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Research Article

Comparative biochemical analysis in vegetative thallus and archegoniophores of *Marchantia papillata* subspecies *grossibarba*

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ABSTRACT

The present work was aimed at comparative study of the contents of chlorophyll 'a', chlorophyll 'b', carotenoids, proteins, free amino acids and specific activity of enzymes α- amylase, β-amylase and invertase in the vegetative thalli and in the archegoniophores of *Marchantia papillata* subspecies *grossibarba.* The protein (25.61±0.86 mg g^{-1} fw), carbohydrate (30.72±0.46 mg g^{-1} fw) and amino acid (12.43±0.56 mg g^{-1} fw) contents were found to be much higher in archegoniophores than in vegetative thalli. Chlorophyll 'a' (0.26 ±0.001 mg g^{-1} fw) and carotenoids (0.012±0.002 mg g^{-1} fw) were found to be more in vegetative thalli, but chlorophyll 'b' (0.07±0.006 mg g^{-1} fw) content more in archegoniophores. In case of enzymes, activity of invertase (7.33±0.10 µg min^{-1} mg^{-1} protein) was higher in archegoniophores but activities of α-amylase (26.12±1.15 µg min^{-1} mg^{-1} protein) and β-amylase (20.55±3.44 µg min^{-1} mg^{-1} protein) were higher in vegetative thalli than in archegoniophores. Present study showed that the carbohydrates and their hydrolyzing enzymes coupled with the high content of proteins and free amino acids play the most important role in the onset of sexuality and favour archegonial formation. The data indicate that significant changes in biochemical compounds occur during the transition from vegetative to reproductive state.

Key words: Vegetative thalli, Archegoniophores, Biochemical analysis, *Marchantia* and Variation.

INTRODUCTION

The bryophytes are a group of plants including liverworts, hornworts and mosses. Alternation of generations is a fundamental concept in plant biology. Each plant species undergoes two distinct stages in its life cycle: the gametophyte- a haploid stage capable of generating gametes and the sporophyte- a diploid stage distinguished by the ability of the plant to generate spores via meiosis. The bryophytes are unique among land plants in having relatively large, perennial, photosynthetic and free-living, haploid gametophyte, and annual, relatively simple, unbranched diploid sporophyte that remains attached to the maternal gametophyte throughout its lifespan.

Marchantia papillata subspecies *grossibarba* is a large liverwort with a wide distribution around the world. The plant body is a gametophytic thallus. The thallus is dorsiventral, dichotomously branched, with an apical notch at the growing point of each branch. From the apical notches arise upright branches which bear the sex organs. Reproduction in *Marchantia* takes place by means of asexual as well as sexual methods. In liverworts, the haploid gametophyte is the dominant phase of the life cycle. Archegonial initiation is accomplished by intense metabolic activities, and in certain liverworts there is an increase in the contents of carbohydrates, auxins, RNA and proteins, whereas the level of total nitrogen drops down[1].

The purpose of the present work is to report basic information on the variation in various storage compounds and enzyme activities related to these storage compounds in vegetative thalli and the archegoniophores of *M. papillata* supspecies *grossibarba*.

MATERIALS AND METHODS

Collection of plant material: Plant material was collected from Vikasnagar (Uttrakhand). The thallus was first cleaned by removing adhering material and thoroughly washing with clean water and finally with distilled water. Archegoniophores are excised from gametophyte and subjected to biochemical analysis separately.

Quantitative analysis:

Proteins: The content of proteins was determined by following the method of Lowry *et al.*[2] using Bovin serum albumin as standard.

Free amino acids: The quantity of free amino acids was determined according to the method of Lee and Takahashi[3] using ninhydrine reagent and glycine as standard.

Carbohydrates: The quantity of carbohydrates was determined according to the method of Yemm and Willis[4] by taking glucose as standard and using anthrone reagent.

α – amylase: Its activity was measured by the method of Mutenz.[5] Starch is used as standard.

β – amylase: β- amylase activity was estimated according to the procedure given by Bernfeld[6] using maltose as standard.

Invertase: Invertase was measured by the estimation of total reducing sugars by Dinitrosalicylic reagent by Sumner[7] method using glucose as standard.

Photosynthetic pigments: Chlorophyll 'a' and Chlorophyll 'b', were estimated by the method of Arnon[8] and the content of carotenoids was estimated by the method of Kirk and Allen[9].

RESULTS AND DISCUSSION

The results obtained from the present study are given in Table 1 and Table 1a. The results depicted the variation in the contents of chlorophyll 'a', chlorophyll 'b', carotenoids, proteins, free amino acids and specific activity of the enzymes α- amylase, β-amylase and invertase, the contents of almost all the compounds being higher in archegoniophores as compared to vegetative thalli.

Douin[10] studied the pigments of gametophytes of forty species of mosses belonging to twenty-eight genera and reported the presence of chlorophyll a and chlorophyll b, similar to higher plants. In our study, the contents of chlorophyll 'a', and carotenoids were higher in vegetative thalli but chlorophyll 'b' and total chlorophyll content were found to be higher in archegoniophores. Earlier observations showed no qualitative differences in chlorophylls 'a', chlorophyll 'b' and carotenoids during comparative study of the sporophyte and the gametophyte of five species of mosses[11].The analysis of pigments in mosses (qualitative and quantitative) carried out during various growth stages i.e. gametophyte, sporophytes and germinating spores revealed significantly higher amount of total chlorophyll in sporophyte than that in gametophyte, whereas during spore germination the content of carotenoids was found to be the least[12-14]. The higher chlorophyll a/b ratio might be responsible for a degree of adaptation or acclimation in plants[15]. Chlorophyll a/b ratio is a measurement of the proportion of light harvesting complex to other chlorophyll components[16]. In the present study, chl a/b ratio is higher in the vegetative thalli than in the archegoniophores.

Among chemical factors, the carbohydrates, in general, enhance gametangial formation[17]. In the present study, carbohydrate content was much higher in the sporophyte indicating the utilization of gametophytic carbohydrates in the formation of sporophyte. In *Amblystegium riparium* - an aquatic moss, accumulation of carbohydrate assimilates in gametophytes leads to the development of sporophytes[18]. Carbohydrate/nitrogen ratio showed a marked increase when a transition occured from vegetative to reproductive phase in female plants of *Fimbriaria angusta*[19]. Recently, sugars are also reported to help to regulate the timing of developmental phase changes, such as the progression from juvenile to adult phases, flowering and senescence[20].

Presently, contents of proteins and free amino acids are also found to be higher in archegoniophores than in vegetative thalli. A transition from the vegetative to the reproductive phase is accompanied by significant intracellular metabolic changes during reproductive development responsible for its higher protein content[21]. In bryophytes, amino acids supplied exogenously influence gametangial formation. In *Riccia crystallina, in vitro* production of archegonia and antheridia is enhanced by different amino acids which do not show inhibitory effect in any case[22]. Amino acids can act as alternate source of energy in seed development, whereas the vegetative organs can obtain energy through photosynthesis[23]. Some amino acids like proline play a vital role in the reproduction by initiating the formation and development of various reproductive parts[24, 25]. Higher content of free amino acids observed in the archegoniophores of the presently studied taxon may also fulfill the extra energy requirements and help in the reproductive development. Proteins play significant roles in vegetative as well as reproductive growth during the development of plant[26]. During reproductive growth the proteins act as the building material for seed and fruit development and are also required for the rapid growth of vegetative tissues after the unfavorable period.

Enzymes α- amylase, β-amylase and invertase catabolize carbohydrates and provide energy for various developmental processes. Of all the tested sugars in *Riccia frostii* only glucose and sucrose favour archegonial formation[27]. Utilization of sucrose as a source of carbon and energy depends on its cleavage into hexoses catalyzed by the enzyme invertase. Various processes like reproductive development and respiration are correlated with the enhanced activities of amylases to supply energy by hydrolyzing sugars[28].

In the present study, activities of α- amylase and β-amylase were found to be higher in the vegetative thalli leading to catabolism of the carbohydrates to fulfill the energy needs for the formation of archegoniophores. The activity of enzyme invertase to split the sucrose molecules was seen higher in the archegoniophores. Invertases may be indirectly involved in the control of cell differentiation and plant development, since the sugars in plants are not only the nutrients but also play an important role as a regulator of gene expression[29]. The action of invertases in the cell may be helpful in establishing the appropriate levels of sugars, which by interacting with other components, participate in the regulation of developmental processes[30]. Invertase plays a vital role in plant growth and development[31] as it could affect differentiation by regulating the level of sucrose which helps in cell morphogenesis. Carbohydrates are the main source of energy for the development of reproductive parts and they must be supplied by the photosynthetic parts like leaves to the reproductive tissues[32]. Therefore, lower amount of carbohydrates and higher activities of its degrading enzymes in the vegetative thalli than the archegoniophores of *M. papillata* indicates supply of sugars to the archegoniophores to meet the energy requirements.

CONCLUSION

On the basis of present studies, we can conclude that the onset of the reproductive phase involves metabolic changes in the differentiating tissues. The present study reveals that intense metabolic activities occur during archegoniophore formation indicated by higher contents of proteins and free amino acids during the gametangial phase. Carbohydrates and their splitting enzymes- α- amylase, β –amylase and invertase play the most important role in the onset of sexuality in plants. Glucose and sucrose formed after the action of α- amylase, β-amylase and invertase on carbohydrates, favour archegonial formation. Invertases help in cell morphogenesis, differentiation and also regulate transition from one phase to another during development. Carbohydrate splitting enzymes also play an important role in the regulation of gene expression.

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Table1
Variation in the content of photosynthetic pigments in the two stages of *Marchantia papillata* subspecies *grossibarba*

Part of plant	Chl 'a' in mg/g fw	Chl 'b' in mg/g fw	Chl a/b ratio in mg/g fw	Total Chl in mg/g fw	Carotenoids in mg/g fw
Vegetative thalli	0.26 ± 0.001	0.01 ± 0.002	36.37 ± 14.48	0.27 ± 0.002	0.012 ± 0.002
Archegoniophores	0.21 ± 0.003	0.07 ± 0.006	3.01 ± 0.23	0.28 ± 0.004	0.003 ± 0.001

Results are represented as mean of triplicates ± standard error

Table1a
Variation in the content of storage compounds and enzymes in the two stages of *Marchantia papillata* subspecies *grossibarba*

Part of plant	Carbohydrates in mg/g fw	Proteins in mg/g fw	Free amino acids in mg/g fw	α-amylase in µg $min^{-1}mg^{-1}$ protein	β-amylase in µg min^{-1} mg^{-1} protein	Invertase in µg min^{-1} mg^{-1} protein
Vegetative thalli	11.24 ± 0.21	13.58 ± 0.35	8.33 ± 0.19	26.12 ± 1.15	20.55 ± 3.44	5.40 ± 0.23
Archegoniophores	30.72 ± 0.46	25.61 ± 0.86	12.43 ± 0.56	14.24 ± 1.04	9.95 ± 1.89	7.33 ± 0.10

Results are represented as mean of triplicates ± standard error

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