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Acid phosphatase activity in some Indian Liverworts

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Abstract – Seasonal changes in specific activity of acid phosphatase have been examined in 12 taxa of liverworts collected from different areas of Himachal Pradesh. The collection period was divided into three seasons relevant to bryophyte life history: rainy, winter and end of the growing season. The specific activity of acid phosphatase was highest during the rainy season in all except *Asterella angusta* and the lowest at the end of the growing season, thus indicating that the activity of the enzyme varies seasonally.

Liverworts / acid phosphatase activity / seasonal variations

INTRODUCTION

Liverworts comprise nearly 6000 species globally (Asakawa *et al.*, 2013), with 121 genera with 675 species in the Indian subcontinent (Dandotiya *et al.*, 2011). They grow on moist, cool and shaded habitats, and are ecologically important in plant succession and production of phytomass. With the change in seasons, the plants experience long and short photoperiods, low and high temperatures and changes in moisture content leading to the changes in physiological and biochemical processes, resulting in variations in the storage compounds. In India, Kapila & Dhawan (2000), Kapila *et al.* (2014), Kapila & Thakur (2016) and Thakur & Kapila (2016) have worked on carbohydrate content, protein, free amino acids and enzymatic activities (α-amylase, β-amylase, invertase, protease) of West Himalayan bryophytes, but there are no reports on seasonal variations of acid phosphatase in this country.

Acid phosphatase involved in phosphate uptake and transferring phosphate across cell membranes (Besford & Syred, 1979) catalyzes the removal of inorganic phosphate(orthophosphate) from organic phosphate esters in acidic medium (Vincent *et al.*, 1992). It can also provide an indication of P deficiency in plants (Kolari & Sarjala, 1995). Acid phosphatase plays an important role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Mishra & Dubey, 2008). Activity has been shown to increase under low phosphorus availability e.g. in leaves of tomato (Besford, 1979a; 1979b), cucumber and wheat (Besford, 1979c) and cultured cells of rice (Igaue *et al.*, 1973).

In flowering plants, acid phosphatases provide inorganic phosphate to the growing plant during germination and many different phosphate esters of sugars and substrates stored in the seed and seedling need to be hydrolyzed during germination and growth (Schultz & Jensen, 1981).

In bryophytes, Maier & Maier (1972) reported the phosphatases from *Polytrichum piliferum*. Higher phosphatase activities were recorded in the active

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zone of transport of solutes between gametophyte and sporophyte, while these activities declined sharply as the seta matured and the spores dispersed (Hébant & Suire, 1974). Pakarinen (1978) studied the concentration of phosphorus in *Sphagnum* and revealed that oligotrophic species (poor solute habitats) show low concentration, whereas mesotrophic and eutrophic species (rich solute habitats) show higher concentration of phosphorus. Phosphate supply and enzyme activity reported in *Sphagnum* has been found to be similar to that in flowering plants (Bieleski, 1973; Clark, 1975). Acid phosphatase activity was negatively correlated with phosphate content in 11 *Sphagnum* species (Press & Lee, 1983).

Christmas & Whitton (1998a, 1998b) examined the surface phosphatase activity was in two mosses *Rhynchostegium riparioides* and *Fontinalis antipyretica*. There was a low level of surface PMEase (phosphomonoesterase) activity when the moss is phosphate-rich, whereas PDEase (phosphodiesterase) activity is apparently absent under phosphate-rich conditions and is induced later than PMEase activity. Tessler *et al.* (2014) observed the influence of pH on bryophyte distribution and reported higher PMEase activity at lower pH than at higher pH in stream bryophytes.

Seasonal variations in acid phosphatase were noted in the aquatic mosses of northern England by Turner *et al.* (2003) and Ellwood *et al.* (2007). There are no reports on the seasonal activity of phosphatase in the Indian liverworts. The objective of the present study was to observe seasonal fluctuation in acid phosphatase activity of West Himalayan liverworts during the three seasons relevant to the liverwort life history and to investigate whether any such changes could be related to phosphorus status of liverworts.

MATERIAL COLLECTION

To study the seasonal variation of acid phosphatase in liverworts, the collection period was divided into three seasons relevant to bryophyte life histories with different temperature ranges and rainfall as mentioned in Kapila *et al.* (2014)**:** July-September (rainy season), October-December (winter season) and January-March (end of growing season).

The materials were collected from Himachal Pradesh**,** Western Himalaya (Fig. 1). The voucher specimens are deposited in the herbarium of Department of Botany, Panjab University, Chandigarh (PAN). Details of taxa, nature of substrata and their PAN numbers are given in Table 1. The genera of liverworts are arranged according to the classification by Crandell-Stotler and Stotler (2000).

EXPERIMENTAL METHOD

Freshly collected whole plants of each species were thoroughly washed with distilled water to remove all adhering soil particles and any associated organisms. The material was dried in the folds of sterilized blotting paper, and 500 mg homogenized in a pestle and mortar with 10 ml distilled water. It was centrifuged at 3000 rpm for 20 min to use the supernatant for analysis. Acid phosphatase activity was measured by the method of Bessey *et al.* (1946). To 0.5 ml substrate solution

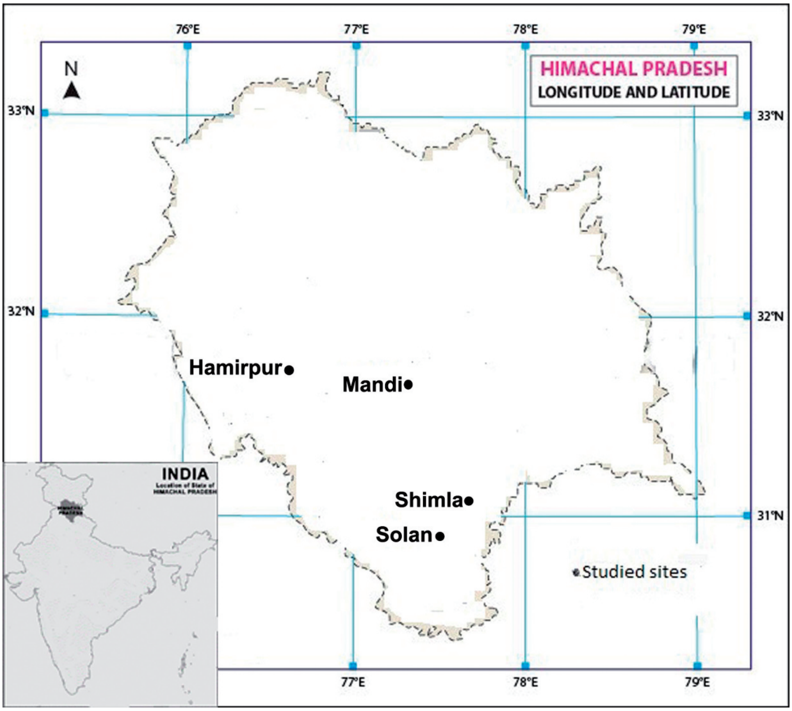

Fig. 1. Areas of collection.

Table 1. The names of studied taxa, their substrata, habitats and herbarium reference numbers

Name of Taxon	*Substratum and Habitat*	*Herbarium Reference Number*
AYTONIACEAE		
Asterella angusta Steph.	Wet soil	PAN 6110
Asterella pathankotensis Kash.	Wet soil on stone wall	PAN 6111
Plagiochasma appendiculatum Lehm. et Lindenb.	Wet soil on stone wall	PAN 6105
Plagiochasma articulatum Kash.	Wet soil	PAN 6106
Plagiochasma intermedium Lindenb. et Gottsche	Wet soil	PAN 6107
Reboulia hemisphaerica (L). Raddi	Wet soil	PAN 6109
CONOCEPHALACEAE		
Conocephalum conicum (L.) Dum.	Wet soil near stream, shaded	PAN 6112
MARCHANTIACEAE		
Dumortiera hirsuta (Sw.) Nees	Wet soil near stream, shaded	PAN 6104
Marchantia nepalensis L. et L.	Wet soil	PAN 6102
Marchantia palmata Nees	Wet soil	PAN 6103
TARGIONIACEAE		
Targionia indica Udar et Gupta	Wet soil	PAN 6113
PELLIACEAE		
Pellia endiviifolia Dicks.	Wet soil near stream, shaded	PAN 6108

(10 ml distilled water + 50 mg p-Nitrophenol phosphate + 25 ml 0.1M acetate buffer pH 4.8) 0.5 ml enzyme extract was added into the test tubes and incubation was done at 35°C for 30 minutes. Then the reaction was terminated by adding 3.5 ml 0.1 NaOH. The absorbance was read at 420nm. A standard was prepared by taking known amount (100μg) of p-nitrophenol. Enzyme activity was evaluated from the amount of p-nitrophenol produced and expressed as μg $min^{-1}mg^{-}1$ protein. For each analysis, the data obtained from three replicates were represented as mean values and standard error (S.E.).

RESULTS

The duration of the first experimental period was from July to September (rainy season) – a period characterized by maximum rainfall and slightly high temperature than normal and liverworts are in the young luxuriantly growing vegetative stage. The duration of the second experimental period was from October to December (winter season) which is the most favorable reproductive period of these plants since the temperature is suitable and rainfall is lower than rainy season. The third experimental period from January to March is the end of the favorable period of growth when the plants reach maturity after completing their life cycle and release spores.

In the rainy season the highest specific activity of acid phosphatase was in *Plagiochasma appendiculatum* and the lowest in *Asterella angusta* (Table 2). In the winter season, *A. angusta* had the highest activity and *P. articulatum* the lowest. At the end of the growing season activity was maximum in *Targionia indica* and minimum in *Pellia endiviifolia.*

Table 2. Specific activity of enzyme Acid phosphatase (μg $min^{-1}mg^{-1}$ protein) in three seasons relevant to bryophyte life history

Name of Taxon	*Rainy*	*Winter*	*End of growing*
Asterella angusta	0.35 ± 0.01	1.13 ± 0.01	0.23 ± 0.01
A. pathankotensis	0.62 ± 0.01	0.30 ± 0.01	0.18 ± 0.01
Plagiochasma appendiculatum	3.29 ± 0.17	0.62 ± 0.03	0.42 ± 0.01
P. articulatum	1.23 ± 0.03	0.26 ± 0.02	0.19 ± 0.01
P. intermedium	2.62 ± 0.57	0.44 ± 0.01	0.23 ± 0.01
Reboulia hemisphaerica	1.08 ± 0.05	0.95 ± 0.02	0.46 ± 0.01
Conocephalum conicum	0.76 ± 0.01	0.66 ± 0.01	0.34 ± 0.02
Dumortiera hirsuta	0.76 ± 0.01	0.66 ± 0.01	0.35 ± 0.06
Marchantia nepalensis	1.01 ± 0.01	0.36 ± 0.02	0.21 ± 0.01
M. palmata	1.24 ± 0.01	0.29 ± 0.01	0.14 ± 0.01
Targionia indica	1.27 ± 0.32	0.71 ± 0.01	0.49 ± 0.01
Pellia endiviifolia	0.49 ± 0.01	0.48 ± 0.01	0.13 ± 0.01

Three species, *Pellia endiviifolia, Conocephalum conicum* and *Dumortiera hirsuta*, were collected near streams in highly humid and hydric conditions, while the rest were from mesic conditions. Among hydric species, *Pellia endiviifolia* showed the least activity in all three seasons.

DISCUSSION

The specific activity of acid phosphatase followed the seasonal variation, with rainy season (except for *Asterella angusta)* > winter season > end of the growing season (Fig. 2). In higher plants, Biwas & Cundiff (1991) and Thomas (1993) related the enhanced activity of this enzyme to the release of reserve materials for the growing embryo. Presumably activity in the rainy season releases the nutrients permitting rapid growth of the liverworts. Turner *et al.* (2003) reported the greatest phosphatase activity in winter and the least during summer in mosses from northern England. During the present study, the end of the growing season witnessed the least activity of acid phosphatase leading to the accumulation of food reserves as the liverworts shift to the dormant phase.

Many reports have demonstrated that acid phosphatase is a rapid and sensitive indicator of the phosphorus status of a plant (e.g. Besford, 1978) and this was shown for *Sphagnum* by Press and Lee (1983) found in a study on 11 species of *Sphagnum* that acid phosphatase activity reduces in phosphate enrichment and increases in phosphate starvation. Our own study on liverworts in India shows clearly that activity varies seasonally in the species studied. The phosphate concentration in a plant tissue can be used to assess the nutritional status of the environment (Pakarinen, 1978) and this has been shown for mosses by Christmas & Whitton (1998a) and Turner *et al.* (2001, 2003). However, data need to be assessed

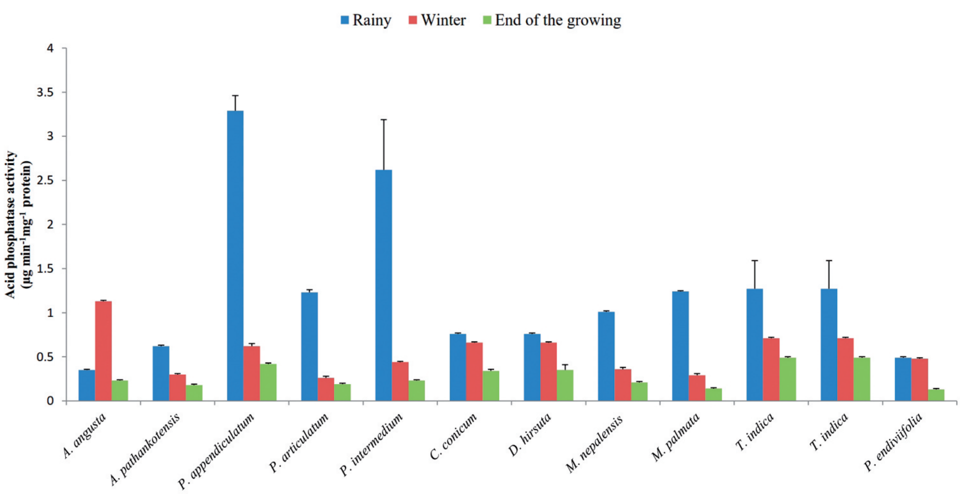

Fig. 2. Specific activity of enzyme Acid phosphatase (µg $min^{-1}mg^{-1}$ protein) in three seasons relevant to bryophyte life history. Values are means of three replicates ± S.E.

critically, For instance, in the leaves of some higher plants (e.g.cowpeas, Takaoki, 1968; cotton, Vieira-Da-Silva, 1969; wheat, Barrett-Lennard *et al.*, 1982) water stress increases acid phosphatase activity, whereas in the present study decreased activity of this enzyme occurred when water availability was less.

No marked differences were observed in acid phosphatase activities of the hydric liverworts (*P. endiviifolia, C. conicum*, *D. hirsuta)* than the mesic liverworts.

Ellwood & Whitton, (2007) reported PMEase activity of an axenic culture of the aquatic moss *Warnstorfia fluitans* than the PDEase activity when grown with organic P.

As the present study shows that the activity of acid phosphatase in liverworts is highest in the rainy season and lowest towards the end of the growing season it can be concluded that phosphatase activity is an indicator of environmental changes. A similar broad study on surface activity of acid phosphatase is also required for the mosses growing in different habitats under different seasons.

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REFERENCES

ASAKAWA Y., LUDWICZUK A. & NAGASHIMA F., 2013 — Phytochemical and biological studies of bryophytes. *Phytochemistry* 91: 52-80.

BARRETT-LENNARD E.G., ROBSON A.D. & GREENWAY H., 1982 — Effect of phosphorus deficiency and water deficit on phosphatase activities from wheat leaves. *Journal of experimental botany* 33 (135): 682-693.

BESFORD R.T. & SYRED A.D., 1979 — Effect of phosphorus nutrition on the cellular distribution of acid phosphatase in the leaves of *Lycopersicon esculentum* L. *Annals of botany* 43: 431-435.

BESFORD R.T., 1978 — A phosphatase as a potential indicator of the phosphorus status of the glasshouse cucumber (*Cucumis sativus*). *Journal of the science of food and agriculture* 29 (2): 87-91.

BESFORD R.T., 1979a — Quantitative aspects of leaf acid phosphatase activity and the phosphorus status of tomato plants. *Annals of botany* 44 (2): 153-161.

BESFORD R.T., 1979b — Nutrient imbalances in tomato plants and acid phosphatase activity in the leaves. *Journal of the science of food and agriculture* 30 (3): 275-280.

BESFORD R.T., 1979c — Phosphorus nutrition and acid phosphatase activity in the leaves of seven plant species. *Journal of the science of food and agriculture* 30 (3): 281-285.

BESSEY O.A., LOWERY O.H. & BROCK, M.J., 1946 — A method for the determination of alkaline phosphatase with five cubic millimeters of serum. *Journal of biological chemistry* 164: 321-329.

BIELESKI R.L., 1973 — Development of an externally-located alkaline phosphatase as a response to phosphorus deficiency. *In:* Bieleski R.L., Ferguson A.R. & Cresswell M.M. (eds), *Mechanism of Regulation of Plant Growth*. Wellington, The Royal Society of New Zealand, pp. 165-170.

BIWAS T.K. & CUNDIFF C., 1991 — Multiple forms of acid phosphatase in germinating seeds of *Vigna sinensis*. *Phytochemistry* 30 (7): 2119-2125.

CHRISTMAS M. & WHITTON B.A., 1998a — Phosphorus and aquatic bryophytes in the Swale-Ouse river system, north-east England. 1. Relationship between ambient phosphate, internal N: P ratio and surface phosphatase activity. *Science of the total environment* 210/211: 389-399.

CHRISTMAS M. & WHITTON B.A., 1998b — Phosphorus and aquatic bryophytes in the Swale-Ouse river system, north-east England. 2. Phosphomonoesterase and phosphodiesterase activities of *Fontinalis antipyretica*. *Science of the total environment* 210/211: 401-409.

CLARK R.B., 1975 — Characterization of the acid phosphatase of intact maize roots. *Journal of agricultural and food chemistry* 23(3): 458-460.

CRANDALL-STOTLER, B. & STOTLER, R. E., 2000 — Morphology and classification of the Marchantiophyta. *In:* Shaw A.J. & Goffinet B. (eds), *Bryophyte Biology*. Cambridge University Press, pp. 21-70.

DANDOTIYA D., GOVINDAPYARI H., SUMAN, S. & UNIYAL P. L., 2011 — Checklist of the bryophytes of India. *Archive for bryology*, 88: 1-126.
ELLWOOD N. T. W. & WHITTON B. A., 2007 — Phosphatase activities of the aquatic moss *Warnstorfia fluitans* (Hedw.) Loeske from an acidic stream in North-East England. *Hydrobiologia* 575: 95-107.
ELLWOOD N.T.W., TURNER B.L., HAILE S.M. & WHITTON B.A., 2007 — Seasonal changes in the surface phosphatase kinetics of aquatic mosses in northern England. *Journal of bryology* 29: 174-182.
HÉBANT C. & SUIRE C., 1974 — Mise en évidence d'activités enzymatiques au niveau de la zone de transfert gamétophyte-sporophyte chez quelques bryophytes. *Revue bryologique et lichénologique* 40: 171-181.
IGAUE I., NISMO M. & KURASAWA F., 1973 — Occurrence of acid phosphatase-isozymes repressible by inorganic phosphate in rice plant cell cultures. *Agriculture and biological chemistry* 37: 941-943.
KAPILA S. & DHAWAN A., 2000 — Preliminary biochemical studies on some west Himalayan bryophytes. *Punjab university research bulletin* 50: 107-113.
KAPILA S., DEVI K., RAO A. & MAHAJAN A., 2014 — Seasonal variations in carbohydrate, protein, free amino acids and enzyme activities in three species of Marchantiaceae. *Lindbergia* 37: 85-89.
KAPILA S. & THAKUR S., 2016 — Seasonal changes in the chemical constituents of a liverwort *Targionia hypophylla* L. *Hikobia* 17: 137-143.
KOLARI K.K. & SARJALA T., 1995 — Acid phosphatase activity and phosphorus nutrition in Scots pine needles. *Tree physiology* 15: 747-752.
MAIER K. & MAIER U., 1972 — Localization of beta-glycerophosphatase and Mg^{++}-activated adenosine triphosphatase in a moss haustorium, and the relation of these enzymes to the cell wall labyrinth. *Protoplasma* 75: 91-112.
MISHRA S. & DUBEY R.S., 2008 — Changes in phosphate content and phosphatase activities in rice seedlings exposed to arsenite. *Brazilian journal of plant physiology* 20 (1): 19-28.
PAKARINEN P., 1978 — Element content of Sphagna: variation and its sources. *Bryophytorum bibliotheca* 13: 751-762.
PRESS M.C. & LEE J.A., 1983 — Acid phosphatase activity in *Sphagnum* species in relation to phosphate nutrition. *New phytologist* 93: 567-573.
SCHULTZ P. & JENSEN W.A., 1981 — Pre-fertilization ovule development in *Capsella*: ultrastructure and ultracytochemical localization of acid phosphatase in the meiocyte. *Protoplasma* 107: 27-45.
TAKAOKI T., 1968 — Relationship between drought tolerance and ageing in higher plants. II. Some enzymes activities. *Botanical magazine* 81: 297-309.
TESSLER M., TRUHN K.M., BLISS-MOREAU M. & WHER J.D., 2014 — Diversity and distributions of stream bryophytes: does pH matter? *Freshwater science* 33 (3): 778-787.
THAKUR S. & KAPILA S., 2016 — Comparative biochemical analysis in vegetative thallus and archegoniophores of *Marchantia papillata* subspecies *grossibarba*. *International journal of advances in pharmacy biology and chemistry* 5: 100-104.
THOMAS T.L., 1993 — Gene expression during plant embryogenesis and germination: an overview. *Plant cell* 5 (10): 1401-1410.
TURNER B.L., BAXTER R., ELLWOOD N.E.W. & WHITTON B.A., 2001 — Characterization of the phosphatase activity of mosses in relation to their environment. *Plant, cell and environment* 24: 1165-1176.
TURNER B.L., BAXTER R., ELLWOOD N.E.W. & WHITTON B.A., 2003 — Seasonal phosphatase activities of mosses from Upper Teesdale, northern England. *Journal of bryology* 25: 203-214.
VIEIRA-DA-SILVA J.B., 1969 — Comparaison entre cinq espèçes de Gossypium quant à l'activité de la Phosphatase acide après un traitement osmotique. Étude de la vitesse de solubilisation' et de formation de l'enzyme. *Zeitschriftfür Pflanzenphysiologie* 60 (5): 385-387.
VINCENT J.B., CROWDER M.W. & AVERILL B.A., 1992 — Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. *Trends in biochemical sciences* 17: 105-110.