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FATTY ACID COMPOSITION OF MUTANTS OF THE MOSS *PHYSCOMITRELLA PATENS*

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Key Word Index—*Physcomitrella patens*; Funariaceae; moss; fatty acids; mutant; glycolipid; phospholipid.

Abstract—The fatty acid composition of various mutant strains of the moss *Physcomitrella patens* has been compared to the wild-type. These included strains defective in their responses to auxins and/or cytokinins, one which releases much more cytokinin into the medium than the wild-type, and two aphototropic strains. The lipids of the aphototropic mutants were also studied after culture in different light regimes. Although some differences in fatty acid composition have been found between strains, these alone are probably not responsible for their physiological differences. Considerable changes occur in many fatty acids in senescent or dark-grown material, including changes in the proportion of C_{20} polyenoic fatty acids.

INTRODUCTION

The moss *Physcomitrella patens* was chosen to study the process of development in plants [1]. The life cycle is predominantly haploid, can be completed under sterile culture conditions, and mutants may be obtained relatively easily following chemical treatment of spores. Auxins, cytokinins and light are important at many stages of development, as in higher plants. Auxin-resistant (NAR) and cytokinin-resistant (BAR) strains can be isolated by screening clones of mutagenized spores on high concentrations of auxin or cytokinin [2]. OVE strains release *ca* 100 times more cytokinin (N^6-(Δ^2-isopentenyl)adenine) than the wild type into the growth medium [3]. In the course of previous work, many biochemically or developmentally abnormal strains have been collected, and some of these have been analysed genetically, either conventionally by crossing [1] or parasexually using protoplast fusion [2, 4, 5]. However, very little is known about the biochemical basis of these mutations, and in this paper we present a study of the fatty acid (FA) patterns of some types of mutants. One type fails to respond to the direction of strong white light [6], whilst others may be altered in the uptake of, response to, or release of auxins or cytokinins. All of these mutant types may be defective in some aspect of membrane structure. The FAs of some species of mosses have already been studied [7, 8] and the occurrence of long chain polyunsaturated FAs, such as arachidonic acid (all *cis* 5,8,11,14-eicosotetraenoic acid, abbreviated 20:4ω6), which are not found in seed plants, is well known.

RESULTS AND DISCUSSION

One of the main problems of physical methods of cell fractionation is that the chance of enzymatic degradation of some components is increased [9]. By choosing a chemical method involving separation of extracted lipids into three fractions, neutral lipids (NL), glycolipids (GL) and phospholipids (PL), the risk of lipid alterations was reduced. GLs and PLs are thought to be the main structural lipids of chloroplasts and plasma membrane, respectively [10].

Transesterification yielded *ca* 3 μmol FA Me esters per g fr. wt white light- (WL) grown material from both GLs and PLs. GC routinely gave *ca* 30 peaks, but many of these were very small and are not included in the results presented here. Due to the complexity of the FA mixture, some peaks were not resolved, and the FAs shown in the table can therefore only represent tentative identifications of the largest components. However, we found our apparatus suitable for general comparative work as peaks could be reproducibly recognized by their retention times. More detailed FA analyses of mosses have already been reported [8, 11].

Tables 1 and 2 compare the FA of strains altered in auxin or cytokinin metabolism to the control strains *thi*-1 and wild-type. Although there are some differences between test and control strains, for example 20:4ω6 in BAR-383 GL and in OVE-100 PL, these are relatively small quantitative changes and are probably not the cause of the physiological differences between mutant and wild-type. Comparison of Tables 1 and 2 shows some large and consistent differences, for example in the FA 20:4ω6. These arise because *thi*-1, NAR-87, NAR-113 and BAR-2 were 14 days old at the time of extraction, whereas wild-type, NAR-23, BAR-383 and OVE-100 were 19 days old. At 14 days old *thi*-1 is morphologically quite different from the test strains NAR-113, BAR-2 and NAR-87, but the composition of the FAs is similar, indicating that under these experimental conditions changes in types of tissue have little effect on FAs. Differences in FA composition due to age and morphological changes will be the subject of another paper.

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Table 1. Fatty acid composition of *Physcomitrella* mutants

Fatty acid	Strain, % FAs by mol			
	Wild-type control	NAR-23	BAR-383	OVE-100
GLYCOLIPIDS				
12:2ω6	1.3	1.4	2.0	1.4
15:0	0.3	0.3	0.8	0.9
16:0	11.7	11.3	11.6	12.3
16:1ω9	0.8	1.6	2.9	1.4
16:2ω6	6.6	6.5	6.8	6.2
16:3ω3	14.5	15.4	12.5	12.1
18:1ω9	1.0	1.7	2.5	1.1
18:2ω6	10.9	10.0	9.4	6.9
18:3ω6	3.7	3.3	3.8	3.4
18:3ω3	23.6	25.0	20.7	19.2
18:4ω3	0.4	0.5	1.9	0.5
20:3ω6	2.9	1.8	5.0	6.0
20:4ω6	17.0	15.1	10.8	18.9
20:4ω3	—	0.3	1.1	0.8
20:5ω3	1.4	2.1	2.2	2.7
22:3ω6	0.2	0.5	0.7	0.4
PHOSPHOLIPIDS				
12:2ω6	0.7	0.7	0.6	1.5
15:0	0.6	0.5	0.4	—
16:0	33.1	31.9	33.3	36.1
16:1ω9	1.7	1.5	1.0	1.1
16:2ω9	0.3	0.9	—	1.1
16:2ω6	0.9	0.6	0.4	0.4
16:3ω9	0.7	1.1	0.8	0.9
16:3ω3	3.2	2.6	2.6	3.8
18:1ω9	2.8	2.9	2.3	4.5
18:2ω6	13.7	11.3	9.7	10.3
18:3ω6	2.9	2.6	3.3	2.8
18:3ω3	11.9	11.8	10.7	10.2
18:4ω3	0.6	0.8	0.5	0.5
20:3ω6	3.0	2.1	6.4	8.3
20:4ω6	16.1	14.7	19.6	9.5
20:4ω3	—	—	1.3	1.9
20:5ω3	1.6	2.3	3.0	1.5
20:3ω6	0.6	3.1	1.1	0.4

19-day-old WL material was used for extraction. Each figure is the mean from two independent extractions.

In Tables 3 and 4 the PLs and FAs of aphototropic mutants *pyr* A1 and *ptr* B3 (which carry mutations in separate genes [12]) are compared with the control strains wild-type and *thi*-1 under different lighting regimes. No differences in the PLs between the strains could be found. Dark-grown (DG) and dark-adapted (DA) material contained proportionately less phosphatidylglycerol and red light irradiation increased the amount of phosphatidic acid of DG material. Some differences between strains in FA components were found. DG *ptr* B3 GL 12:2ω6 did not respond to the red light stimulus as the other strains did. Wild-type and *ptr* B3 differ from *ptr* A1 and *thi*-1 in the amount of white light (WL) GL 16:0 and also in DA 18:2ω6 and 18:3ω3. These differences could be due to a previously unseen mutation in *thi*-1, which, on crossing the original aphototropic lines with *thi*-1, segregated so that it is present in *ptr* A1 *thi*-1 but not in *ptr* B3 *thi*-1, the strains used in this study. This demonstrated the necessity of including both wild-type and *thi*-1 as control strains. In WL the amount of PL 20:3ω6 in *ptr* A1 and *ptr* B3 was increased two-fold compared to the controls, and it is possible that the increased concentration of this component in the membranes hinders the phototropic response in some way. However, we consider this to be an unlikely explanation since 20:3ω6 is a relatively minor component and the overall FA composition of these mutants is not very different from the wild-type.

The FA composition of DG and DA material is very different to WL-grown material. Although the experiments were not strictly designed to investigate senescence changes, the results do give some information

Table 2. Fatty acid composition of *Physcomitrella* mutants

Fatty acid	Strain, % FA by mol			
	thi-1 control	NAR-87	NAR-113	BAR-2
GLYCOLIPIDS				
12:2ω6	2.1	1.9	3.7	3.7
15:0	0.2	0.1	0.3	0.2
16:0	12.6	9.7	10.1	11.8
16:1ω9	0.6	1.0	1.2	0.8
16:2ω6	3.2	3.4	3.1	3.3
16:3ω3	21.4	24.9	27.0	19.4
18:1ω9	1.1	1.1	1.5	1.0
18:2ω6	5.3	4.4	3.5	4.9
18:3ω6	2.8	2.6	1.2	5.1
18:3ω3	31.2	35.2	32.7	29.3
18:4ω3	0.4	0.6	0.4	0.6
20:3ω6	6.2	5.1	5.4	7.3
20:4ω6	5.9	6.5	4.1	7.8
20:4ω3	0.8	1.1	1.4	1.2
20:5ω3	1.0	1.0	0.5	1.0
22:3ω6	—	0.2	0.1	0.1
PHOSPHOLIPIDS				
12:2ω6	0.9	0.5	0.7	0.9
15:0	0.1	0.2	0.2	0.3
16:0	36.1	36.0	34.6	37.8
16:1ω9	—	0.9	0.6	0.5
16:2ω9	1.1	2.1	2.1	1.3
16:2ω6	0.2	0.3	0.2	0.2
16:3ω9	0.7	0.4	0.5	0.1
16:3ω3	3.7	3.3	4.0	3.8
18:1ω9	2.1	1.8	2.2	1.6
18:2ω6	12.5	11.2	7.3	11.5
18:3ω6	3.5	3.5	1.8	4.4
18:3ω3	11.7	15.4	13.4	12.6
18:4ω3	0.3	0.3	0.6	0.4
20:3ω6	16.2	10.7	15.8	15.0
20:4ω6	4.4	7.8	6.2	4.7
20:4ω3	1.8	1.1	—	0.2
20:5ω3	0.5	1.0	1.4	0.3
22:3ω6	0.9	0.5	0.2	0.2

14-day-old WL material was used for extraction.

about this. WL material was younger than DG or DA material, but this was unavoidable since the moss grows very slowly in the dark. A possible alternative approach would be to compare DG with DG regreening material after transferring some cultures to the light, but this has not yet been attempted. Although the changes in FAs caused by senescence and regreening have been studied in some seed plants [13, 14] and algae [15, 16], as far as we know these changes have not been studied in mosses. Mosses are of particular interest because large changes occur in C_{20} polyunsaturated FAs, as shown by this study. These FAs have not been found in seed plants. In GL FAs the degree of saturation increased in DA material and further in DG material, as shown in Table 5. The increase in saturation was mainly due to increased 16:0 and decreased 16:3ω3, 18:3ω3 and 20:3ω6. DA and DG cultures contained much more 18:2ω6 and 20:4ω6 than WL cultures; DA material contained *ca* twice as much 20:4ω6 than DG GL material, suggesting that as senescence of WL material occurs the proportion of saturation in the chloroplast membranes is preserved to some extent by this FA, which becomes the largest FA component, whereas in DG material less is synthesized. Although the overall saturation of PL FAs was the same for all cultures, considerable changes in some FAs occurred. In the dark 18:2ω6 and 20:4ω6 increased whilst 18:3ω3 and 20:3ω6 decreased.

EXPERIMENTAL

Strains. Further details about the strains used and their origins can be found in the following refs: wild-type [1], *thi*-1 [1], *ptr* A1 *thi*-1 [6, 12], *ptr* B3 *thi*-1 [12], BAR-2 (*thi*-1) [2], BAR-383 (*pab* A3) [17], OVE-100 (*pab* A3) [3], NAR-23 [2], NAR-87 (*thi*-1) [2], NAR-113 (*pab* A3) [2]. The strain nomenclature is described elsewhere [18]. In the text, strains are usually referred to by their relevant partial genotype.

Table 3. Phospholipids of aphototropic and control strains of *Physcomitrella*

	Treatment, strain and % phospholipid by mol											
	White light				Dark-grown				Dark-grown + red light			
Phospholipid	wild type	*thi*-1	*ptr* A1	*ptr* B3	wild type	*thi*-1	*ptr* A1	*ptr* B3	wild type	*thi*-1	*ptr* A1	*ptr* B3
P-inositol	8	9	8	9	5	3	4	6	8	8	8	10
P-choline	54	53	55	53	67	73	71	70	58	57	65	60
P-glycerol	22	21	22	22	10	4	6	5	8	6	6	7
P-ethanolamine	13	12	11	11	16	18	16	15	15	14	14	12
Phosphatidic acid	4	5	4	5	2	3	2	3	11	15	7	10

A yield of 1.0–1.5 μmol P/g fr. wt extracted was obtained.

Table 4. Fatty acids of aphototropic mutants of *Physcomitrella* compared with wild-type and *thi*-1 controls grown under different light regimes

Fatty acid	Strains, treatments, and % FA by mol																			
	Wild type					*thi*-1					*ptr* A1					*ptr* B3				
	White light	Dark-adapted		Dark-grown		White light	Dark-adapted		Dark-grown		White light	Dark-adapted		Dark-grown		White light	Dark-adapted		Dark-grown	
		D	R	D	R		D	R	D	R		D	R	D	R		D	R	D	R
GLYCOLIPIDS																				
12:2ω6	1.5	6.3	3.9	6.8	14.4	1.7	3.1	3.6	9.1	13.0	0.7	2.3	3.3	9.5	18.3	1.4	4.2	4.7	10.0	10.0
14:0	0.9	3.4	2.0	2.9	2.4	0.4	1.1	1.6	2.7	1.5	0.4	1.4	1.0	2.5	2.1	0.4	2.0	1.7	3.0	2.4
16:0	9.2	14.6	15.9	16.6	14.4	13.0	15.9	14.4	17.8	17.0	13.3	16.1	13.4	16.5	14.5	9.3	16.9	14.4	16.6	16.7
16:1ω9	1.1	3.1	2.0	0.7	0.6	1.0	0.8	0.0	1.4	0.7	1.1	1.0	1.1	2.7	2.6	1.1	0.0	2.3	3.0	2.6
16:2ω6	5.4	0.8	0.7	1.4	1.0	4.1	0.9	0.4	1.4	1.2	3.7	0.6	1.2	2.0	1.3	5.2	0.7	0.9	1.2	1.7
16:3ω3	19.4	10.5	10.2	9.9	8.8	18.4	5.6	5.5	11.2	10.4	17.6	6.2	4.9	9.3	6.8	22.3	7.2	7.6	11.2	10.7
18:1ω9	1.5	3.3	3.2	5.7	4.0	1.4	3.3	3.2	6.0	5.0	1.5	4.2	3.1	5.5	4.2	1.5	3.5	3.8	6.0	5.3
18:2ω6	7.9	12.9	13.0	12.7	13.6	6.8	20.4	18.8	12.9	14.1	6.0	19.1	19.5	13.5	12.2	6.9	14.2	14.4	11.0	10.1
18:3ω6	3.2	0.5	0.8	0.0	0.0	4.6	0.8	1.0	0.0	0.3	3.5	0.6	1.5	0.0	1.9	3.0	1.0	0.8	0.0	0.0
18:3ω3	29.7	9.0	9.4	7.0	6.9	28.0	13.2	14.0	6.1	7.9	31.0	15.0	16.2	10.7	9.5	31.4	10.2	10.4	5.8	6.7
20:2ω6	0.0	0.8	0.7	0.7	2.1	0.0	1.0	0.7	1.8	1.1	0.0	1.0	1.0	0.0	0.3	0.0	0.7	0.7	1.0	2.2
20:3ω6	5.7	0.0	0.0	0.6	0.3	5.3	0.3	0.0	0.0	0.0	7.8	0.0	0.0	0.0	0.0	5.8	0.0	0.0	0.5	0.0
20:4ω6	9.9	22.5	23.5	11.1	11.1	11.0	24.1	24.9	12.4	11.2	8.1	22.5	22.4	14.5	14.0	7.0	21.7	23.2	8.8	9.6
20:5ω3	0.9	1.7	2.0	0.8	0.7	1.0	2.0	2.5	0.2	0.1	0.7	1.9	2.0	0.0	1.1	0.8	2.1	2.0	0.2	0.6
PHOSPHOLIPIDS																				
12:2ω6	0.5	0.4	0.4	1.4	1.7	0.5	0.0	0.0	1.3	1.3	0.3	0.4		1.3	1.7	0.2	0.4	0.6	2.2	2.1
14:0	0.4	0.8	0.7	1.2	1.1	0.3	0.3	0.3	1.0	0.8	0.4	0.4		0.9	0.8	0.4	0.6	0.5	0.9	1.1
16:0	35.1	33.8	33.3	28.9	39.9	37.9	33.2	33.2	31.3	31.3	36.0	32.0		27.9	29.5	33.9	33.5	36.0	32.3	29.6
16:1ω9	0.8	0.0	0.0	1.2	0.7	0.8	0.0	0.0	0.6	0.5	1.0	0.0		0.2	0.6	0.9	0.0	0.0	0.1	0.6
16:2ω6	1.2	0.4	0.4	0.8	0.6	0.8	0.2	0.2	0.7	0.7	0.5	0.4		1.2	0.5	0.6	0.5	0.5	0.5	0.6
16:3ω3	2.2	3.0	2.4	3.6	3.9	1.9	1.1	1.1	3.3	3.1	1.6	1.6		4.4	3.4	0.8	2.0	2.5	4.5	4.2
18:1ω9	4.0	3.1	4.1	3.5	3.0	3.6	3.2	2.8	3.1	2.8	3.7	4.9		4.0	3.3	3.7	4.1	3.7	4.4	3.3
18:2ω6	12.6	21.4	21.7	20.6	19.6	12.1	24.1	23.8	22.3	20.5	11.6	25.4		18.5	20.9	11.9	23.3	21.9	17.7	18.7
18:3ω6	3.7	0.3	0.7	0.6	1.4	3.3	0.2	0.5	0.2	1.6	3.6	0.6		1.0	1.3	3.7	0.4	1.0	0.9	1.1
18:3ω3	12.6	6.4	6.4	5.2	5.0	11.6	7.7	7.7	4.5	4.6	12.8	7.6		5.4	4.5	13.3	7.0	6.2	4.5	5.2
20:2ω6	0.0	2.1	2.1	1.8	2.4	0.0	3.3	3.0	2.3	2.5	0.0	2.2		2.7	2.3	0.0	1.8	1.6	2.0	2.4
20:3ω6	6.6	0.4	0.5	0.3	0.5	5.5	1.3	1.1	0.3	0.4	12.9	0.5		1.9	0.4	10.3	0.4	0.3	1.1	0.6
20:4ω6	14.4	20.1	19.4	15.3	17.3	14.2	16.3	17.0	17.7	17.6	10.0	16.8		14.2	17.7	12.7	18.9	18.3	14.0	18.0
20:5ω3	1.6	1.5	1.4	1.4	1.4	1.6	2.5	2.1	0.9	1.5	0.6	1.3		1.5	1.2	1.3	1.6	1.5	1.4	1.9

D: Kept in the dark until extraction; R: irradiation with red light before extraction (see Experimental). Two independent extractions and four chromatograms were run for white light and two independent extractions for dark-grown material were done. The figures shown are means of these. No repeat extraction was done for the dark-adapted treatment, and one sample was lost (blank column).

Table 5. Saturation of fatty acids following different light treatments

Treatment	Mol π-bond per mol FA	
	Glycolipids	Phospholipids
White light	2.46 ± 0.05 ($n = 4$)	1.69 ± 0.05 ($n = 4$)
Dark-adapted	2.13 ± 0.10 ($n = 8$)	1.69 ± 0.05 ($n = 7$)
Dark-grown	1.62 ± 0.14 ($n = 8$)	1.56 ± 0.08 ($n = 8$)

Culture conditions. WL culture conditions have been described in detail previously [1]. 0.5–1.0 g fr. wt of whole moss was used for each extraction, and this amount of sterile, agar-free material could be harvested from one 9 cm Petri dish containing cellophane-overlaid medium after 14–19 days of growth [4]. DA and DG material were obtained by modifying the method of ref. [6]; 9 cm Petri dishes, containing 50 ml medium with appropriate growth supplements and 0.5% (w/v) sucrose, overlaid with cellophane, were inoculated with a row of protonemal fragments, and incubated in WL for 9 days. The cultures were then transferred to the dark for 35 days at 20°, with the Petri dishes standing on edge and the rows of inocula parallel to the floor. Under these conditions new tissue grows negatively geotropically and DG tissue can be separated from DA tissue by cutting. 24 hr before extraction half of the cultures were transferred to 4.8 W/m^2 red light ($\lambda = 660$ nm, Philips fluorescent TL 40W/5 and Plexiglas filter 501, Röhm), Petri dishes horizontal. Each Petri dish yielded *ca* 12 mg fr. wt DG material and *ca* 100 mg fr. wt DA material and 16 were used for each extraction. Transesterification of GLs and PLs yielded 0.5 to 2.0 μmol FA Me ester/g fr. wt extracted tissue.

Separation and analysis of lipids. The moss material was boiled in 10 ml *iso*-PrOH for 1 min to inactivate phospholipases, ground for 3 min with sea sand in a pestle and mortar, 20 ml $CHCl_3$ added, and left to stand 30 min. After filtration and separation of phases by centrifugation, the organic phase was washed with 0.2 vol. 0.73% NaCl, re-separated, dried (Na_2SO_4) and concd to ≤1 ml $CHCl_3$. Re-extraction of the residue was not usually necessary. Three fractions were collected following column chromatography [19] using 0.75 g silicic acid, corresponding to NLs (12 ml $CHCl_3$), GLs (18 ml Me_2CO), and PLs (15 ml MeOH). These vols and wt were halved for extraction of DG material. After concn samples were used for TLC [20] and P determinations [21] or for transesterification in MeOH with 5% H_2SO_4 at 60° in sealed ampoules. For GC, N_2 carrier gas 60 ml/min and 15% DEGS at 190° as a stationary phase was used. The output was analysed with an integrator calibrated with relative molar FID response using ext. standards [22].

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