

Pigment concentration of ten bryophytes from Nainital, Kumaun Himalayas

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Abstract. The chlorophylls, carotenoids and their ratios were determined in 6 liverworts and 4 mosses of Nainital, Kumaun Himalaya. The amount of chlorophylls and carotenoids was higher in liverworts except for *Stephensiella brevipedunculata* compared to mosses. Liverworts studied occurred in shady habitats, whereas the mosses occurred in open situations. The chlorophyll *a/b* ratios were less than 1.5. This is a response of bryophytes to low light environments. The chlorophyll/carotenoid ratio did not show any remarkable difference among liverworts and mosses, as the study was carried out during the rainy season—the luxuriant growth period of bryophytes.

Keywords. Bryophytes; chlorophylls; chlorophyll *a/b* ratio; carotenoids; irradiance.

1. Introduction

Generally, the plants inhabiting at low solar irradiances have a greater chlorophyll concentration and lower chlorophyll *a/b* ratio compared to the plants growing in bright light (Boardman 1977). Thornber *et al* (1976) concluded that chlorophyll *b* is found only in the light-harvesting chlorophyll protein complex; a relatively larger amount of chlorophyll *b*, thus a lower chlorophyll *a/b* ratio is expected for plants inhabiting low light habitats relative to those growing in exposed areas. Martin (1980) and Martin and Churchill (1982) reported the effects of sun and shade on the variation of chlorophyll concentration among the bryophytes. Several other workers have investigated a positive correlation between chlorophyll *a/b* ratio and irradiance (Brown *et al* 1975; Patterson *et al* 1977, 1978; Martin 1980). Carotenoids also assist in light absorption.

Relatively few reports of chlorophyll *a* and *b* concentrations and chlorophyll *a/b* ratio of bryophytes are known (Wolf 1955; Miyata and Hosokawa 1961; Rao *et al* 1979; Dager *et al* 1980; Martin 1980; Muller and Ruch 1981; Glime 1984; Penuelas 1984a). The luxuriant growth of bryophytes was observed during the rainy season (from July to mid September); therefore, the present study was undertaken to assess the chlorophyll *a* and *b* and the carotenoid concentrations in certain bryophytes, at their maximum growth period growing in the Himalayan region.

2. Methods

Bryophytes were collected in August 1980 and 1981 respectively from various habitats in Nainital (29°24'N lat, 79°28'E long) at an altitude ranging from 1950–2200 m. The liverworts and mosses collected were *Marchantia polymorpha* from moist stony walls,

Conocephalum conicum from scree deposit of gravel and sandy soil, *Dumortiera hirsuta* from shady, wet clayey soil, *Stephensiella brevipedunculata* from dripping calcareous rocks, *Cryptomitrium himalayense* from caverns, *Pellia endiviaefolia* from aquatic shady habitats, *Hymenostylium recurvirostrum* from stone walls, *Rhodobryum roseum* and *Plagiomnium cuspidatum* from the forest-floor and *Leucodon secundus* from the tree bark. All the species were found growing in shadows cast by surrounding plants and boulders except *Hymenostylium recurvirostrum*.

A sample of 0.4 g green parts of the plants (in triplicate) was cleaned and blotted dry. Each sample was then divided into two halves, one of these was used for the determination of dry weight, whereas the other half was ground in a glass mortar with 80% acetone. For the determination of dry weight, another half was oven dried at 80°C and weighed.

The extract was centrifuged at 3000 rpm for 5 min and supernatant liquid was separated out for spectrophotometric analysis. Final volume of the extract was made upto 10 cc. The absorptions were made on a Beckmann's DK₂ spectrophotometer at 663 nm and 645 nm for chlorophylls *a* and *b* and at 480 nm and 510 nm for total carotenoids (Arnon 1949). The equation given by Maclachlan and Zalik (1963) was used for calculating the chlorophylls *a* and *b* concentrations, and from these the *a*:*b* ratios. Total carotenoids were computed from the expression given by Duxbury and Yentsch (1956). Total chlorophyll was calculated as the sum of chlorophyll *a* and *b* components.

3. Results and discussion

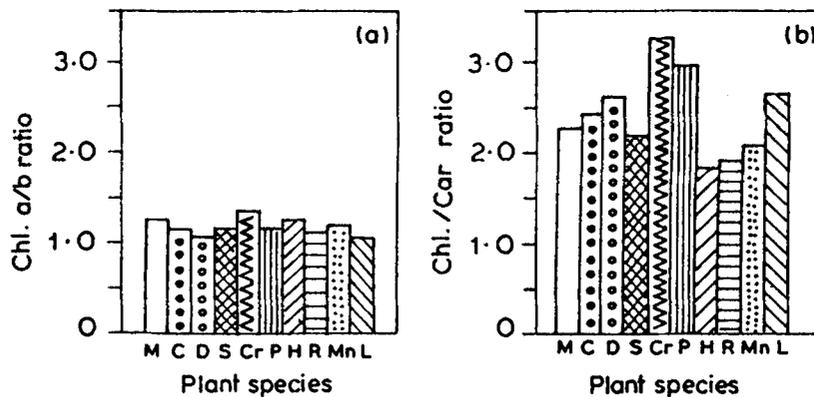
Mean total chlorophyll concentrations for the species examined ranged from 1.72–6.44 mg g⁻¹ dw (table 1). The greatest concentration occurred in *D. hirsuta*, whereas the lowest was in *H. recurvirostrum*. It may perhaps be due to the fact that *D. hirsuta* was growing under low solar irradiance (10 Klux) compared to *H. recurvirostrum*, which was found in exposed habitats (70 Klux). This was in agreement with the results of Martin and Churchill (1982). Mosses had a lower total chlorophyll concentration compared to liverworts except *S. brevipedunculata* (1.89 mg g⁻¹ dw).

Likewise, chlorophylls *a* and *b* concentrations varied significantly from one species to another. It was maximum for *D. hirsuta* (chlorophyll *a* = 3.35 mg g⁻¹ dw; chlorophyll *b* = 3.09 mg/g⁻¹ dw) and minimum for *H. recurvirostrum* (chlorophyll *a* = 0.96 mg/g⁻¹ dw; chlorophyll *b* = 0.76 mg/g⁻¹ dw).

The carotenoid concentration ranged from 0.87–2.44 mg g⁻¹ dw, the highest being in *D. hirsuta* and lowest in *S. brevipedunculata*. Although the mean total chlorophyll concentration of *H. recurvirostrum* was less than that of the liverworts, the carotenoid concentration was slightly higher (0.94 mg g⁻¹ dw). The value of total carotenoids for the 10 bryophytes was low relative to values reported for higher plants (Paule 1977) indicating that these plants might be adapted to the environments of low irradiance (Martin 1980; Valanne *et al* 1981; Martin and Churchill 1982). The results, therefore, suggested that generally bryophytes thriving in dark (low solar irradiance) and shady habitats accumulated greater chlorophyll and carotenoid concentrations. For example, *D. hirsuta* growing in shady habitats with low solar irradiance (10 Klux) had a mean total chlorophyll concentration of 6.44 mg g⁻¹ dw and mean total carotenoid of 2.44 mg g⁻¹ dw, while *H. recurvirostrum* growing in exposed habitats with high solar irradiance (70 Klux) had mean total chlorophyll

Table 1. Concentration of different pigments (mg g^{-1} dw) in bryophytes (mean \pm SE; sample size = 3 in each case).

Species	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Total carotenoid	Solar irradiance (Klux)
<i>Cryptomitrium himalayense</i> Kash.	3.33 \pm 0.24	2.58 \pm 0.22	5.91 \pm 0.47	1.81 \pm 0.15	8
<i>Dumortiera hirsuta</i> (Reinw. Bl. et Nees)	3.35 \pm 0.07	3.09 \pm 0.13	6.44 \pm 0.18	2.44 \pm 0.24	10
<i>Pellia endiviaefolia</i> (Dicks.) Dum.	3.16 \pm 0.09	2.72 \pm 0.08	5.88 \pm 0.13	1.97 \pm 0.10	10
<i>Conocephalum conicum</i> (L.) Necker	2.86 \pm 0.32	2.39 \pm 0.37	5.25 \pm 0.69	2.15 \pm 0.29	12
<i>Leucodon secundus</i> (Harv.) Mitt.	1.43 \pm 0.09	1.32 \pm 0.10	2.75 \pm 0.19	1.03 \pm 0.04	15
<i>Stephensoniella brevi-</i> <i>pedunculata</i> Kash.	1.02 \pm 0.09	0.87 \pm 0.07	1.89 \pm 0.15	0.87 \pm 0.03	18
<i>Marchantia polymorpha</i> L.	1.57 \pm 0.17	1.25 \pm 0.13	2.82 \pm 0.27	1.24 \pm 0.08	20
<i>Rhodobryum roseum</i> (Hedw.) Limpr.	1.57 \pm 0.17	1.41 \pm 0.02	2.98 \pm 0.04	1.54 \pm 0.37	50
<i>Plagiomnium cuspidatum</i> (Hedw.) Kop.	1.67 \pm 0.06	1.38 \pm 0.01	3.05 \pm 0.06	1.48 \pm 0.17	60
<i>Hymenostylium recurvi-</i> <i>rostrum</i> (Hedw.) Dix	0.96 \pm 0.05	0.76 \pm 0.01	1.72 \pm 0.05	0.94 \pm 0.04	70

**Figure 1.** Pigment concentration in bryophytes. (a) Chlorophyll *a/b* ratio, (b) Chlorophyll Carotenoid ratio. M, *M. polymorpha*; C, *C. conicum*; D, *D. hirsuta*; S, *S. brevipedunculata*; Cr, *C. himalayense*; P, *P. endiviaefolia*; H, *H. recurvirostrum*; R, *R. roseum*; Mn, *P. cuspidatum*; L, *L. secundus*.

concentration of 1.72 mg g^{-1} dw and mean total carotenoid of 0.94 mg g^{-1} dw. Sanger (1971), in angiosperms has reported higher chlorophyll and carotenoid concentrations in plants growing under shade.

The chlorophyll *a/b* ratio varied between 2.0 and 4.0 for most of the vascular plants (Chang and Troughton 1972; Alberte *et al* 1976). The chlorophyll *a/b* ratio in the present bryophytes was less than 2.0 (figure 1a). The maximum mean value (1.3) occurred in *C. himalayense*. Similar values for chlorophyll *a/b* ratio (1.1–1.2) were

reported in 3 species of liverworts by Rao *et al* (1979). Kallio and Valanne (1975) and Valanne (1977) also reported low values (1.3–2.4) for 4 moss species, grown under various light conditions. Tieszen and Johnson (1968) in their observation on some arctic tundra communities noted very low values for the chlorophyll *a/b* ratio in the moss layer (0.99–1.89). The low ratios are likely to be a response of these plants to a low light intensity as also suggested by Martin (1980) for 11 mosses of North Carolina and Martin and Churchill (1982) for 31 moss species.

The chlorophyll/carotenoid ratio varied between 1.82 and 3.28, the highest being in *C. himalayense* and the lowest in *H. recurvirostrum* (figure 1b).

In summary, it can be concluded that chlorophyll and carotenoid concentration was greater in liverworts relative to mosses, irrespective of solar irradiance. Bryophytes thriving in habitats with bright light, in general, have a lower chlorophyll concentration and higher chlorophyll *a/b* ratio compared to those occurring in shaded habitats.

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References

- Alberte R S, McClure P M and Thornber J P 1976 Photosynthesis in trees. Organization of chlorophyll and photosynthetic unit size in isolated gymnospermous chloroplasts; *Plant Physiol.* **58** 341–344
- Arnon D I 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*; *Plant Physiol.* **24** 1–15
- Boardman N K 1977 Comparative photosynthesis of sun and shade plants; *Annu. Rev. Plant Physiol.* **28** 355–357
- Brown J S, Alberte R S and Thornber J B 1975 Comparative studies on the occurrence and spectral composition of chlorophyll protein complexes in a wide variety of plant material; in *Proc. III International Congress on Photosynthesis* (ed) M Avron (Amsterdam: Elsevier) pp 1951–1952
- Chang F H and Troughton J H 1972 Chlorophyll *a/b* ratios in C₃ and C₄ plants; *Photosynthetica* **6** 57–65
- Dager J C, Awasthi A C and Singh V P 1980 Effect of light quality on the growth and photosynthetic pigments of *Riccia discolor* L. et L.; *Cryptogamic Bryol et Lichen.* **1** 305–309
- Duxbury A C and Yentsch G S 1956 Plankton pigment monograph; *J. Marine Res.* **15** 19–101
- Glime J M 1984 Theories on adaptations to high light intensity in the aquatic moss *Fontinalis*; *J. Bryol.* **13** 257–262
- Kallio P and Valanne N 1975 On the effect of continuous light on photosynthesis in mosses; *In Fennoscandian Tundra Ecosystems Part I* (ed) S E Wielgolaski; *Ecol. Stud.* **16** 149–162
- Maclachlan S and Zalik S 1963 Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley; *Can. J. Bot.* **41** 1053–1062
- Martin C E 1980 Chlorophyll *a/b* ratios of Eleven North Carolina Mosses; *Bryologist* **83** 84–87
- Martin C E and Churchill S P 1982 Chlorophyll concentrations and *a/b* ratios in mosses collected from exposed and shaded habitats at Kansas; *J. Bryol.* **12** 297–304
- Miyata I and Hosokawa T 1961 Seasonal variations of the photosynthetic efficiency and chlorophyll content of epiphytic mosses; *Ecology* **42** 766–775
- Muller and Ruch 1981 Polymorphism in the thallus morphogenesis of *Sphaerocarpous donnelli* Aust. as a result of different substrate combinations; *J. Bryol.* **11** 555–558
- Patterson D T J, Bunce A, Alberte R S and Van Volkenburgh 1977 Photosynthesis in relation to leaf characteristics of cotton from controlled and field environments; *Plant Physiol.* **59** 384–387
- Patterson D T J, Duke S O and Hoagland R E 1978 Effects of irradiances during growth on adoptive photosynthetic characteristics of velvet leaf and cotton; *Plant Physiol.* **61** 402–405

- Paule L 1977 Contents of pigments in assimilatory organs of silver fir; *Biologia* **32** 729–739
- Penuelas J 1984a Pigments of aquatic mosses of the river of silver fir; *Biologia* **32** 729–739
- Penuelas J 1984a Pigments of aquatic mosses of the river muga NE Spain and their response to water pollution; *Lindbergia* **10** 127–132
- Rao K R, Kumar N R and Reddy A N 1979 Studies of photosynthesis in some liverworts; *Bryologist* **82** 286–289
- Sanger J E 1971 Quantitative investigations of leaf pigments from their inception in buds through autumn colouration to decomposition in falling leaves; *Ecology* **52**
- Thorner J P, Alberte R S, Hunter F A, Shiozawa J A and Kan K S 1976 The organisation of chlorophyll in the plant photosynthetic unit. (eds) J M Olson and G Hind Chl.-Proteins, Reaction Centres and Photosynthetic Membranes, Brookhaven Symposia in Biology **27** 132–148
- Tieszen L L and Johnson P L 1968 Pigment structure of some arctic tundra communities; *Ecology* **49** 270–373
- Valanne N 1977 Effect of continuous light on CO₂ fixation, chlorophyll content growth and chloroplast structure in *Ceratodon purpureus* (Hedw.) Brid., *Zeit Schr-Pflanzen Physiol.* **81** 347–357
- Valanne N E-Maró and Niemi H 1981 Photosynthetic apparatus of mosses; *Abstr XIII Int. Bot. Congr.* 145
- Wolf F T 1955 Comparative chlorophyll content of the two generations of Bryophytes; *Nature (London)* **181** 579–580