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QUANTITATIVE CHANGES IN FREE AMINO ACIDS IN *POLYTRICHUM FORMOSUM* DURING DEVELOPMENT

C. UHEL (GUYOMARC'H)

Laboratoire de Physiologie Cellulaire, ERA 323, 12 Rue Cuvier, 75005 Paris, France

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Key Word Index—*Polytrichum formosum*; Musci; free amino acids; quantitative variations; sporophyte development.

Abstract—The concentrations of each free amino acid in *Polytrichum formosum* have been determined for female gametangia at different developmental stages of the sporophyte. On a dry weight basis, glutamic and aspartic acids are the predominant compounds but arginine is also often present in significant amounts. Furthermore, the quantitative changes which this amino acid undergoes are of interest; especially in the female leafy shoot during the exit and the period of seta elongation of the young sporophyte.

INTRODUCTION

The nitrogen metabolism of mosses has not received much attention, and most studies have been concerned with the nitrogen requirements of mosses grown in a chemically defined medium. Thus, Burkholder [1] noted the role of arginine in the development of leafy shoots of *Atrichum*, and Gagnon and Bernier [2] showed that alanine is the predominant amino acid in *Calliergonella schreberi*. However, there is little data on the amino acid content of all parts of these plants. Until our investigations on *Dicranum* [3], the free amino acids present in the sporophyte and gametangia of mosses were unknown. In that work, we noted seasonal changes in the levels and proportions of soluble nitrogen compounds.

We now report the physiological significance of various changes in nitrogen metabolism which occur during sexual development of *Polytrichum formosum*. We have already reported that the onset of meiosis in the sporophyte is accompanied by a significant decrease in glycine content, and that the mature spores contain a considerable amount of arginine [3]. The present paper is concerned with the nature and amounts of free amino acids present at different stages in the female gametangia bearing the sporophyte.

RESULTS

Polytrichum formosum is a dioecious moss which is frequently found in the fruiting condition under natural conditions of growth. The first samples for analysis were collected as soon as the calyptra, as a definite structure of the sporophyte, emerged from the perichaetial bracts (stage GF-1a, Fig. 1). All the developmental stages were recognized by the macroscopic characteristics of the whole sporophyte during its ontogenesis (Fig. 1). Further samples (SP) were collected at the period of maximum frequency of any stage. At stage 1 and after, the seta can be easily separated from the female gametangia (GF) for separate

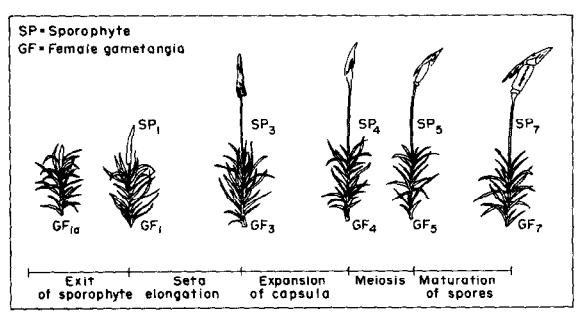

Fig. 1. Different developmental stages in *Polytrichum formosum*.

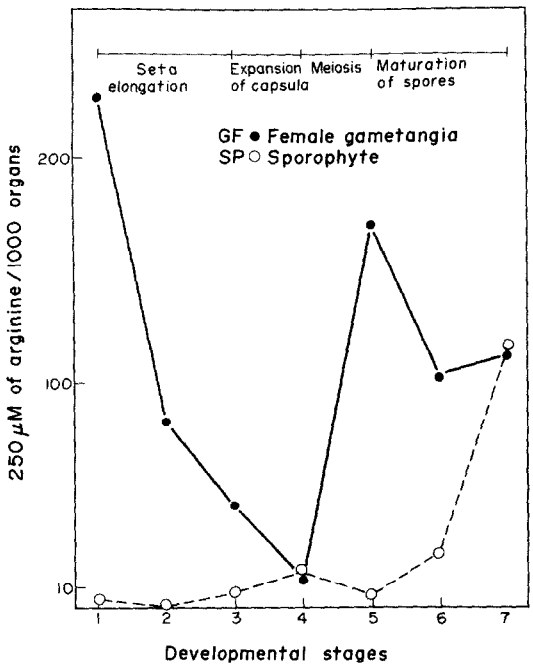

Fig. 2. Changes in the concentration of arginine during the development of the sporophyte and the female gametangia.

analysis (Tables 1 and 2). The changes in the main amino acids, both in gametangia and sporophyte, are shown in Figs. 2 and 3.

The amino acids identified in *Polytrichum formosum* are those common in higher plants. The low level of total amino acids should be noted: the highest found are 1·0 and 1·5 mg of amino nitrogen per g dry weight in the female shoot and the sporophyte, respectively.

Proline and hydroxyproline are not present in the two organs and methionine is sometimes present, but only in trace amounts (below 0·3 μg per g). The other amino acids found in low concentrations (below 14 μg/g) are: citrulline, phenylalanine, leucine, isoleucine and valine. Tyrosine is also found in low amounts except when the female gametangia is just above the exit of the young sporophyte, when a relatively high level (50 μg/g) occurs.

Histidine, lysine and ornithine are also present in low concentrations, but change markedly during development; thus, a relatively high level of ornithine was observed just before the expansion of capsula in the sporophyte (SP_3, Table 2). Glycine also is generally found at low concentrations, but a considerable increase was observed during seta elongation of the sporophyte (from SP_1 to SP_2, Table 2) and a similar significant increase is also found in the female gametangia when the sporophyte becomes mature (from GF_6 to GF_7).

Significant changes in the levels of alanine were recorded during onset of meiosis in the sporophyte: when the capsula was expanding and the total amino acids increasing, the amount of alanine decreased greatly; on the other hand, during the division of mother cells of spores (SP_4 to SP_5) which is correlated with a decrease of amino acid content, the alanine content rose abruptly (Table 2). We had not detected ethanolamine in *Dicranum* [3]; in *Polytrichum formosum*, it was present at high levels in both organs at all different stages: in the sporophyte, an increase was found during the maturation of the spores (Tables 1 and 2).

Glutamic acid, aspartic acid and arginine were the three predominant metabolites in the pool of free amino acids, both in the female gametangia after fertilization and in the sporophyte during its ontogenesis. We noted that the low level of total amino acid content in the sporophyte at

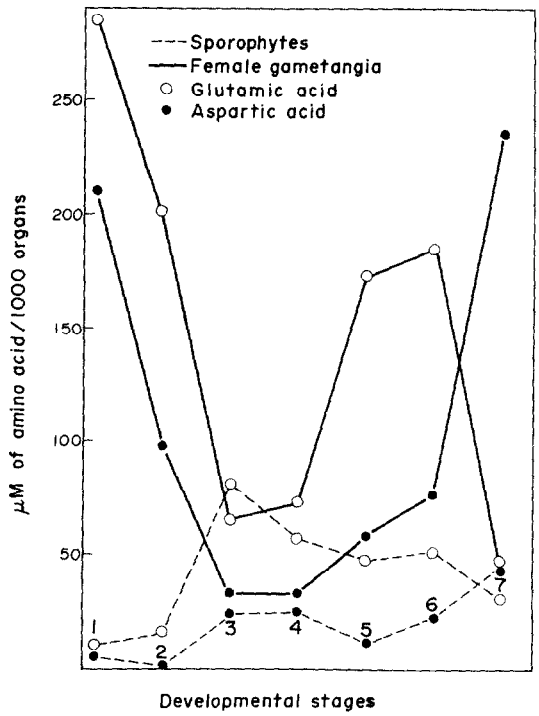

Fig. 3. Changes in the concentrations of the major amino acids: glutamic and aspartic acid, in each organism.

Table 1. Free amino acids in the soluble nitrogen fraction extracted from the female gametangia of *Polytrichum formosum* in weight

Developmental stages	GF_0	GF_{1a}	GF_1	GF_2	GF_3	GF_4	GF_5	GF_6	GF_7
Total amino acid (μg of N/g dry wt)	996	955	995	734	362	181	857	749	734
Histidine	52·9	25·6	58·4	23·5	16·8	8·4	18·5	19·7	41·2
Lysine	40·9	23·2	33·3	13·7	8·7	5·3	12·0	16·5	18·5
Ornithine	14·8	10·6	5·3	3·9	1·7	1·4	15·7	14·0	16·8
Arginine	57·1	435·7	435·1	235·8	154·0	38·1	460·3	365·7	342·7
Aspartic acid	150·2	69·7	101·9	77·7	28·7	23·0	40·5	54·2	50·1
Threonine	17·4	6·3	18·1	23·5	0·3	3·2	27·9	11·5	11·3
Glutamic acid	230·2	140·2	138·2	155·3	55·7	50·7	119·7	131·0	100·8
Glycine	6·2	6·0	3·2	2·5	2·0	2·1	3·4	2·1	36·3
Alanine	67·5	34·6	44·7	20·9	12·3	12·6	13·0	12·6	11·9
Valine	8·9	*T*	8·3	2·8	1·8	1·8	4·6	4·5	3·6
Isoleucine	13·3	1·4	3·2	3·4	1·8	1·0	3·4	4·8	2·1
Leucine	10·4	2·8	13·2	13·1	10·6	4·9	9·4	9·1	7·0
Citrulline	4·7	*T*	*T*	2·8	*T*	*T*	10·8	5·8	11·8
Tyrosine	50·1	1·1	6·7	1·1	0·7	0·6	2·0	1·8	1·7
Phenylalanine	7·0	12·7	4·2	4·6	2·0	2·1	3·4	5·3	3·8
Serine + amides	185·9	107·9	97·4	90·3	24·9	11·3	80·8	59·8	61·5
Ethanolamine	83·2	76·9	23·9	61·9	41·4	14·7	42·4	36·3	25·1

stage 2 was accompanied by an exceptional low concentration of aspartic acid. The amounts of arginine were usually higher than those of glutamic acid in the female shoot and, quantitatively, aspartic acid usually came third (Figs. 2 and 3). In the sporophyte, the level of arginine rose during the expansion of the capsula and after meiosis (Fig. 2); at stage 7, the mature stage, arginine accumulated greatly presumably as a nitrogen reserve.

DISCUSSION

It should first be noted that the procedures used did not allow an examination of the changes

Table 2. Free amino acids in the soluble nitrogen fraction extracted from the sporophyte of *Polytrichum formosum* during ontogenesis

Developmental stages	SP_1	SP_2	SP_3	SP_4	SP_5	SP_6	SP_7
Total amino acid (μg of N/g dry wt)	383·0	198·1	1016·4	641·9	490·2	731·9	1494·1
Histidine	33·6	7·6	51·2	47·9	44·9	73·5	45·8
Lysine	12·0	5·9	28·6	24·4	37·8	28·3	48·2
Ornithine	6·7	3·1	39·5	35·6	12·6	29·7	9·5
Arginine	87·4	30·8	98·0	138·3	40·3	170·0	973·4
Aspartic acid	35·3	4·2	91·4	58·8	21·0	41·7	11·2
Threonine	7·0	2·1	15·0	18·5	11·3	18·8	2·7
Glutamic acid	79·5	77·6	299·6	134·5	90·4	90·6	50·3
Glycine	5·2	0·8	97·3	43·1	8·8	11·5	4·9
Alanine	20·4	6·2	36·4	4·9	53·2	33·7	64·4
Valine	2·5	0·3	6·3	8·3	10·9	8·7	10·9
Isoleucine	0·6	*T*	4·2	10·6	8·1	13·7	2·7
Leucine	0·8	*T*	3·2	6·2	7·6	10·6	8·7
Citrulline	16·8	*T*	8·4	10·7	9·5	3·9	7·7
Tyrosine	1·7	*T*	2·1	3·1	5·2	5·7	6·0
Phenylalanine	0·8	*T*	7·8	8·3	4·9	14·4	4·3
Serine + amides	46·1	31·1	197·3	71·8	57·0	73·5	89·0
Ethanolamine	43·4	28·4	38·5	27·6	76·2	107·5	162·1

T = <0·015 μM/g dry wt (<0·2 μg N).

in amides which are essential mobile amino compounds in most vascular plants; these compounds are recorded as a common peak with serine. Tryptophan was also not determined in this work.

From the analytical data, we can conclude that the relative concentrations of free amino acids in *Polytrichum formosum* are similar to those found in higher plants [4]. It is not easy to draw definite conclusions from the changes in the amounts of histidine and ornithine, but among the basic amino acids, arginine obviously plays an important physiological role. This amino acid has been found to accumulate in several plants [5,6] and appears to be one of the soluble nitrogen reserves which can be mobilized when it is required for growth. This view is supported by the data in the present study (Fig. 2). The high percentage of arginine in spores just before dispersal could indicate that it may serve nitrogen requirements during germination. On the other hand, it also accumulates in the female shoot and the greatest concentration of arginine is found at the stage preceding the exit of the sporophyte (GF_1). The amount of arginine then decreases in the leafy shoot while it is increasing in the sporophyte (Fig. 2). These results suggest that, the emerged sporophyte receives nitrogen in the form of arginine from the mature leaves of the female gametangia in the same way that numerous metabolites of the field pea are translocated from mature to young organs [7].

It was recently established that the sporophyte of the moss *Funaria* was fully autotrophic at two developmental stages, especially during the expansion of the capsula [8]. Thereafter, this organ started to synthesize certain amino acids.

EXPERIMENTAL

Samples were air dried and finely ground. Dry weight was determined at 105°.

Preparation of extracts. Powder was suspended in 10% trichloracetic acid (TCA, 40 ml/g) and stirred for 15 min at 0°. After 15 h at 0° supernatant was removed by centrifugation and the ppt washed 2 × with 2·5% TCA and once with H_2O.

Analytical procedures. Total soluble N_2 (NS) was determined by a micro-kjeldahl procedure. Excess TCA was extracted with Et_2O and the soln evaporated to dryness at 35°. Residue, in 2 ml of citrate buffer at pH 2·2 was chromatographed on ion exchange resin columns with an automatic amino acid analyser.

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