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EFFECT OF SEASONAL CHANGES ON SOME BIOCHEMICAL PARAMETERS IN THE THALLI OF THREE SPECIES OF AYOTONIACEAE (MARCHANTIOPHYTA)

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ABSTRACT

This study investigates the effects of seasonal changes on biochemical parameters in the thalli of three liverwort species: *Asterella angusta* Steph., *Asterella pathankotensis* Kash., and *Reboulia hemisphaerica* (L.) Raddi, collected from various regions of Himachal Pradesh, India. Samples were collected across different seasons and the analysis focused on several key biochemical components, including carbohydrates, proteins, amino acids, total chlorophyll, carotenoids and chlorophyll/carotenoid ratio, as well as the activities of enzymes such as α-amylases, β-amylase, invertase and protease. The findings revealed that carbohydrate levels peaked at the end of the growing season (January-March) across all the species. In contrast, protein concentrations were highest during the winter for both the *Asterella* species, while *R. hemisphaerica* exhibited elevated protein levels during the rainy season. Additionally, free amino acids showed increased concentrations in the rainy season for all the species. The total chlorophyll content was highest in the end of the growing season in all the three studied liverwort species, whereas carotenoid content was found highest during the rainy season in *A. angusta* and *R. hemisphaerica*. The chlorophyll to carotenoid ratio (chl/car.) was lowest in the rainy season for both these species whereas in *A. pathankotensis* it is minimum in winter season. Enzymatic activity also varied with the seasons. Amylase activity was significantly higher in winter for all the species, whereas invertase activity peaked during the rainy season in *A. angusta* and *R. hemisphaerica*. Protease activity was observed maximal in the rainy season and minimal in the winter season, indicating seasonal fluctuations in enzyme function. Overall, the study concludes that seasonal changes have a significant impact on the quantity of storage compounds and enzymatic activities in the thalli of *Asterella* and *Reboulia*.

***Keywords* :** Seasonal changes, Aytoniaceae, *Asterella, Reboulia*

Introduction

Bryophytes are the first land plants to experience seasonal changes after terrestrialization. Although small in size, these show significant biochemical adaptability to environmental changes (During, 1992; Klavina, 2015). They are influenced by several ecological factors such as photoperiod, temperature, precipitation, and nutrient availability. These factors vary in different seasons of the Indian calendar year (Yadav *et al*., 2021). In early successional phases, these plants function as colonizers, accumulating debris, retaining water and aiding in soil deposition and stabilization. They reduce erosion and create microhabitants for the establishment of vascular plants (Zamfir, 2000; Streitberger *et al*., 2017).

Liverworts are biochemically distinct from mosses due to the presence of oil bodies that contain secondary metabolites. These metabolites primarily consist of lipophilic terpenoids, bis-bibenzyls, and small aromatic compounds (Asakawa *et al*., 2013). The study on the liverwort *Dumortiera hirsuta* by Yadav *et al*. (2021) showed how seasonal changes affect oxidative stress markers and antioxidant activities. It also indicated the highest levels of malondialdehyde and hydrogen peroxide during the fruiting season, while protein and pigment contents were maximal in the monsoon season. Antioxidant activities, except for ascorbate peroxidase and ascorbic acid were elevated during the fruiting season, suggesting that the antioxidative defense system plays a crucial role in helping liverworts to adapt to the oxidative stress induced by seasonal climatic variations.

There are many studies that show a relationship between abundance and the distribution of bryophytes with the changing environmental conditions (Smith, 1982; Jagerbrand *et al.*, 2012; Aranda *et al*., 2014; Gupta and Asthana, 2016). However, there are only few studies that analyzed the seasonal variations in the storage compounds, photosynthetic pigments and enzymatic activities in the liverworts (Martinez-Abaigar *et al.*, 1994, Kapila *et al.*, 2014, Devi *et al*., 2015, Thakur *et al.*, 2017; Devi *et al.*, 2018). These reports indicate that the changes in temperature, photoperiod and relative humidity due to the seasonal changes have a significant effect on the biochemical constituents. But no reports exist for the genera *Asterella* and *Reubolia*, making this study important in filling this knowledge gap.

Materials and Methods

Collection of plant material:

The whole plants along with the fertile shoots were collected from different sites of Himachal Pradesh in different seasons and used for the biochemical analysis (Table 1). Voucher specimens of the liverworts have been deposited in the herbarium maintained at Department of Botany, Panjab University, Chandigarh.

Table 1: The names of the taxa, time and place of collection, altitude and herbarium reference numbers

Name of taxa	Month of collection, locality and altitude	Substratum	Herbarium Reference No.
Asterella angusta Steph.	August, Mandi; 911m November, Mandi; 911m January, Hamirpur; 738m	Wet soil	PAN 6110
Asterella pathankotensis Kash.	August, Mandi; 911m October, Mandi; 911m January, Hamirpur; 738m	Wet soil on stony wall	PAN 6111
Reboulia hemisphaerica (L.) Raddi	September, Mandi; 911m October, Mandi; 911m January, Mandi; 911m	Wet soil	PAN 6109

Preparation of plant extract: The plants with rhizoids were extensively washed with distilled water and dried in the folding of the blotting paper. The dried specimens were used for the biochemical analysis.

Determination of physiochemical parameters: Total water-soluble carbohydrates were estimated by Anthrone method of Yemm and Willis (1954) with glucose as standard. The estimation of total proteins was done by Lowry *et al*. (1951) method by using bovine serum albumin as the standard. The concentration of free amino acids was measured by the method of Lee and Takahashi (1966) using ninhydrin reagent and glycine as standard. The activity of α-amylase was measured by the method of Muentz (1977) and the activity of β-amylase was determined by Bernfeld (1955). The activity of invertase was assayed by the method of Sumner (1935) and the activity of protease was measured according to the method given by Basha and Beevers (1975). The contents of Chlorophyll and Carotenoids were estimated by Lichtenthaler and Wellburn (1983).

All the three studied species were found growing during July-March period, whereas they were not found during the April-June months of the year due to dry and hot weather conditions. Therefore, the growing period was divided into three bryological seasons i.e. July-September (rainy season) when the plants are in the young growing stage, October-December (winter season) which is the most favorable period with most congenial weather conditions for the growth of these plants and January-March (end of growing season)**.** The amount of total rainfall and the range of temperature during the three seasons are given in Table 2.

Table 2 : The range of temperature and rainfall during the three periods of collection.

Site of collection \ Period of collection	July- September (Rainy Season)	October-December (Winter season)	January- March (End of growing season)
Mandi			
Temp. (ºC)	25.5-25.3	23.1-17.4	16.8-21
Rainfall (mm)	240-130	25-10	30-22
Hamirpur			
Temp. (ºC)	26-25	23-17	17-21
Rainfall (mm)	223-138.4	33-15.2	31.3-35.7

Statistical analysis: Values were obtained in triplicates and represented as mean±SE (standard error). Data from three replicates were subjected to analysis of variance using SPSS for all statistical analyses. Differences between means at 5% ($P<0.05$) level were considered as significant.

Results

The results revealed significant variations in all the studied parameters in all the three species during different seasons. The variations generally appeared to be strongly affected by changes in environmental conditions during different seasons. Significant variations in the concentrations of carbohydrate were observed (Figure 1). The highest concentrations of carbohydrates (88.68 ± 2.66 mg/g fw in *A. angusta* and 74.89 ± 0.39 mg/g fw in *R. hemisphaerica*) were found during the end of the growing season and the lowest in the rainy season (12.63±0.62 mg/g fw in *A. angusta*, 8.42±1.1 mg/g fw in *A. pathankotensis*, 60.05±0.58 mg/g fw in *R. hemisphaerica)* in all the three liverworts. The activities of enzymes (α-amylase, β-amylase and invertase) related to the carbohydrate's metabolism were also observed seasonally in all the three taxa (Figures 2,3,4). The activities of all three enzymes were found lowest in the end of the growing season except β-amylase in *A. pathankotensis* which reached its lowest levels in the rainy season (5.95±0.15 µg/min/mg protein). During the rainy season the activities of α-amylase and β-amylase were noticed highest in all the three studied species, whereas invertase was at peak during rainy season in *A. angusta* (8.44±0.14 µg/min/mg protein) and *R. hemisphaerica* (5.62±0.28 µg/min/mg protein) and in winter season in *A. pathankotensis* (7.55±0.36 µg/min/mg protein).

The higher concentration of carbohydrates in all the three taxa of Plagiochasma towards the end of the growing season is similar to our previous re-ports on Marchantia and Dumortiera (Kapila and Dhawan, 2000; Kapila *et al*., 2014).

The higher concentration of carbohydrates in all the three taxa of Plagiochasma towards the end of the growing season is similar to our previous re-ports on Marchantia and Dumortiera (Kapila and Dhawan, 2000; Kapila *et al*., 2014).The activities of enzymes associated with carbohydrates metabolism were also studied seasonally. The α-amylase and β-amylase in all the three genera were noticed highest in the winter season whereas lowest during the end of the growing season (Fig.....).

The protein levels (Figure 5) were higher during winter season in *A. angusta* and *A. pathankotensis* (12.65±0.32 mg/g fw and 26.91±0.58 mg/g fw respectively) and in rainy season in *R. hemisphaerica* (29.0 ± 0.57 mg/g fw). The content of free amino acids was found highest in the rainy season (43.28±0.2 mg/g fw in *A. angusta*, 35.9±0.59 mg/g fw in *A. pathankotensis* and 24.42±0.81 mg/g fw in *R. hemisphaerica*) and lowest in the winter season in (8.54±0.31 mg/g fw in *A. angusta*, 20.49±0.28 mg/g fw in *A. pathankotensis* and 8.08±0.22 mg/g fw in *R. hemisphaerica*) in all the three taxa (Figure 6). The activity of protease enzyme was maximal in the rainy season and minimal in winter like the content of free amino acids (Figure 7).

Total chlorophyll content analysed in all the three seasons of bryological growth indicated significant seasonal changes. The total chlorophyll content an all the three thalloid liverworts was found to be maximal at the end of the growing season (Figure 8) i.e. January–March (1.22±0.001mg/g fw in *A. angusta*, 1.05±0.008 mg/g fw in *A. pathankotensis*, 1.11±0.006 mg/g fw in *R. hemisphaerica*) and minimal in the winter season i.e. October-December (0.71±0.003 mg/g fw in *A. angusta*, 0.70±0.005 mg/g fw in *A. pathankotensis*, 0.81±0.008 mg/g fw in *R. hemisphaerica*). In the rainy season, *R. hemisphaerica* showed the highest carotenoid content (0.24±0.005 mg/g fw). The lowest content was observed in (*A. angusta* (0.03±0.003 mg/g fw) at the end of the growing season and *A. pathankotensis* (0.03±0.003

mg/g fw in rainy season) (Figure 9). The chlorophyll to carotenoid (Chl./Car.) ratios varied significantly across seasons for the studied three species (Figure 10).

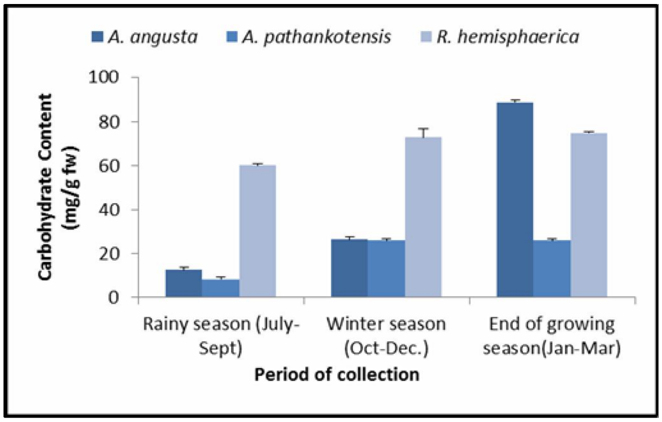

Fig. 1: Total carbohydrate content in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

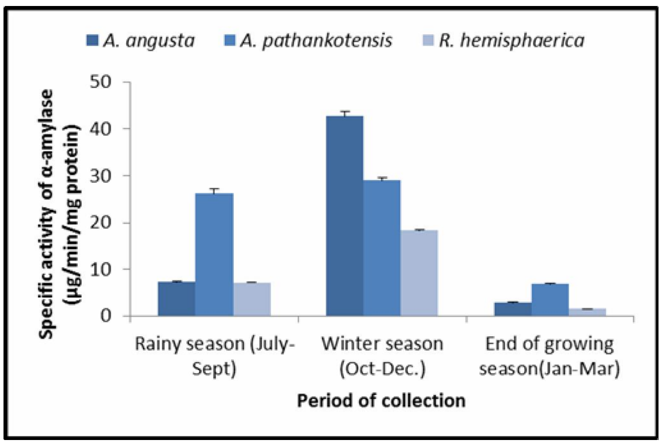

Fig. 2: Specific activity of α-amylase in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

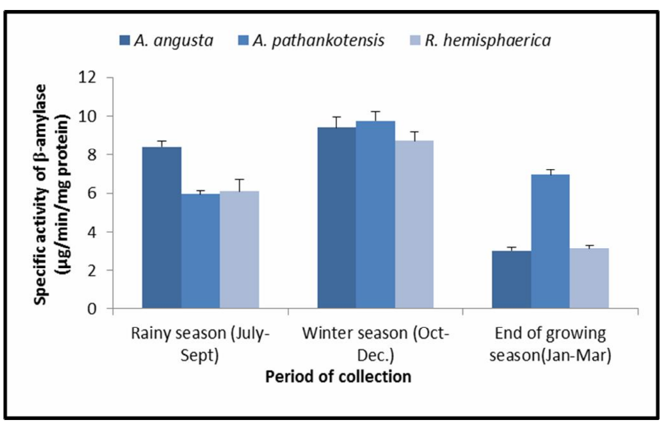

Fig. 3: Specific activity of β-amylase in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

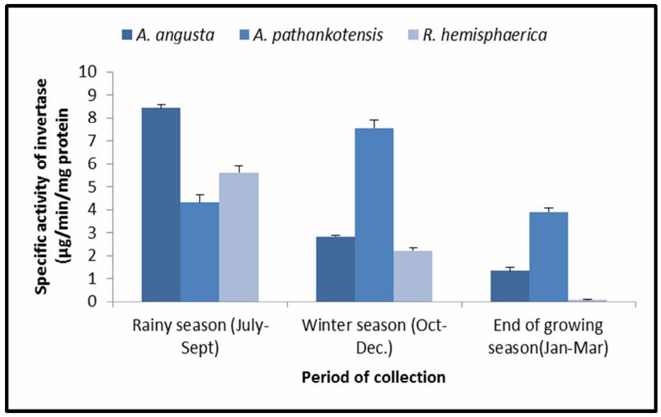

Fig. 4: Specific activity of invertase in three liverworts in three periods of collection. Values are means of three replicates±standard error (SE).

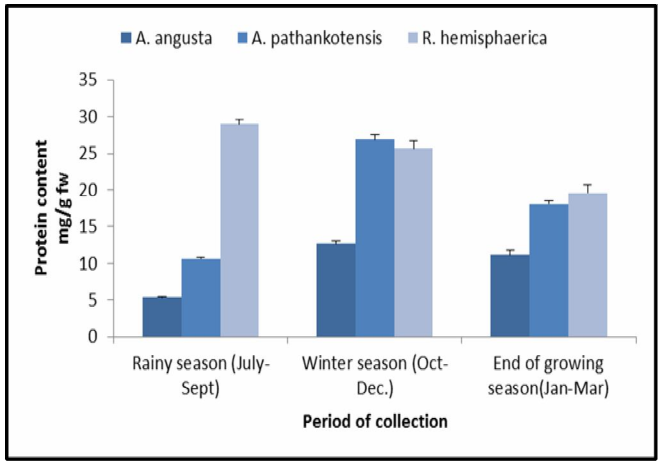

Fig. 5: Protein content in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

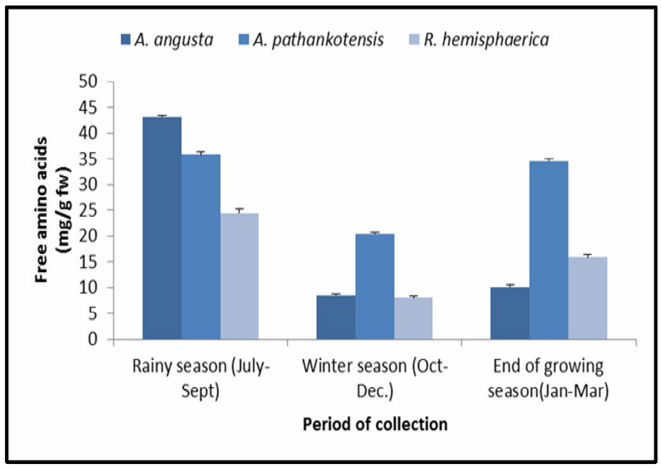

Fig. 6: Free amino acids content in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

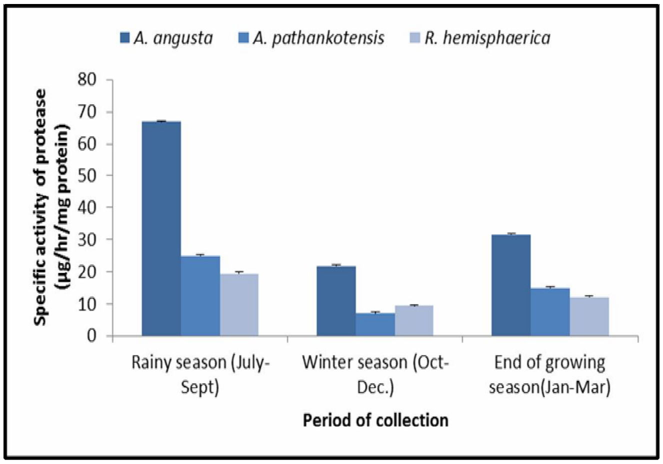

Fig. 7: Specific activity of protease in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

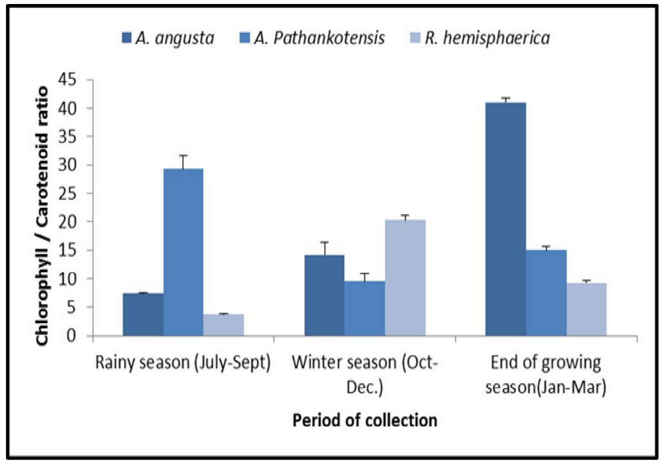

Fig. 10: Chlorophyll/Carotenoid ratios in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

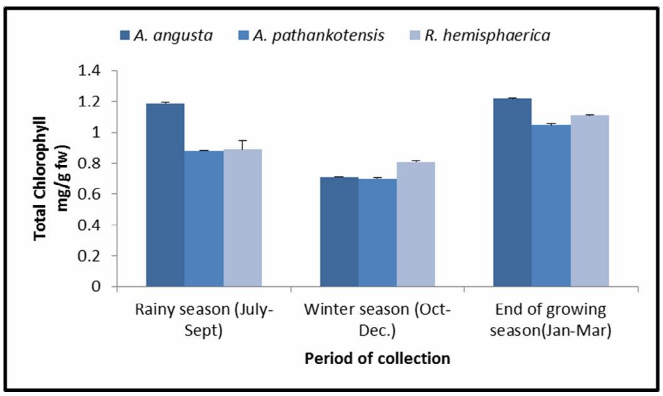

Fig. 8: Total Chlorophyll in three liverworts in three periods of collection. Values are means of three replicates±standard error (SE).

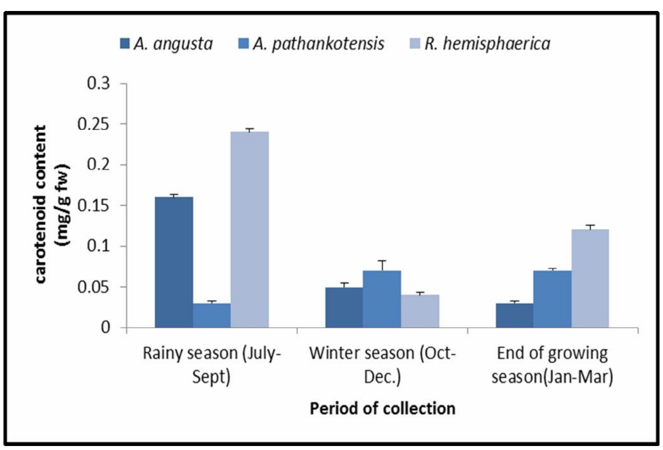

Fig. 9: Carotenoid content in three liverworts in three periods of collection. Values are means of three replicates ± standard error (SE).

Discussion

The highest concentration of carbohydrates was found during the end of the growing season in line with previous report on *Marchantia* and *Dumortiera* (Kapila *et al.*, 2014) and three species of *Plagiochasma* (Devi *et al.*, 2018) which suggests that with rise in temperature after the winter period, the rate of photosynthesis increases to store carbohydrates to provide energy to tide over harsh unfavourable conditions, and for thalli regeneration. *R. hemisphaerica* showed higher carbohydrate content as compared to both of the species of *Asterella* and less seasonal variations indicating that it might be less dependent on seasonal carbohydrate availability possibly due to its ability to adapt to various ecological conditions. Overall, the variations in carbohydrate content amongst the three taxa emphasize the distinct ecological strategies of different taxa in response to seasonal changes.

There has been a relationship between amylase activities and carbohydrates storage. Highest carbohydrates content and lowest amylase activity at the end of the growing season may indicate reduced needs for enzymatic breakdown as carbohydrates are already abundant, favoring their storage instead. Todaka *et al*. (2000) reported enhanced the β–amylase activity water stressed conditions.

Invertase showed a clear seasonal pattern, with *A. angusta* and *R. hemisphaerica* presenting the highest activity during the rainy season and decline in the winter and at the end of the growing season corresponding with a decrease in carbohydrate mobilization needs, as the excess carbohydrates are likely to be stored. This relationship indicates that invertase may play a critical role in the initial stages of carbohydrate breakdown for metabolism following habitat moisture fluctuations.

The content of free amino acids in all the three taxa was found highest in the rainy season, thus highlighting a possibly enhanced metabolic response during optimal growth conditions. Plant proteases are crucial enzymes that play significant role in various biological processes within plants, they are responsible for breaking down proteins into smaller peptides and amino acids by hydrolyzing peptide bonds (Palma *et al*., 2002; Vander Hoorn, 2008; Sharma and Gayen, 2021). The highest protease activity during the rainy season in all three taxa indicates an important role in nitrogen mobilisation vital for growth.

Photosynthesis is a vital process that powers energy flow within ecosystems and offers a valuable, quick and practical approach to understand how plant and ecosystem function (Węgrzyn *et al*., 2021) Seasonal climate changes can greatly affect how photosynthesis works in plants (Veres *et al*., 2020). Chlorophyll content in leaves is heavily influenced by various environmental factors, particularly light conditions. Higher light intensity typically enhances chlorophyll synthesis, while lower light levels can lead to diminished chlorophyll content as the plants adjust to their surroundings (Gond *et al*., 2012; Atar *et al*., 2013). Climate elements like temperature, humidity and CO_2 concentration also indirectly impact chlorophyll by influencing plant growth and metabolic functions (Dai *et al*., 2009; Çetin, 2017). In the present study, chlorophyll content followed the seasonal variation i.e. end of the growing season > rainy season > winter season. This could be related to the growth period and temperature fluctuations during the studied bryological seasons. Many studies (Martínez-Abaigar *et al*. 1994; Gerdol, 1996; Martínez-Abaigar and Núñiez-Olivera, 1998; Çetin, 2017; Shi *et al*., 2023) demonstrate that the amount of chlorophyll varies during the growth period, light availability and various environmental stresses. Gaberščik and Martinčič (1987) recorded a positive correlation between chlorophyll concentration and net photosynthesis in *Sphagnum*. Presently a correlation has been observed between the increased chlorophyll levels during the end of the growing season with higher carbohydrate levels during this season for all the species. This finding supports the hypothesis that chlorophyll concentration significantly influences carbohydrate synthesis, as the enhanced photosynthetic activity typically leads to increased carbohydrate production.

Carotenoids are essential pigments in photosynthesis that absorb blue-green light and transfer energy to chlorophylls, enhancing photosynthetic efficiency. They also protect plants from excess light through triplet–triplet energy transfer, preventing the damage from harmful reactions (Hashimoto *et al.*, 2016). Little seasonal variation found in the carotenoid content of all the studied species is in agreement with our previous finding on *Marchantia palmata* and *M. nepalensis* (Devi *et al.*, 2015). Liverworts possess higher concentrations of chlorophyll and carotenoids as compared to mosses, regardless of sunlight exposure (Pande and Singh, 1987). Like carotenoids, Chl./Car. ratio did not follow any seasonal pattern. For *A. angusta*, the mean Chl./Car. ratio was lowest during the rainy season, little increased in the winter season and rose sharply by the end of the growing season. *A. pathankotensis* exhibited highest ratio during the rainy season, which decreased in winter and then increased at the end of the growing season. Meanwhile, *R. hemisphaerica* showed the lowest overall ratios during the rainy season, increased sharply during to winter, and then again decreased by the end of the growing season. These results indicate distinct seasonal adaptations and physiological responses among the species, reflecting their strategies for acclimatization in varying environmental conditions.

Conclusion

The present findings highlight the seasonal variations in biochemical constituents and the activity of associated enzymes. These variations are important for understanding their functional significance in plant growth and metabolism throughout the bryological growth period. In summary, we conclude that the changes observed in biochemical compounds and enzyme activities across different seasons play a vital role in the adaptation of all the three presently studied thalloid liverworts species.

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