Research Article

STUDY ON SOME OF THE CONTENTS OF SOME BRYOPHYTES-I ANTHOCEROTAE AND HEPATICAE

S. Kaur, Anju Rao* and S.S. Kumar
*Department of Botany, Panjab University, Chandigarh, India. PIN-160014
***Corresponding author's E-mail:** anjuraoanju@rediffmail.com

Received on: 02-10-2010; **Finalized on:** 29-11-2010.

ABSTRACT

Amount of chlorophyll content, Proteins, Carbohydrates and RNA has been studied in ten taxa of hepaticae and *Anthoceros erectus*. The study also embodies the specific activity of enzymes α-amylase, ß-amylase, Proteases and polyphenol oxidases. It has been found that carbohydrate content is more than protein content in *Anthoceros erectus*, *Plagiochila asplenioides, Targionia indica*, *Cryptomitrium himalayense* and *Conocephalum conicum* whereas, total protein content is more than carbohydrates in *Pellia endivifolia*, *Plagiochasma appendicultum Marchantia palmata*, *Weisnerella denudata* and *Dumortiera hirsuta* although the difference is only marginal. Total RNA content in all the investigated taxa is lesser than protein and carbohydrates. Total chlorophyll has found to be maximum in *Conocephalum conicum* and minimum in *Targionia indica*. The four enzymes tested with regard to their specific activity show the following sequential order in all the studied taxa. Polyphenyl oxidases > proteases > β amylase > α amylase.

Keywords: Chlorophyll, Proteins, Carbohydrates, RNA, α-amylase, ß-amylase, Proteases and polyphenol oxidases.

INTRODUCTION

Bryophytes are a preferred material for experimental studies because of their small size, simple morphology and structural organisation, extremely high regeneration potential, low nutritional requirements, easy culturability and rapid completion of life cycle.

Although studies on chemical constituents of bryophytes were initiated in the beginning of the last century only (Lohmann[1] 1903), yet some significant work was done by Japanese workers only in the later part of twentieth century (cf Asakawa[2] 1983).

The available data shows that a very little attention was paid to carbohydrates, proteins, RNA, enzymes, enzyme activity and chlorophyll contents of bryophytes (Freeland[3], 1957, cf Asakawa, 1983; Taylor *et al*,[4] 1995; Bendz *et al*,1962[5], 1966 a[6], b[7], 1967[8], 1968[9]). The present study is undertaken with a view to provide more information on these little known aspects of bryophytes.

MATERIALS AND METHODS

Materials were collected from different locations in Shimla (Western Himalayas). Precise data concerning the locality, altitude and nature of the substratum for each collection is given in table I. The material of each taxon were first purified (removed adhering plants, particularly mosses) and thoroughly washed with clean water and then with distilled water. It was then dried at room temperature. The materials were then crushed in pestle and mortar and sieved for biochemical analysis.

The voucher specimens are deposited in the Herbarium, Department of Botany, Punjab University, Chandigarh under reference numbers (given in Table I), assigned to each sample.

The methods given below were followed for various estimations.

Total Chlorophyll Content: The chloroplastic pigments were extracted in Dimethyl Sulphooxide (DMSO) following the method given by Hiscox and Israelstam[10] (1979) and improved by Daizy and Kohli[11] (1991).

Estimation: 100 mg of fresh material was suspended in 10 ml of DMSO and incubated at 65°C for one hour. The DMSO was recovered by decantation and final volume corrected to 10 ml with fresh DMSO. The value of chlorophyll thus recovered in DMSO was measured at dual wave lengths of 645 and 663 nm of spectronic 1201 spectrophotometer using DMSO as blank. The total amount of chlorophyll was calculated from the extinction values following the equation of Arnon (1949) as suggested by Hiscox and Israelstam (1979).

Chl 'a' (µg/ml) = $10.63 \times {}^{A}663 - 2.39 \times {}^{A}645$

Chl 'b' (µg/ml) = $20.11 \times {}^{A}645 - 5.18 \times {}^{A}663$

Total Ch1 (µg/ml)= $6.45 \times {}^{A}663 + 17.72 \times {}^{A}645$

where ${}^{A}645$ and ${}^{A}663$ represent extinction values at 645 nm and 663 nm respectively. The total content was expressed in terms of dry wt.

Macromolecules: Preparation of material for macromolecular estimation

For estimation of macromolecules, material was crushed in acetone and freed of pigments by repeated washings in acetone for about 3 days. The crushed tissue was then put in 1:1 mixture of acetone and petroleum ether for 24 hr followed by further suspension in petroleum ether for 24 hr and then air dried. The dried powder was used for estimation of total proteins, RNA, different enzymes and water and acid soluble carbohydrates.

Table 1: Showing different taxa studied, locality from where collected, altitude of locality, substration and herbarium number

S.No.	Name of Taxon	Order and Family	Locality	Altitude of locality	Substratum	Herbarium reference number
1.	*Anthoceros erectus* St.	Anthocerotales Anthocerotaceae	Way to Glen, Shimla	1900 m	On rocks	4479
2.	*Plagiochila asplenioides (L.)* Dum.	Jungermanniales Plagioc-hilaceae	Glen. Shimla	1830 m	Soil on rocks	4480
3.	*Pellia endivifolia* Dicks.	Metzgeriales Pelliaceae	Glen, Shimla	1830 m	Soil gathered on rocks	4477
4.	*Targionia indica* sp. nov.	Marchantiales Targioniaceae	Glen, Shimla	1830 m	On rocks	4478
5.	*Plagiochasma appendiculatum* Lehm. et Lindb.	Marchantiales Aytoniaceae	Chedwick Fall, Shimla	1586 m	Moist soil gathered on rocks	4474
6.	*Cryptomitrium himalayense* Kash.	Marchantiales Aytoniaceae	Way to Glen, Shimla	1900 m	Wet soil on rocks	4475
7.	*Marchantia palmata* Nees.	Marchantiales Marchantiaceae	Chedwick Fall, Shimla	1586 m	Moist soil on rocks	4471
8.	*Weisnerella denudata (Mitt.)* Steph.	Marchantiales Marchantiaceae	Glen, Shimla	1830 m	Wet Soil on rocks	4473
9.	*Conocephalum conicum (L.)* Necker	Marchantiales Conocephalaceae	Glen, Shimla	1830 m	On wet soil	4476
10.	*Dumortiera hirsuta* Sw. R. Bl. et. Nees.	Marchantiales Marchantiaceae	Glen, Shimla	1830 m	Wet Soil on rocks	4472

Estimation of Proteins: Proteins were estimated by Lowry's method using Folin's reagents (Lowry *et al,*[12] 1951).

Estimation of Enzymes: For estimation of enzymes thalli and leaves of different taxa were washed, surface dried between the folds of the filter paper and kept in freezer for chilling, then homogenized in a pre-chilled pestle and mortar using a pinch of acid washed (pH = 7.0) sand and little amount of extraction buffer (1.19 gm Na_2HP0_4/100 ml D.W. and 1.04 gm NaH_2PO_4/100ml D.W., mixed in the ratio of 1:1 (v/v pH = 7.0). The homogenate was centrifuged at 8000 × g for 7 minutes, followed by recentrifugation at 17,000 × g for 11 minutes and finally at 27,000 × g for 5 minutes. All the three centrifugations were done at 4°C of the rotor temperature. The clear supernatent, thus obtained, was collected for estimation of enzymes.

a. **α-amylase:-** It was estimated by Muentz's [13]method (1977).

b. **β-amylase:-** β-amylase activity was measured following the method of Bernfeld[14] (1951), as modified by Dure (1960).

c. Protease:- The activity of the enzyme was measured according to the method given by Basha and Beevers[15] (1975).

d. Polyphenol oxidase:- The activity of polyphenol oxidase was measured by adopting the methodology of Van Leyveld and Pretorius[16] (1973).

Estimation of Carbohydrates:-

Carbohydrate content was estimated according to the method devised by Loweus[17] (1952).

Estimation of Total RNA content:-

The RNA content was extracted in 3N perchloric acid following the method of Majbaum[18] (1939).

RESULTS AND DISCUSSION

The findings of the present investigations on proteins, carbohydrates, RNA, chlorophyll 'a', 'b', total chlorophyll and a study of the activity of enzymes (α-amylase, β-amylase, protease, polyphenol oxidase) on the growth of some liverworts are insufficient to draw any definite conclusions. Nevertheless, these results together with few earlier studies do provide a basis for drawing some inferences that are given under each table.

A perusal of the present data (given in Table 2.) on the total carbohydrate, proteins and RNA content shows that carbohydrate content is more than protein content in *Marchantia palmata, Dumortiera hirsuta, Cryptomitrium himalayense, Conocephalum conicum, Targionia indica. Plagiochila aspleniodes* and *Anthoceros erectus,* whereas

total protein content is more than carbohydrates in *Weisnerella denudata, Plagiochasma appendiculatum* and *Pellia endivifolia.* The total RNA content in all the investigated taxa is observed to be lesser than the protein and carbohydrate contents.

Fertile materials, contained relatively more carbohydrate content than found in the sterile materials of *Dumortiera hirsuta*. This observation is in line with an earlier finding in *Targionia hypophylla,* in which starch, soluble sugars, non-reducing sugars and fats were found to be more in the fruiting thalli than in the vegetative thalli (Chaudhary, Singh and Bapna,[19] 1985).

Unlike the carbohydrate content, the total protein content and the total RNA content in the sterile materials of *Dumortiera hirsuta* was found to be more than that observed in the fertile speciments of this taxaon. This observation is also in line with an earlier report (Chaudhary, Singh and Bapna, 1985) on the chemical analysis of the vegetative and fruiting thalli of *Targionia hypophylla.* Besides proteins, terpenes, alcohols and RNA were also found to be in higher concentration in the vegetative thalli than in the fruiting thalli of *Targionia hypophylla.*

Of the studied taxa, *Dumortiera hirsuta* (sterile) is the richest in the total RNA content (242 µg/mg dry wt.) and also in the total protein content (318 µg/mg dry wt.), while *Cryptomitrium himalayense* is the poorest in the total RNA content (79.9 µg/mg dry wt.) as well as in the total protein content (150 µg/mg dry wt.). A closer look on Table 2. shows that in other taxa also, where there is higher content of total RNA, there is also a correspondingly higher content of total proteins and vice versa. It seems to suggest, that in bryophytes there may be a strong correlation between their total RNA content and their total protein content. Further studies on liverworts and mosses are essential in order to confirm or review this generalisation.

The amount of Chl 'a', Chl 'b', Chl 'a': Chl 'b' ratio and total chlorophyll of the presently studied taxa is given in Table 3.

Table 2: Showing total carbohydrate, total protein and total RNA content in ten taxa of liverworts and Anthoceros

S. No	Name of the taxon	Total carbohydrate content in µg/mg dry wt.	Total Protein content in µg/mg dry wt.	Total RNA content in µg/mg dry wt.
1	*Anthoceros Orectus*	246	211	132
2	*Plagiochila asplenioides*	224	201	124
3	*Pellia endivifolia*	200	220	160
4	*Targionia indica*	220	155	102
5	*Plagiochasma appendiculatum*	233	237	212
6	*Cryptomitrium himalayense*	230	150	79.9
7	*Marchantia palmata*	196	244	237
8	*Weisnerella denudata*	120	161	110
9	*Conocephalum conicum*	239	227	202
10	*Dumortiera hirsuta*			
	a. Sterile	313	318	242
	b. Fertile	349	258	212

Table 3: Amount of Chl 'a', Chl 'b', Chl 'a': Chl 'b' ratio and total chlorophyll in all the studied taxa.

S. No	Taxa	Chl 'a' in µg/ml	Chl 'b' in µg/ml	Chl 'a': Chl 'b' ratio	Total chlorophyll in µg/ml
1	*Anthoceros erectus*	8.8	10.7	0.82	20.4
2	*Plagiochila asplenioides*	6.5	11.6	0.56	18.7
3	*Pellia endivifolia*	28.8	23.8	1.21	55.8
4	*Targionia indica*	6.38	11.3	0.56	18.5
5	*Plagiochasma appendiculatum*	20	4.9	4.08	26.8
6	*Cryptomitrium himalayense*	8.2	14.7	0.55	23.8
7	*Marchantia palmata*				
	a. Sterile	24.0	12.0	2.0	38.6
	b. Fertile	20.2	13.0	1.55	35.4
8	*Weisnerella denudata*	6.6	11.7	0.56	19.2
9	*Conocephalum conicum*	31.9	28.9	1.10	64.5
10	*Dumortiera hirsuta*				
	a. Sterile	31.0	19.0	1.63	45.6
	b. Fertile	27.2	20.2	1.34	42.4

Table 4: Specific activities of enzymes (α-amylase, β-amylase, protease and polyphenol oxidase) in various studies taxa

S. No.	Taxa	α-amylase in µg/min/mg protein	β-amylase in µg/min/mg protein	Protease in µg/hr/mg protein	Polyphenol oxidase in µ kats/min/gm protein
1	*Anthoceros erectus*	0.022	0.043	0.63	1.09
2	*Plagiochila asplenioides*	0.12	0.64	1.23	0.90
3	*Pellia endivifolia*	0.019	0.026	0.56	1.12
4	*Targionia indica*	0.014	0.039	0.60	1.15
5	*Plagiochasma appendiculatum*	0.060	0.120	0.50	0.90
6	*Cryptomitrium himalayense*	0.035	0.29	0.86	1.25
7	*Marchantia palmata*				
	a. Sterile	0.023	0.016	0.19	0.70
	b. Fertile	0.11	0.31	0.80	1.25
8	*Weisnerella denudata*	0.31	0.37	0.87	1.20
9	*Conocephalum conicum*	0.029	0.085	0.29	0.80
10	*Dumortiera hirsuta*				
	a. Sterile	0.019	0.031	0.13	0.65
	b. Fertile	0.27	0.49	0.88	1.30

On the basis of the nature of the chlorophyll content, the studied taxa fall into two groups:

Group A: Chlorophyll 'a' content is more than the chlorophyll 'b' content: *Marchantia palmata, Conocephalum conicum, Dumortiera hirsuta, Plagiochasma appendiculatum, Pellia endivifolia.*

Group B: Chlorophyll 'b' content is more than the chlorophyll 'a' content: *Weisnerella denudata, Cryptomitrium, himalyense, Targionia indica, Plagiochila asplenioides and Anthoceros erectus.*

A perusal of Table-3 reveals that of the investigated taxa, *Conocephalum conicum, Dumortira hirsuta* and *Pellia endivifolia* are relatively richer in chlorophyll content. This richness in the chlorophyll content of these taxa may be due to their occurence in very favourable habitat which promote the activity of chlorophyll synthesising enzymes.

Conocephalum Conicum is richest in chlorophyll content (64.5µg/ml) while *Targionia indica* is poorest in chlorophyll content (18.5 µg/ml).

It would be desirable to determine and compare the 'Zn' content of mosses and liverworts, as this element is an essential constituent of enzymes synthesizing chlorophyll pigments particularly chlorophyll 'a' which in turn may affect the total chlorophyll content (Rai and Dey[20], 1980).

A study of the specific activity of some enzymes i.e. α-amylase, β-amylase, protease and polyphenol oxidase in 10 taxa (Table 4) reveals considerable differences among the taxa.

The specific activity of α-amylase of *Dumortiera hirsuta* (female) is maximum i.e. 0.27 µg/min/mg protein and of *Targionia indica* minimum i.e. 0.014 µg/min/mg protein. This finding is explained by the carbohydrate content of these taxa, which is higher in *D. hirsuta* (fertile) i.e. 349 µg/mg dry wt. and lesser in *T. indica* i.e. 220 µg/mg/dry wt. However, this increase in specific activity with increase in substrate concentration is not true in all the studied taxa, because the velocity of an enzymatic reaction usually increases with increase in the concentration of the substrate up to a certain maximum level, after which the relative amount acted upon per unit of time decreases with increase in the substrate concentration. The retarding effect of relatively high concentrations of the substrate upon enzyme activity may be caused in part by the more rapid accumulation of the end products of the reaction.

The specific activity of α-amylase is maximum i.e. 0.64 µg/min/mg protein in *Plagiochila asplenioides* and minimum i.e. 0.016 µg/min/mg protein in sterile plants of *Marchantia palmata* although there is a little difference in the carbohydrate content of these two taxa (224 µg/mg dry wt in *P. aspleniodes* and (196 µg/mg dry wt.) in *M. palmata.* It has been observed that the specific activity does not increase with the increase in the protein content in case of protease, where the substrate is protein.

Udar and Chandra[21,22] (1960 a,b) studied the amylase activity in the sterile and fertile (♀ & ♂) materials of *Riccia discolor* and found that the male (♂) plants excelled the female (♀) plants in respect of the specific activity of amylase. However, studies are essential to see if any correlation exists between amylase activity and sexual nature of plant.

The present observations on the specific activity of protease in *Plagiochila asplenioides* (1.23 µg/hr/mg protein) and in *Dumortiera hirsuta* (0.13 µg/hr/mg protein) when analysed in relation to their protein content (201 µg/mg dry wt. in *Plagiochila asplenioides* and 318 µg/mg dry wt. in *Dumortiera hirsuta*), which is a substrate for protease, clearly suggest that the specific activity of this enzyme does not necessary increase with the increase in the protein content of the taxa beyond a

certain level. After a maximum level is achieved, it may rather show a progressive decline.

A comparison of the present results in some of the few taxa earlier studied by Kapila and Dhawan[23] (2000) in some of the present respects (protein, water soluble carbohydrate, RNA, activity of α- and β- amylase invertase) indicates wide variations. These variations appear to be governed by the microclimatic conditions of the area, collection time and also the seasons and period of active or inactive growth.

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