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Research Article

Seasonal variations in photosynthetic pigments of three species of Marchantiaceae

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ABSTRACT

The present study provides information on the seasonal variations in Chlorophyll a, Chlorophyll b, total Chlorophyll (a+b), Carotenoids and Chlorophyll a/b ratio in three species of family Marchantiaceae, two collected from mesic habitat *(Marchantia palmata* and *M. nepalensis*) and one collected from hydric habitat *(Dumortiera hirsuta)*. Chlorophyll a was observed higher than Chlorophyll b in all the three periods of collection in all the three species. Chlorophyll a is found to be least in the winter period, whereas seasonal changes in Chlorophyll b did not follow any pattern. Total Chlorophyll content is more in the end of the growing season. A very less seasonal variation in the Carotenoid content was observed in both the species of *Marchantia,* but *Dumortiera hirsuta* exhibited much lower Carotenoid content (0.06 ± 0.005 mg/g fresh weight) in winter season than in rainy season (0.16 ± 0.005 mg/g fw) and at the end of the growing season (0.15 ± 0.005 mg/g fw).

KEYWORDS: Chlorophyll, Carotenoids, *Marchantia palmata, M. nepalensis, Dumortiera hirsuta.*

INTRODUCTION

This paper is in continuation with our earlier work on seasonal variations in carbohydrate, protein, free amino acids and enzyme activities in three species of Marchantiaceae[1].

Plant pigments Chlorophyll and Carotenoids play a significant role in photosynthesis as well as in plant protection from extensive radiations. They convert the light energy into chemical energy[2, 3]. Chlorophyll plays unique role in the physiology and productivity of green plants and its concentration per unit area is an indication of photosynthetic capacity and productivity of a plant[4]. Its content is one of the indices of photosynthetic activity[5]. Carotenoids absorb light in the blue region of the spectrum and play role in the organization of photosynthetic membranes, energy transfer, quenching of Chlorophyll excited states and singlet oxygen, and participation in light harvesting[6-8]. The physiological state of plants during development, senescence, acclimation and adaptation to different environments and stresses can be assessed by the changes of leaf Carotenoid content and its proportion to the Chlorophyll content[9-11].

Photosynthesis in the leaves varies with the season[12-14]. Variation in the photosynthetic capacity is related to the potential of Photosystems I and II to transport electrons, and also to the variations in specific leaf area as well as total Chlorophyll content and Chlorophyll a/b ratio[15]. The physiological conditions of plants such as nitrogen status of leaf and photosynthetic capacity can be assessed by the Chlorophyll concentration of leaf[16, 17]. The seasonal variations in the chlorophyll content indicate the physiological state of the plant[18] and are related to the seasonal water deficiency, high ambient irradiance during summer and very low temperature in winter[19]. Mosses as well as liverworts grow in low-light environments and show lower Chlorophyll a/b ratio than higher plants indicating that these groups are well suited to such environments[20-21]. Pande and Singh[22] reported higher content of Chlorophyll and Carotenoids in liverworts than in mosses the former being prominent in shaded areas and the latter in sunlight. Deora and Chaudhary[23] determined the Chlorophyll content in 16 species of mosses and four species of liverworts and concluded that bryophytes exhibit low Chlorophyll concentration and high a:b ratios in high solar irradiances. Martínez-Abaigar and Núñez-Olivera[24] reported that on the basis of both area and weight, bryophytes have lower chlorophyll content than tracheophytes. Martin and Adamson[25] observed the photosynthetic capacity of mosses to be lower than that of the higher vascular plants.

In bryophytes, the available information about the seasonal variations in the photosynthetic pigments is presented by Miyata and Hosokawa[26] in the epiphytic mosses, by Kershaw and Webber[27] in the moss *Brachythecium rutabulum*, by Martı´nez-Abaiger *et al*.[28] in the aquatic bryophytes and by Melick and Seppelt[29] in Antarctic bryophytes. The present study was focussed to compare the pigments of liverworts growing under different habitats and to observe their seasonal variation in the taxa under study.

MATERIALS AND METHODS

Materials 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.

The plant material of each 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 20 ml of 80% acetone. The suspension was centrifuged at 3000 rpm for 20 minutes and then absorbance of the sample was measured at 470, 646 and 663nm using double beam spectrophotometer. Chlorophyll a, Chlorophyll b and Carotenoids were calculated using the method of Lichtenthaler and Wellburn[30].

The data for various parameters were subjected to Two-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

To study the seasonal variation in liverworts, the collection period was divided into three bryological seasons with different temperature and rainfall conditions[1]**:** July-September (rainy season), October-December (winter season) and January-March (end of growing season). In the first season i.e. July-September, plants are in the young growing stage and temperature is slightly more than the normal and rainfall is maximum. The second season i.e. October-December is the most favorable period for the growth of liverworts when temperature is suitable and rainfall is much lower than in rainy season. The third season i.e. January-March is the end of the favorable period of growth and the temperature in this period is slightly cooler and the rainfall is slightly more than that in second season.

The results obtained from the present study are given in Tables 2-4. The present study using two way ANOVA revealed that the studied liverwort taxa show significant seasonal variation ($p<0.05$) in the studied parameters.

Photosynthesis is related to Chlorophyll content[31, 32], therefore, the content of chlorophyll gives an idea about the photosynthetic efficiency of a plant. Variations occur both seasonally and between the species among the studied taxa. Seasonal variations in Chlorophyll a, Chlorophyll b, total Chlorophyll (Chl.), total Carotenoid (Car.) and Chlorophyll a/b ratio were observed to be apparent. The Chlorophyll a found to be higher than the Chlorophyll b showed significant seasonal variation in different periods of collection ($p<0.05$) (Table 2). Among the two species of *Marchantia*, Chlorophyll a was found to be higher in *M. nepalensis* than in *M. palmata*. In the hydric taxon *D. hirsuta*, the Chlorophyll a was observed lower than in the mesic taxa *M. nepalensis* and *M. palmata* in the all three bryological seasons. The content of Chlorophyll a was found to be lowest in the October-December period of collection in all the three species.

The Chlorophyll b content in all the three bryological seasons showed significant seasonal trend ($p<0.05$). *Dumortiera hirsuta* showed higher values of Chlorophyll b as compared to the both of the species of *Marchantia* in the rainy season, lower than *M. nepalensis* in winter season and lower than *M. palmata* at the end of the growing season. Kaur *et al.*[33] categorized the liverworts into two groups: Group A- Chlorophyll a content is more than Chlorophyll b and in Group B- Chlorophyll b is more than Chlorophyll a. In the present study, however, in all the taxa Chlorophyll a is more than Chlorophyll b in all the seasons with Chl a/b ratio ranging between 1.82 ± 0.01 to 2.96 ± 0.09, expect in *D. hirsuta* in winter season (Chl a/b ratio 0.92 ± 0.02). This is in agreement with earlier reports[34,21] that the bryophytes growing in shaded habitats and low light conditions show increased Chlorophyll b concentration but some studies reveal that Chlorophyll a/b ratio is lower in shaded plants than in sunny plants[35, 36].

The total Chlorophyll was significantly higher ($p<0.05$) in the end of the growing season than in rainy and winter seasons (Table 3). During seasonal changes, the bryophytes experience long and short photoperiods along with the change in temperature and moisture. In the presently studied bryological seasons, January-March period of collection (end of the growing season) shows longer day length than July-September (rainy season) and October-December (winter season) periods of collection. This may be the possible cause for higher chlorophyll content during January-March period of collection in agreement with an earlier report[27] due to increased irradiation and longer day length[29].

During winter, Chlorophyll content is lowered probably due to the degeneration of chloroplasts and due to the oxidative degradation of Chlorophyll by chlorophyll degrading peroxidase enzymes[37]. The reduction in total Chlorophyll in Antarctic bryophytes and lichens during winter was also reported by Melick and Seppelt[29]. Martin and Adamson[25] attributed the lower photosynthetic rates and lower chlorophyll concentrations of mosses as compared to those of the higher plants to the smaller number of photosynthetic units per cell, although the light utilization capacity of each unit in mosses may be equal to that of the higher plants. The photosynthetic apparatus of modern extant bryophytes, thus, seems to have evolved to the extent equal to that of higher plants.

It is of interest to note that Chlorophyll content of hydric *D. hirsuta* was found to be lower than that of both the mesic species of *Marchantia* suggesting that the liverworts growing along water streams and in more shaded areas contain lower Chlorophyll content than the liverworts that grow on wet soil in mesic conditions. This is contrary to the findings of Martin and Churchill[21] that the mosses growing in shaded habitats have higher Chlorophyll content than the mosses growing in exposed conditions.

Light also plays significant role in the Chlorophyll content by changing the pigment concentration due to different climatic conditions[38]. High or low light affects the Chlorophyll molecules leading to change in its content. High light damages Chlorophyll and DNA structure that results in decrease in Chlorophyll content. Low light increases Chlorophyll b concentration resulting in the decrease of Chlorophyll a/b ratios.

In the present study, Analysis of Variance of Chlorophyll a/b ratios indicated significant seasonal changes during three bryological seasons ($p<0.05$). Shaded plants exhibit lower Chlorophyll a/b ratios than the sunny plants[34, 21]. Same observation was recorded in the presently studied taxa except in the mesic *Marchantia palmata* (1.48 ± 0.03) that exhibited less Chlorophyll a/b ratio in the end of the growing season. It is not always true that Chlorophyll a/b ratio is lower in shaded plants than in sunny plants [35, 36].

Seasonal changes in the Chlorophyll a/b ratio were apparent in all the studied taxa. Chlorophyll a/b ratio was observed lower in the winter than in the rainy and end of the growing seasons except in *M. palmata* (Table 4). The Chlorophyll a/b ratio reduces as the irradiance decreases[39, 40]. This might be the reason of the low Chlorophyll a/b ratio in the winter season.

The species collected from the shaded habitats and from near the water streams did not show any remarkable difference in Carotenoid content from the ones from exposed and mesic habitat. This is in line with an earlier report by Martínez-Abaigar and Núñez-Olivera[24] who did not find any major differences in the carotenoids of aquatic and terrestrial bryophytes. Carotenoids serve as accessory light harvesting pigments that protect the chlorophyllous pigments against photo-damage and oxygen[41]. A very less seasonal variation in the carotenoid content was found in the two species of *Marchantia* but *Dumortiera hirsuta* showed a very low carotenoid content (0.06 ± 0.005 mg/g fw) in winter season than in rainy season (0.16 ± 0.005 mg/g fw) and at end of the growing season (0.15 ± 0.005 mg/g fw). Low seasonal changes in the Carotenoids of both the species of *Marchantia* and apparent seasonal changes in the Carotenoids of *Dumortiera hirsuta* are suggestive of the role of habitat in these variations.

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

S.No.	Name of Taxon	Month of collection , locality and altitude	Substratum and Habitat	Herbarium reference No.
1.	*Marchantia nepalensis* L. et L.	August, Mandi; 750m 0ctober, Mandi; 750m January, Mandi;750m	On exposed wet soil	PAN 6102
2.	*Marchantia palmata* Nees	August, Mandi; 750m October, Mandi; 750m January, Mandi; 750m	On exposed wet soil	PAN 6103
3.	*Dumortiera hirsuta* (Sw.) Nees	July, Dharampur, Solan; 1483m October, Chadwick fall, Shimla;1580m March, Chadwick fall, Shimla;1580m	On wet soil, near water streams, shaded habitat	PAN 6104

Table 2
Content of Chlorophyll a and Chlorophyll b (mg/g fw) in three liverwort species during three periods of collection

Name of Taxon	July- September		October-December		January-March	
	Chl. a	Chl. b	Chl. a	Chl. b	Chl. a	Chl. b
Marchantia nepalensis	0.84 ± 0.01	0.3 ± 0.01	0.7 ± 0.005	0.43 ± 0.01	0.87 ± 0.01	0.29 ± 0.01
Marchantia palmata	0.79 ± 0.005	0.32 ± 0.01	0.66 ± 0.005	0.32 ± 0.01	0.7 ± 0.005	0.47 ± 0.01
Dumortiera hirsuta	0.62 ± 0.005	0.34 ± 0.005	0.36 ± 0.002	0.39 ± 0.005	0.62 ± 0.005	0.34 ± 0.02

Data represented as mean ± S.E. (Standard Error) of three replicates.

Table 3
Content of Chlorophyll and Carotenoids (mg/g fw) in three liverworts during three periods of collection

Name of Taxon	July- September		October-December		January-March	
	Chl.	Car.	Chl.	Car.	Chl.	Car.
Marchantia nepalensis	1.14 ± 0.01	0.15 ± 0.005	1.13 ± 0.01	0.16 ± 0.01	1.16 ± 0.02	0.15 ± 0.005
Marchantia palmata	1.12 ± 0.01	0.14 ± 0.005	0.98 ± 0.01	0.16 ± 0.01	1.17 ± 0.01	0.12 ± 0.01
Dumortiera hirsuta	0.97 ± 0.01	0.16 ± 0.005	0.76 ± 0.01	0.06 ± 0.005	0.97 ± 0.01	0.15 ± 0.005

Data represented as mean ± S.E. (Standard Error) of three replicates.

Table 4
Chlorophyll a/b ratio in three periods of collection

Name of Taxon	July- September	October-December	January-March
Marchantia nepalensis	2.8 ± 0.11	1.62 ± 0.41	2.96 ± 0.09
Marchantia palmata	2.46 ± 0.1	2.07 ± 0.05	1.48 ± 0.03
Dumortiera hirsuta	1.82 ± 0.02	0.92 ± 0.02	1.82 ± 0.01

Data represented as mean ± S.E. (Standard Error) of three replicates.

CONCLUSION

The results obtained from the present study indicate that the seasonal variations in content of Chlorophyll and Carotenoid may be due to the high irradiance during summer, extreme low temperature in winter, and shady humid conditions during rainy season. The variations in the Chlorophyll and Carotenoid content help to explain the variations in photosynthetic capacity in different phases of the growth of bryophytes in different seasons of the year.

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