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Seasonal variations in carbohydrate, protein, free amino acids and enzyme activities in three species of Marchantiaceae

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This study provides information on the seasonal variations in storage compounds and enzyme activities related to these storage compounds in three species of the family Marchantiaceae: *Marchantia palmata, M. nepalensis* and *Dumortiera hirsuta*. *Dumortiera hirsuta* growing near water streams or hydric habitat shows higher carbohydrate as well as protein content and exhibits low seasonal changes as compared to *M. palmata* and *M. nepalensis* which grows in mesic conditions. In all the species the activity of α-amylase, β-amylase and invertase were decreasing towards the end of the primary growth season due to carbohydrate accumulation in their thalli in this period. The relationship between protein and free amino acids (FAA) was found to be inverse. The activity of protease, which is associated with the metabolism of proteins, was noticed to peak in the rainy season.

As compared to other groups of plants, studies on seasonal variation of storage compounds and enzyme activity in bryophytes have not received much attention. A diverse range of soluble carbohydrates including sucrose, fructan and polyols such as sorbitol, mannitol and volemitol was reported in some liverworts (Suleiman et al. 1979, Suleiman and Lewis 1980). All these compounds are not found universally, so their absence or presence can be used as a taxonomic character (Suleiman et al. 1980). In the Antarctic bryophytes, insignificant seasonal changes were observed in soluble carbohydrates (Melick and Seppelt 1994). Galloway and Black (1989) studied the enzymes associated with sucrose metabolism i.e. sucrose synthase, glucokinase, fructokinase, UDP-glucopyrophosphorylase and phosphoglucomutase in eight species of bryophytes and reported that the enzymes of sucrose synthase pathway are present in bryophytes to synthesize sucrose for their cellular metabolism.

In India, a preliminary biochemical study was done on western Himalayan liverworts by Kapila and Dhawan (2000). Quantitative analyses of carbohydrate, protein and chlorophyll and specific activity of enzymes; α-amylase, β-amylase, protease and polyphenol oxidase have been also carried out for some bryophytes (Kaur et al. 2010a, b).

In our study, emphasis is given to the variation between species, between populations of the same species, and between species belonging to the same family. The purpose of this study is to obtain basic information on the seasonal variations in storage compounds and enzyme activities related to these storage compounds.

Material and methods

Material were collected from different areas of Himachal Pradesh (western Himalaya). The names of taxa, month of collection, locality, altitude and nature of substratum are given in Table 1.

First each specimen taxon was thoroughly washed with distilled water to remove all adhering soil particles and organisms. The material was dried in the folds of sterilized blotting paper and subsequently 500 mg of dry material was homogenized in 10 ml of distilled water. The suspension was centrifuged at 3000 rpm for 20 min and the supernatant used for various analyses. For the estimation of free amino acids extract was made in 80% ethanol.

Biochemical estimation and enzyme activities

Total water-soluble carbohydrate content was estimated by anthrone reagent as per the method of Yemm and Willis (1954). The amount of proteins was determined by the

Table 1. The names of taxa, month of collection, locality, altitude and nature of substratum.

Spec. no.	Name of taxon	Month of collection, locality and altitude	Substratum	Herbarium reference no.
1	*Marchantia nepalensis* L. et L.	August, Mandi; 750 m 0ctober, Mandi; 750 m January, Mandi;750 m	wet soil on stony wall on wet soil on wet soil	PAN6102
2	*Marchantia palmata* Nees	August, Mandi; 750 m October, Mandi; 750 m January, Mandi; 750 m	wet soil on stony wall on wet soil on wet soil	PAN 6103
3	*Dumortiera hirsuta* (Sw.) Nees	July, Dharampur, Solan, 1483 m October, Chadwick fall, Shimla, 1580 m March, Chadwick fall, Shimla, 1580 m	on wet soil on wet soil on wet soil	PAN 6104

method of Lowry et al. (1951) using bovine serum albumin as the standard. The amount of free amino acids was determined by the method of Lee and Takahashi (1966) using ninhydrin reagent and glycine as standard.

The activity of α-amylase was determined by starch as standard following the method of Muentz (1977). The activity of β-amylase was determined by maltose as standard following the method of Bernfeld (1951). The activity of invertase was measured according to the method given by Sumner (1935). Protease was assayed by the method of Basha and Beevers (1975).

The data were analyzed by two-way analysis of variance (ANOVA).

Results

To study the seasonal variation in these plants, the collection period has been divided into three bryological seasons: July–September (rainy season), October–December (winter season) and January–March (end of growing season) with different climatic conditions. In the first season i.e. July–September, plants are in the young growing stage and the temperature is slightly higher than normal. The second season i.e. October–December is most favorable period for their growth and the temperature for the growth of these plants is suitable. The third season i.e. January–March is the end of the favorable period of growth and the temperature in this period is slightly lower than that in second season. The range of temperature and rainfall during the three seasons are given in Table 2.

The results obtained from the present study are given in Fig.1–7.

The present study using two-way ANOVA revealed that the studied liverwort taxa show significant seasonal variation ($p < 0.05$) in all the studied parameters except protease which shows non-significant seasonal variation ($p > 0.05$) in the studied taxa.

Carbohydrate concentrations were significantly higher ($p < 0.05$) towards the end of the growing season than rainy and winter seasons (Fig. 1). The carbohydrate content of the two species of *Marchantia* is almost the same in rainy season as well as in winter *(M. nepalensis* 25.14 ± 0.47 mg g^{-1} fresh weight (fw) in rainy season and 39.11 ± 2.36 mg g^{-1} fw in winter season, *M. palmata* 24.32 ± 3.63 mg g^{-1} fw in rainy season and 21.91 ± 0.85 mg g^{-1} fw in winter season). At the end of the growing season i.e. Jan–March, carbohydrate content of both the species of *Marchantia* abruptly increased *(M. nepalensis* 105.95 ± 2.81 mg g^{-1} fw and *M. palmata* 48.24 ± 2.89 mg g^{-1} fw).

Table 2. The range of temperature and rainfall during the three seasons.

Period of collection	July–September	October–December	January–March
Mandi			
Temp. (ºC)	25.5–25.3	23.1–17.4	16.8–21
Rainfall (mm)	240–130	25–10	30–22
Shimla			
Temp. (ºC)	20.6–19.4	17.2–10.6	8.3–13.9
Rainfall (mm)	424–160	33–28	60–61
Solan			
Temp. (ºC)	25.2–24.5	22.9–15.8	13.2–19.8
Rainfall (mm)	393–186	52–29	87–73

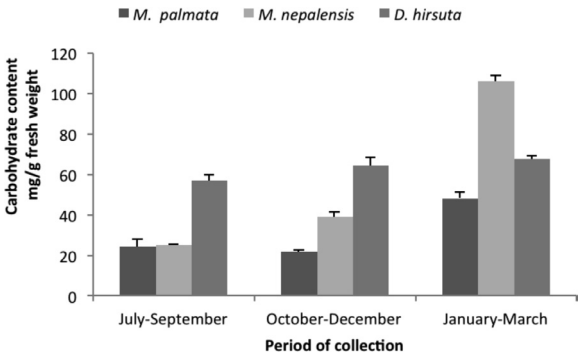

Figure 1. Carbohydrate content in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

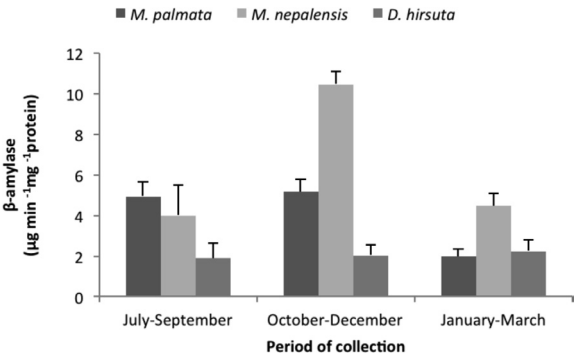

Figure 3. Specific activity of β-amylase in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

The seasonal changes were also studied in the enzymes α-amylase, β-amylase and invertase which are associated with carbohydrate metabolism. Amylase initiates the starch degradation. α-amylase is widely distributed in plants, fungi and bacteria, whereas β-amylase occurs in cereal seeds and sweet potato. During ripening of fruit, it breaks starch into maltose, resulting into the sweet flavor of fruit.

Activities of both the amylases and the invertase varied significantly in the three periods of collection ($p < 0.05$). Among the two species of *Marchantia*, the specific activity of α-amylase was found to be stronger in *M. palmata* than *M. nepalensis* (Fig. 2). Reduced α-amylase activity was noticed during the months of January–March (1.69 ± 0.08 μg min^{-1} mg^{-1} protein in *M. palmata*, 1.47 ± 0.05 μg min^{-1} mg^{-1} protein in *M. nepalensis* and 1.48 ± 0.18 μg min^{-1} mg^{-1} protein in *D. hirsuta*) i.e. towards the end of favourable period of their growth because of carbohydrate accumulation in their thalli. All the three species showed high activity of α-amylase in winter period of collection i.e. October to December (23.2 ± 3.33 μg min^{-1} mg^{-1} protein in *M. palmata*, 17.18 ± 0.83 μg min^{-1} mg^{-1} protein in *M. nepalensis* and 16.28 ± 0.3 μg min^{-1} mg^{-1} protein in *D. hirsuta*) suggestive of high metabolic rate of this enzyme during this season.

The activity of β-amylase was low compared to α-amylase (Fig. 3) but the same seasonal pattern was observed in both the enzymes. *D. hirsuta* showed lower activity of β-amylase (1.89 ± 0.73 μg min^{-1} mg^{-1} protein in rainy season, 2.02 ± 0.51 μg min^{-1} mg^{-1} protein in winter season and 2.23 ± 0.54 μg min^{-1} mg^{-1} protein at the end of the growing season) as compared to the both species of *Marchantia*.

Invertase is involved in the hydrolysis of sucrose. The specific activity of invertase was peaking in the rainy season and reaching its minimum towards the end of the growing season i.e. Jan–March (Fig. 4).

Analysis of variance of total free amino acids concentrations indicated significant seasonal changes during three bryological seasons ($p < 0.05$). The content of free amino acids was found to be lowest in the October–December period of collection (11.18 ± 1.85 mg g^{-1} fw in *M. palmata*, 7.7 ± 1.97 mg g^{-1} fw in *M. nepalensis* and 11.61 ± 1.38 mg g^{-1} fw in *D. hirsuta*) but low seasonal change was observed in any other of the two periods of collection i.e. July–September (14.87 ± 1.73 mg g^{-1} fw in *M. palmata,*

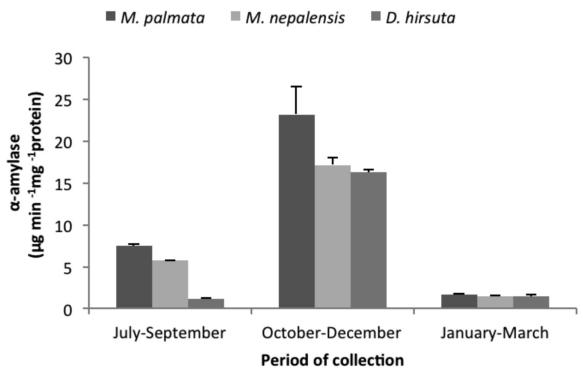

Figure 2. Specific activity of α-amylase in three liverworts in three periods of collection. Values are means of three replicates ± SE.

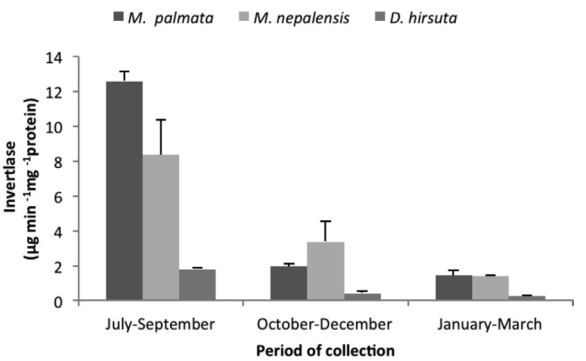

Figure 4. Specific activity of invertase in three liverworts in three periods of collection. Values are means of three replicates ± SE.

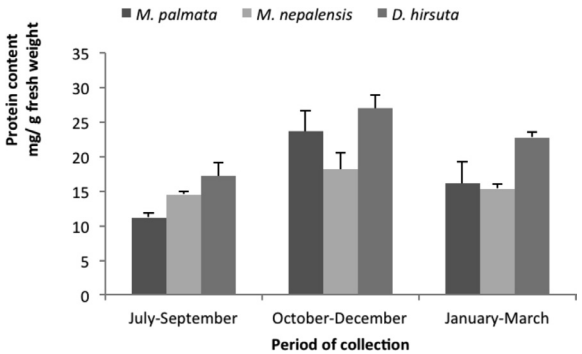

Figure 5. Protein content in three liverworts in three periods of collection. Values are means of three replicates ± SE.

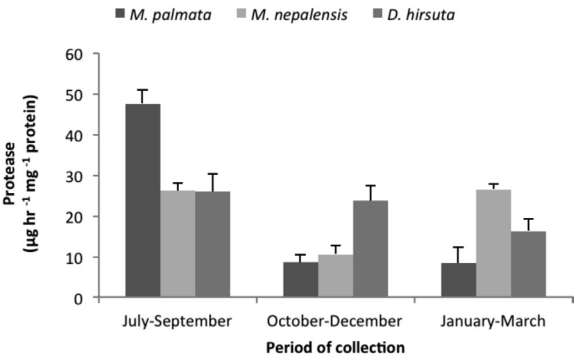

Figure 7. Specific activity of protease in three liverworts in three periods of collection. Values are means of three replicates ± SE.

10.29 ± 5.28 mg g^{-1} fw in *M. nepalensis* and 14.02 ± 0.52 mg g^{-1} fw in *D. hirsuta*) and January–March (21.8 ± 2.21 mg g^{-1} fw in *M. palmata,* 16.39 ± 2.95 mg g^{-1} fw in *M. nepalensis* and 12.22 ± 0.63 mg g^{-1} fw in *D. hirsuta*).

The activity of protein hydrolyzing enzyme, protease in all the three bryological seasons had non-significant seasonal trend ($p > 0.05$), with peak values in the July–September period of collections (Fig. 7). Protease breaks down the protein into amino acids. The high activity of protease and lower content of protein in the rainy season revealed an inverse relationship between protease and protein concentration. We also observed an inverse relationship between protein and free amino acid in the October–December period of collection.

Discussion

Higher content of carbohydrates recorded during the months of January–March (Fig. 1) indicates that these species store carbohydrates in their thalli towards the end of favourable period of their growth as also reported by Kapila and Dhawan (2000) in *Dumortiera hirsuta* and *Conocephalum conicum*. Very low seasonal change in the carbohydrate level observed in *D. hirsuta* suggests that the liverworts growing near water streams in highly humid and hydric conditions show high carbohydrate level in all the seasons. This observation is contrary to an earlier investigation of maritime Antarctic bryophytes by Davey (1999) who reported lower content of carbohydrate and higher protein, nitrogen and phosphorus contents in mosses from hydric habitats than in those from drier habitats. However, those results are reflect the importance of water and the primacy of physical factors in the ecology of Antarctic mosses with extreme climatic and rapid temperature fluctuations (Melick and Seppelt 1994).

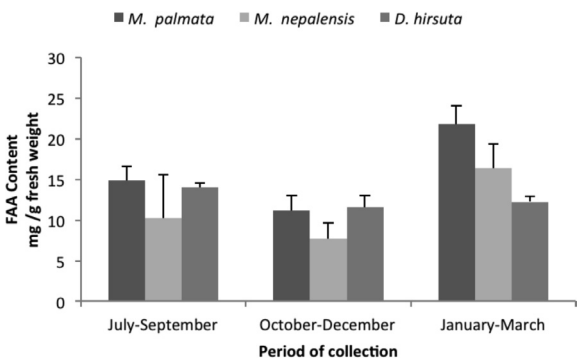

Figure 6. Free amino acids content in three liverworts in three periods of collection. Values are means of three replicates ± SE.

The observation that liverworts store carbohydrates in their thalli towards the end of their favourable period of growth, is compliant with lower activity of α-amylase, β-amylases and invertase during this period in all the studied taxa. *Dumortiera hirsuta* showed lower values of specific activity for all the three enzymes (α-amylase, β-amylase and invertase) as compared to both of the species of *Marchantia* and low seasonal variations. Udar and Chandra (1960a, b) reported more amylase activity in male plants of *Riccia discolor* as compared to that in female plants. Marschall et al. (1998) observed the reduction of invertase activity to 60% in rehydrating plants of *Porella platyphylla.*

The protein concentrations were significantly higher ($p < 0.05$) in the winter season than in the rainy season and at the end of the growing seasons (Fig. 5). Low temperature results in the synthesis of proteins (Mohapatra et al. 1987, Hughes and Dunn 1996, Koc et al. 2010). This might be the reason for a higher protein content observed in the presently studied liverworts during winter season as compared to the other two seasons. It is of interest to note that protein content of *D. hirsuta* was found to be higher than for the two species of *Marchantia* which is suggesting that the liverworts growing along water streams and in more shaded areas contain higher protein content than the liverworts that growing on wet soil in mesic conditions. Davey (1999) recorded similar observation in the protein content of hydric mosses as compared to that of

drier habitat Antarctic mosses. The continuous flushing of nutrients in hydric habitats may be one of the reasons of higher protein content in these plants.

Protease changes the rate of protein synthesis, degrade the altered, damaged or temporarily functional proteins and regulate the gene expression to protect the cell from environmental changes (Adam 2000). Presently observed highest activity of protease during the rainy season reveals its vital role in the breakdown of proteins to release the amino acids for the synthesis of new proteins as also reported by (Vierstra 1996, Palma et al. 2002).

The present study showed wide seasonal variations in the content of carbohydrates, proteins and free amino acids and specific activity of α-amylase, β-amylase, invertase and protease which may be due to the habitat conditions, the growing stage of plant and the microclimatic conditions at the time of collection.

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