyces cerevisiae*. Genetics, 80: 23-40.

[9] Dasgupta, S. Adhikari, J. and Majumdar, A.L. 1984. *Myo*-inositol–1–phosphate synthase from lower plant groups: purification and properties of the enzyme from *Euglena gracilis*. Plant Physiology, 61: 412-416.
[10] Donahue, T.F. and Henry, S.A. 1981b. *Myo* inositol 1-phosphate synthase: Characteristics of the enzyme and identification of its structural gene in Yeast. The Journal of Biological Chemistry, 256: 7077-7085.
[11] Funkhouser, E.A. and Loewus, F.A. 1975. Purification of *myo*-inositol-1-phosphate synthase from rice cell culture by affinity chromatography**.** Plant Physiology, 56: 786-790.
[12] Gumber, S.C. Loewus, M.W. and Loewus, F.A. 1984. *Myo*-inositol-1-phosphate synthase from pine pollen: Sulphahydryl involvement at the active site. Archives of Biochemistry and Biosphysics, 231: 372–377.
[13] Kido, E.A. Neto, J.R.C. Silva, R.L.O. Belarmino, L.C. Neto, J.P.B. Soares-Cavalcanti, N.M. Pandolfi, V. Silva, M.D. Nepomuceno, A.L. and Benko-Iseppon, A.M. 2013. Expression dynamics and genome distribution of osmoprotectants in soybean: identifying important components to face abiotic stress. BMC Bioinformatics, 14: 1-11.
[14] Loewus, F.A. 2006. Inositol and plant cell wall polysaccharide biogenesis. Sub-Cellular Biochemistry, 39: 21-45.
[15] Loewus, F.A. Chen, M.S. and Loewus, M.W. 1973. Inositol and plant polysaccharide biogenesis. Loewus FA (ed), pp 1-28, Academic Press, New York.
[16] Loewus, F.A. and Loewus, M.W. 1983. *Myo*–Inositol: its biosynthesis and metabolism. Annual Review of Plant Physiology, 34: 137-161.
[17] Loewus, F.A. Loewus, M.W. Maity, I.B.and Rosenfield, C.L. 1978. Aspects of *myo*-Inositol metabolism and biosynthesis in higher plants. In: Wells WW, Eisenberg F Jr. (eds) Cyclitols and Phosphoinositides, pp. 249-267 Academic Press, New York.
[18] Loewus, M.W. and Loewus, F. 1971. The isolation and characaterization of D-Glucose 6-phosphate cycloaldolase(NAD dependent) from *Acer pseudoplatanus* L. cell cultures. Plant Physiology, 48: 255-260.
[19] Lohia, A. Hait, N.C. and Majumdar, A.L. 1999. L-*Myo* Inositol 1-phosphate synthase from *Entamoeba histolytica*. Molecular and Biochemical Parasitology, 98: 67-79.
[20] Maeda, T. and Eisenberg, F. Jr. 1980. Purification, structure and catalytic properties of the 1 L-*myo*-inositol-1-phospahte synthase from rat testis. Journal of Biological Chemistry, 255: 8458–8464.
[21] Majumder, A.L. Johnson, M.D. and Henry, S.A. 1997. 1L-*myo*-inositol-1-phosphate synthase. Biochimica et Biophysica Acta, 1348: 245–256.
[22] Mauck, L.A. Wong, Y.H. and Sherman, W.R. 1980. L-*myo*-Inositol-1-phosphate synthase from bovine testis: purification to homogeneity and partial characterization Biochemistry, 19:3623-3629.
[23] Murthy, P.P.N. 1996. Inositol phosphates and their metabolism in plants. In: Biswas BB, Biswas S (eds) Sub-cellular Biochemistry, Vol 26: pp 227–255. Plenum Press, New York.
[24] Ogawa, S. 1999. Chemical components of Rice bran: *Myo* Inositol and related compounds, a review. Anticancer Research, 19: 3635-3644.
[25] Pittner, F. and Hoffmann-Ostenhof, O. 1979. Preparation of homogeneous crystals of myo-inositol 1-phosphate synthase from rat testicles--further data on the chemical and catalytic properties of the enzyme (studies on the biosynthesis cyclitols, XXXIX). Molecular and Cellular Biochemistry, 28: 23-26.
[26] RayChoudhury, A. Hait, N.C. Dasgupta, S. Bhaduri, T.J. Deb, R. and Majumdar, A.L. 1997. L-*myo*-Inositol 1-phosphate synthase of plant sources. Plant Physiology, 115: 727-736.
[27] RayChoudhury, A. and Majumdar, A.L. 1996. Salinity induced L-*myo*-Inositol–1-phosphate synthase in rice (*Oryza sativa*). Plant Cell and Environment, 19: 1437–1442.
[28] Sengupta, S. Patra, B. Ray, S. and Majumder, A.L. 2008. Inositol methyl transferase from a halophytic wild rice, *Porteresia coarctata* Roxb. (Tateoka): regulation of pinitol synthesis under abiotic stress. Plant Cell and Environment, 31: 1442-1459.
[29] Shinozaki, K. and Yamaguchi-Shinozaki, K. 2006. Gene networks involved in drought stress response and tolerance. Journal of Experimental Botany, 58: 221–227.
[30] Taji, T. Takahashi, S. and Shinozaki, K. 2006. Inositols and their metabolites in abiotic and biotic stress responses. Sub-Cellular Biochemistry, 39: 239-264.
[31] Voglmaier, S.M. Bembenek, M.E. Kaplin, A.I. Dorman, G. Olszewski, J.D. Prestwich, G.D. and Snyder, S.H. 1997. Purification and assay of inositol hexakisphosphate kinase and diphosphoinositol pentakisphosphate kinase. In: Shears S (ed) Signalling by Inositides, pp. 195-201. Oxford University Press, New York**.**