Research Article

STUDY ON SOME OF THE CONTENTS OF SOME BRYOPHYTES-II MUSCI

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Received on: 29-10-2010; **Finalized on:** 16-12-2010.

ABSTRACT

Amount of chlorophyll content, Proteins, Carbohydrates and RNA has been studied in ten taxa of Mosses. 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 *Atrichum pallidum, Thuidium recognitum*, *Brachythecium kamounense* and *Funaria hygrometrica*, whereas, total protein content is more than carbohydrates in *Rhodobryum roseum, Pohlia elongata* and *Fissidens taxifolius*. Total RNA content in all the investigated taxa is lesser than protein and carbohydrates. Total chlorophyll has found to be maximum in *Pohlia elongata* and minimum in *Fissidens taxifolius* although the difference is only marginal. The chlorophyll a and chlorophyll b ratio was observed to be highest in *Brachythecium kamounense.* The four enzymes tested with regard to their specific activity show the following sequential order in all the studied taxa. Protease > Polyphenol oxidase > α-amylase > β-amylase.

Keywords: Bryophytes, Taxa of Mosses, RNA content, Chlorophyll.

INTRODUCTION

Bryophytes constitute a preferred material for experimental studies because of their small size, simple morphology and structural organization, extremely high regeneration potential, easy culturability, low nutritional requirements and very short life cycle.

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

The available data shows that a very little attention was paid to carbohydrates, proteins, RNA, enzymes, enzyme activity and chlorophyll contents of bryophytes[3-8]. 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[9] (1979) and improved by Daizy and Kohli[10] (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 thorough 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.

Estimation of Proteins: Proteins were estimated by Lowry's method using Folin's reagents[11].

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 supernatant, thus obtained, was collected for estimation of enzymes.

a. α-amylase:- It was estimated by Muentz's[12] method (1977).

b. β-amylase:- β-amylase activity was measured following the method of Bernfeld[13] (1951), as modified by Dure[14] (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 Loewus [17](1952).

Estimation of Total RNA content:-

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

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

S. No.	Name of Taxon	Order Family	Locality	Altitude of locality	Substratum	Date of collection	Herbarium reference number
1.	*Rhodobryum roseum* (Hedw.) Limpr.	Eubryales Bryaceae	Way to Glen, Shimla.	1900 m	Wet soil on rocks	9.9.93	4481
2.	*Pohlia elongata* Hedw.	Eubryales Bryaceae	Way to Glen, Shimla.	1900 m	Soil on rocks	9.9.93	4482
3.	*Atrichum pallidium* Ren. et Card.	Polytrichales Polytrichaceae	Summer Hill, Shimla	2000 m	Soil on rocks	10.9.93	4483
4.	*Thuidium recognitum* (Hedw.) Lindb.	Hypnobryale, Thuidiaceae	Summer Hill, Shimla	2000 m	Soil on rocks	10.9.93	4484
5.	*Fissidens taxifolius* Dix.	Fissidentales Fissidentaceae	Way to Chewick Fall, Shimla	1586 m	On Soil	10.9.93	4485
6.	*Brachythecium kamounense* (Harv.) Jaeq	Hypnobayales Brachytheciaceae	Way to Glen, Shimla	1830 m	Soil on rocks	9.9.93	4486
7.	*Funaria hygrometrica* Hedw.	Funariales Funariaceae	Glen, Shimla	1830 m	Soil on rocks	9.9.93	4487

Table 2: Showing total carbohydrate, total protein and total RNA content in seven taxa of Mosses

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.	*Rhodobryum roseum*	112	167	97
2.	*Pohlia elongata*	188	215	135
3.	*Atrichum pallidum*	324	220	157
4.	*Thuidium recognitum*	236	215	135
5.	*Fissidens taxifolius*	216	235	169
6.	*Brachythecium kamounense*	360	260	211
7.	*Funaria hygrometrica*	355	206	127

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

S. No.	Taxon	Chl. 'a' in µg/ml	Chl. 'b' in µg/ml	Chl 'a': Chl 'b' ratio	Total chlorophyll in µg/ml
1.	*Rhodobryum roseum*	7.3	13.1	0.55	21.3
2.	*Pohlia elongata*	7.5	13.4	0.55	21.7
3.	*Atrichum pallidum*	7.4	13.4	0.55	21.6
4.	*Thuidium recognitum*	6.4	11.3	0.56	18.5
5.	*Fissidens taxifolius*	1.04	0.38	0.36	1.52
6.	*Brachythecium kamounense*	4.7	1.07	4.39	5.94
7.	*Funaria hygrometrica*	7.5	13.4	0.55	21.7

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

S. No.	Taxon	Specific activities of enzymes			
		α-amylase in µg/min/mg protein	β-amylase in µg/min/mg protein	Protease in mg/hr/mg protein	Polyphenol oxidase in m µ kats/min/gm protein
1.	*Rhodobryum roseum*	0.026	0.020	0.61	0.52
2.	*Pohlia elongata*	0.050	0.034	0.64	0.50
3.	*Atrichum pallidum*	0.041	0.029	0.67	0.40
4.	*Thuidium recognitum*	0.027	0.018	0.58	0.39
5.	*Fissidens taxifolius*	0.032	0.012	0.38	0.30
6.	*Brachythecium kamounense*	0.041	0.033	0.69	0.60
7.	*Funaria hygrometrica*	0.052	0.035	0.66	0.40

RESULTS AND DISCUSSION

The results of the present investigations on proteins, carbohydrates, RNA, chlorophyll 'a', 'b', total chlorophyll together with a study of the activity of enzymes (α-amylase, β-amylase, protease and polyphenol oxidase) of mosses embodied in the preceding pages, 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 of *Rhodobryum roseum, Pohlia elongata, Atrichum pallidum, Thuidium recognitum, Fissidens taxifolius, Brachythecium kamounense* and *Funaria hygrometrica* reveals that in most of the taxa i.e. *Atrichum pallidum, Thuidium recognitum*, *Brachythecium kamounense* and *Funaria hygrometrica*, the carbohydrate content was more than their protein content, which in turn was more than the total RNA content. However, in some taxa i.e. *Rhodobryum roseum, Pohlia elongata* and *Fissidens taxifolius* the total protein content was more than the carbohydrate content. The total RNA content in all the investigated taxa is observed to be lesser than the protein and carbohydrate contents.

Of the studied taxa, the maximum carbohydrate content is found in *Brachythecium kamounense* and the minimum is observed in *Rhodobryum roseum*. This wide variation in carbohydrate content may be due to the lesser photosynthetic area available in *R. roseum* because the stems are almost naked and the leaves are aggregated only at the top of the stem, while, in *Brachythecium kamounense*, *Funaria hygrometrica* and *Atrichum pallidum*, the photosynthetic area is considerably enhanced by the increased number of leaves closely distributed on the stems.

A clear 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.

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:

Brachythecium kamounense and Fissidens taxifolius.

Group B: Chlorophyll 'b' content is more than the chlorophyll a' content:

Atrichum pallidum, Rhodobryum roseum, Pohlia elongata, Thuidium recognitum and Funaria hygrometrica.

A perusal of Table 3, reveals that of the studied taxa, most of them have almost same amount of chlorophyll content (i.e. about 21.3 to 21.7 µg/ml). *Fissidens taxifolius* is poorest in chlorophyll content (i.e. 1.52 µg/ml). *Brachythecium kamounense* has 5.94µg/ml while Thudium *recognitum* has 18.5µg/ml.

Chl 'a': Chl 'b' ratio was found to be maximum in *Brachythecium kamounense*(4.39) and minimum in *Fissidens taxifolius* (0.36).

It would be desirable to determine and compare the 'Zn' content of these taxa 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[19], 1980).

It is also desirable to study Zn content found in some liverworts which show higher chlorophyll content as compared to these mosses (cf. our earlier studies).

Further it is interesting to note that the taxa which show higher chlorophyll content grow on moist rock surface with some soil on it while *Fissidense taxifolius* which is very poor in chlorophyll content grows on soil.

A study of the specific activity of some enzymes i.e. α-amylase, β-amylase, protease and polyphenol oxidase in 7 mosses (Table 4) reveals considerable differences among the taxa.

In the seven moss taxa, studied protease showed a relatively greater specific activity while α-amylase showed a relatively lesser specific activity, the four tested enzymes followed the below given sequential order:

Protease > Polyphenol oxidase > α-amylase > β-amylase

Udar and Chandra[20] (1960a,b) studied the amylase activity in the sterile and fertile **(**♂ and ♀**)** materials of *Riccia discolor* and found that the male plants **(**♂**)** excelled the female (♀) plants in respect of the specific activity of amylase.

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