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EFFECTS OF WATER STRESS ON LIPID METABOLISM IN COTTON LEAVES

ANH THU PHAM THI, CLAIRE BORREL-FLOOD, JORGE VIEIRA DA SILVA*, ANNE MARIE JUSTIN and PAUL MAZLIAK†

*Laboratoire d'Ecologie Générale et Appliquée, Université de Paris VII, 2 Place Jussieu, 75221 Paris Cedex 05, France; †Laboratoire de Physiologie Cellulaire, Université Pierre et Marie Curie, 4 Place Jussieu, 75230 Cedex 05, France

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Key Word Index—*Gossypium hirsutum*; Malvaceae; biosynthesis; lipids; fatty acids; drought.

Abstract—After 2, 10 and 24 hr labelling with [1-^{14}C] acetate, radioactivity incorporated into the lipids of cotton leaves is mainly found in phosphatidylcholine, phosphatidylglycerol and neutral lipids. Galactolipids are slowly synthesized and after 24 hr, account for only 10% of the total radioactivity. Under water stress, a marked decrease of precursor incorporation into leaf lipids occurs, particularly in phosphatidylcholine and galactolipids. Relative incorporation into neutral lipids, on the contrary, increases. Water deficits provoke an inhibition of the fatty acid desaturation, resulting in a sharp decrease of linoleic and linolenic acid biosynthesis. The decrease in unsaturated fatty acid biosynthesis occurs in all lipid classes, but is most pronounced in the galactolipid fractions. In the drought-resistant cotton variety (Mocosinho), the variations in lipid and fatty acid metabolism under water stress are less pronounced than in the drought-sensitive variety (Reba), and this attests a greater stability of the membrane systems.

INTRODUCTION

Water stress can provoke modifications in lipid and fatty acid composition of plant cells [1–4]. Most attention is given to the polar lipids and their compositional changes in fatty acids, as they can reflect the cell membrane status. If galactolipid content seems to decrease with water deficits [4], phospholipid variations depend on plant material and drying method [3].

In cotton, it has been shown [5] that, under water stress, polar lipid content of leaves decreases, the galactolipids being more affected than the phospholipid content. A marked decrease in the degree of unsaturation of fatty acids was also observed, due essentially to a decrease in linolenic acid (18:3) percentage. The aim of this study is then to investigate the effects of water deficits on the lipid metabolism of cotton leaves, by supplying [1-^{14}C] acetate to leaves from two varieties of Cotton (Reba and Mososinho) varying in their capacity to withstand drought.

RESULTS*

Kinetics of [1-^{14}C]*acetate incorporation into lipids of well hydrated cotton leaves*

Figure 1 shows that in both cotton varieties (Reba and Mocosinho), the labelled precursor was incorporated into lipids; incorporation proceeded at a constant rate for 24 hr in Reba leaves while a decrease in the rate of incorporation was observed in Mocosinho leaves after 10 hr.

Table 1 shows that the most highly labelled lipid classes, at all times of incubation, were phospholipids, particularly PC (45% labeling at 2 hr and 49% at 24 hr in Reba leaves, 37% at 2 hr and 50% at 24 hr in Mocosinho leaves) and PG (15% at 2 hr in Reba, 18% in Mocosinho). Neutral lipids were also actively synthesized. Radioactivity incorporated in pigments and sterols, which was relatively high at 2 hr (13% in Reba, 17% in Mocosinho) declined with time. Galactolipids, though representing the most abundant polar lipids of the leaves [5], were slowly synthesized and, after 24 hr incorporation, accounted only for 10% of the total lipids synthesized from [1-^{14}C]acetate. This confirms the relative stability of this compartment, at least in well developed leaves [6]. Results of Table 1 do not show significant differences between labeling patterns of lipids from the 2 cotton varieties.

Kinetics of [1-^{14}C]*acetate incorporation into fatty acids of well-hydrated cotton leaves*

In well hydrated cotton leaves, saturated fatty acids were actively synthesized at 2, 10 and 24 hr. Oleic acid was highly labeled at 2 hr, but its relative radioactivity decreased regularly with time of incorporation, while that of linoleic and linolenic acid increased (Table 2). This confirms the progressive desaturation pathway for the biosynthesis of linolenic acid [7]. Interestingly, the newly synthesized linolenic acid was incorporated exclusively into both galactolipids MGDG and DGDG (Tables 3 and 4). In the major phospholipids (PG and PC) fatty acid desaturation only led to the formation of diunsaturated fatty acids, which is in agreement with previous work by Trémolières and Mazliak [8].

Effects of water stress on [1-^{14}C]*acetate incorporation into lipids of cotton leaves*

When submitted to dehydration, leaves incorporated much less radioactivity from [1-^{14}C]acetate in their total lipids (Fig. 1). The decrease in lipid labeling was more

*Abbreviations—DGDG: digalactosyl-diacylglycerol; MGDG: monogalactosyl-diacylglycerol; NL: neutral lipids; PC: phosphatidylcholine; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; PI: phosphatidylinositol.

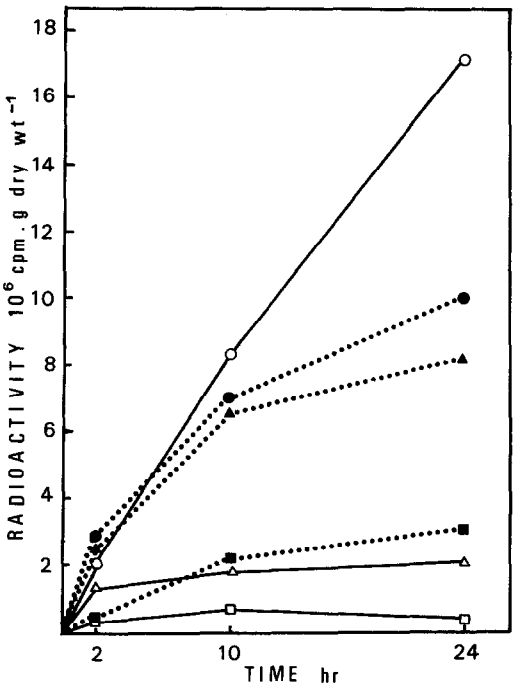

Fig. 1. Effect of water stress on the rate of incorporation of [1-^{14}C]acetate into total lipids from cotton leaves. 5 μmole [1-^{14}C]acetate (specific activity 134.7×10^{10} *Bq*. mole^{-1}) are deposited on the abaxial surface of the leaf. Reba is a drought sensitive cotton cultivar, Mocosinho is a more resistant one. Radioactivity incorporated into total lipids is expressed in cpm per g dry wt. O——O, Reba control (C). △——△, Reba middle water-stressed (S1). □——□, Reba severely stressed (S2). (For water potentials, see the text.) ●· · · ·●, Mocosinho C. ▲· · · ·▲, Mocosinho S1. ■· · · ·■, Mocosinho S2.

pronounced in the drought sensitive cotton variety (Reba) than in the resistant one (Mocosinho). However, it is difficult to know whether this decrease in lipid labeling was due to a decrease in lipid biosynthesis or resulted from a slower penetration of the radioactive precursor: indeed, under water stress, changes in stomatal resistance and modifications of biomembrane permeability could occur. Therefore, only results concerning the distribution of the radioactivity among lipid classes will further be presented.

Under water stress, the relative labeling of PC and galactolipids decreased (Figs. 2 and 3). Due to the very slow biosynthesis of galactolipids, it was not possible to detect any radioactivity in these lipids at severe water deficits. The relative incorporation into neutral lipids, on the contrary, increased with decreasing water potentials. Variations of PG and of other phospholipids (PE and PI) labeling were less clear: it seems that in Mocosinho (Fig. 3) relative incorporation into PG increased with water stress, while in Reba, it remained constant or decreased slightly. Figures 2 and 3 also show that the increase in NL relative labeling and the decrease in galactolipid biosynthesis were more pronounced in Reba than in the drought resistant variety (Mocosinho).

Table 1. Relative incorporation of [1-^{14}C]acetate (in % of total) into lipids from well hydrated cotton leaves after 2, 10 and 24 hr labeling

	Reba % total radioactivity		
	2 hr	10 hr	24 hr
NL	19.6	24.6	15.9
P^{ts} + sterols	12.8	7.0	4.3
PC	45.2	44.8	49.2
PG	14.7	15.1	16.9
PE + PI	3.4	4.8	3.9
MGDG	2.6	2.7	8.1
DGDG	1.7	1.0	1.6
	Mocosinho		
NL	13.7	7.1	18.5
P^{ts} + sterols	17.0	11.8	3.7
PC	37.1	51.2	40.0
PG	18.5	10.9	23.5
PE + PI	1.9	2.8	3.6
MGDG	4.4	5.0	7.6
DGDG	7.4	11.2	3.1

Reba is drought sensitive, Mocosinho is drought resistant.

NL: neutral lipids; MGDG: Monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol; PC: phosphatidylcholine; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; PI: phosphatidylinositol; P^{ts}: pigments.

Table 2. Relative radioactivity (in %) of total fatty acids from well-hydrated cotton leaves, after 2, 10 and 24 hr incorporation of [1-^{14}C]acetate

		% Total radioactivity			
	hr	Saturated*	18:1	18:2	18:3
Reba	2	30.8	61.8	7.2	0.1
	10	43.1	43.0	13.0	0.9
	24	34.5	42.4	19.4	3.6
Mocosinho	2	44.8	54.3	0.9	—
	10	27.6	43.0	18.4	1.0
	24	43.4	35.3	19.3	2.0

*Mostly 16:0, with < 10% 18:0.

Effects of water stress on the incorporation of [1-^{14}C]*acetate into fatty acids of cotton leaves*

Drought provoked an increase in the saturated fatty acid labeling, and a decrease in the unsaturated fatty acid biosynthesis (Fig. 4). These variations were apparent after only 2 hr of acetate incorporation. The decrease in unsaturated fatty acid biosynthesis occurred in all lipid classes, but was most pronounced in galactolipid fractions; 18:3 biosynthesis was particularly affected, as shown in Table 3, where no 18:3 labeling is detectable after 24 hr incorporation in leaves submitted to mild water deficits. In Mocosinho, unsaturated fatty acid biosynthesis

Table 3. Effects of water stress on the relative radioactivity of fatty acids from lipid classes of cotton leaves (cv. Reba) after 2, 10 and 24 hr labelling from [1-^{14}C]acetate

		Plant treatment and duration of 1-^{14}C acetate incorporation								
		C			S1			S2		
Lipids	Fatty acids	2	10	24	2	10	24	2	10	24
PC	Satd.*	10.3	31.9	26.0	23.9	26.3	40.8	38.0	35.8	57.6
	18:1	79.2	59.4	50.2	73.7	68.8	50.9	58.1	51.4	27.5
	18:2	10.5	17.8	23.7	2.4	4.9	8.3	3.9	12.8	14.9
PG	Satd.	74.2	65.5	63.3	72.4	86.1	81.5	83.6	83.2	91.8
	16:1 18:1+	25.8	34.5	36.7	27.6	13.9	18.5	16.4	16.8	8.2
MGDG	Satd.	75.7	43.8	20.5	63.8	58.0	57.8	—	—	—
	18:1	24.0	21.9	18.3	25.7	30.1	26.0	—	—	—
	18:2	0.3	20.3	28.3	10.5	11.9	16.2	—	—	—
	18:3	—	14.0	32.8	—	—	—	—	—	—
DGDG	Satd.	29.6	72.3	47.4	71.2	57.0	81.7	—	—	—
	18:1	70.4	22.7	21.2	22.3	31.5	9.8	—	—	—
	18:2	—	5.0	20.7	6.5	8.1	8.6	—	—	—
	18:3	—	—	10.7	—	3.4	—	—	—	—

*Satd. = mostly 16:0, with < 10% 18:0.
C = control.
S1 = middle water stress.
S2 = severe water stress.
For water potentials, see the text. Same abbreviations as in Tables 1 and 2.

Table 4. Effect of water stress on the relative radioactivity of fatty acids from lipid classes of cotton leaves (cf. Mocosinho) after 2, 10 and 24 hr labeling from [1-^{14}C]acetate

		Plant treatment and duration of 1-^{14}C acetate incorporation (hr)								
		C			S1			S2		
Lipids	Fatty acids	2	10	24	2	10	24	2	10	24
PC	Satd.	25.6	21.0	30.1	55.0	61.2	59.5	47.0	39.7	44.3
	18:1	74.3	58.6	41.7	45.0	32.7	34.0	53.0	54.1	43.1
	18:2	—	20.4	28.1	—	6.2	6.5	—	6.1	12.6
PG	Satd.	80.4	68.6	67.2	90.2	88.7	85.9	92.4	88.7	79.9
	16:1 18:1+	19.6	31.4	32.8	9.8	11.3	14.2	7.6	11.3	20.1
MGDG	Satd.	63.8	31.0	38.8	90.7	72.5	47.3	—	55.5	51.7
	18:1	27.6	26.0	14.6	9.0	17.9	33.5	—	21.5	26.5
	18:2	8.2	27.5	25.9	0.3	9.6	19.2	—	22.9	21.8
	18:3	0.3	15.5	20.8	—	—	—	—	—	—
DGDG	Satd.	30.7	12.7	50.0	28.4	43.0	92.5	—	76.6	81.3
	18:1	66.3	63.9	27.5	71.1	47.0	7.5	—	10.0	12.1
	18:2	3.1	23.4	22.5	0.5	9.9	—	—	13.3	6.6

C = control.
S1 = middle water stress.
S2 = severe water stress.
For water potentials, see the text. Same abbreviations as in Tables 1 and 2.

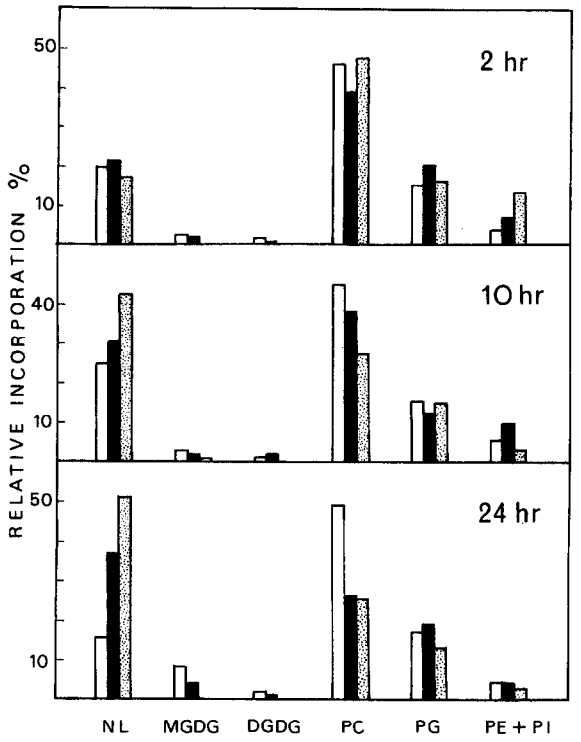

Fig. 2. Effect of water stress on the relative radioactivity (in % of total lipid radioactivity) of lipid classes from cotton cv. Reba leaves, after 2, 10 and 24 hr incorporation of [1-^{14}C]acetate. □, C; ■, S1; ▩, S2. Same abbreviations as in Fig. 1.

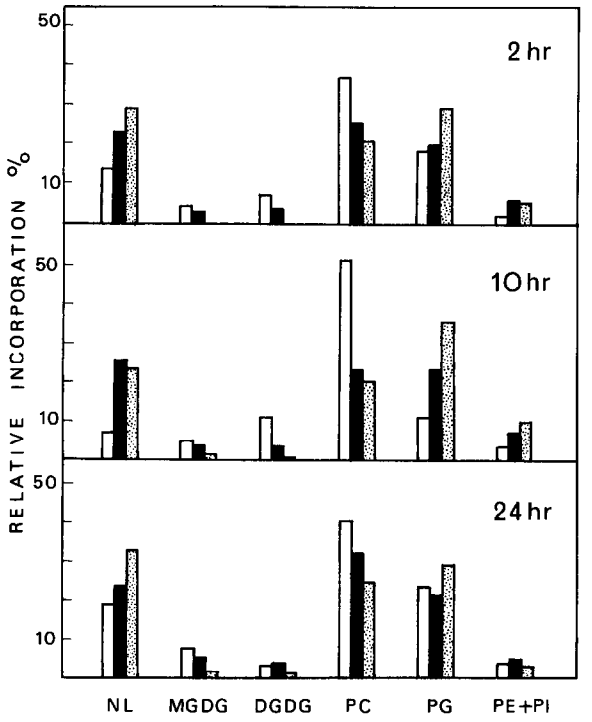

Fig. 3. Effect of water stress on the relative radioactivity (in %) of lipid classes from cotton cv. Mocosinho leaves, after 2, 10 and 24 hr incorporation of [1-^{14}C]acetate. Abbreviations and symbols as in Fig. 2.

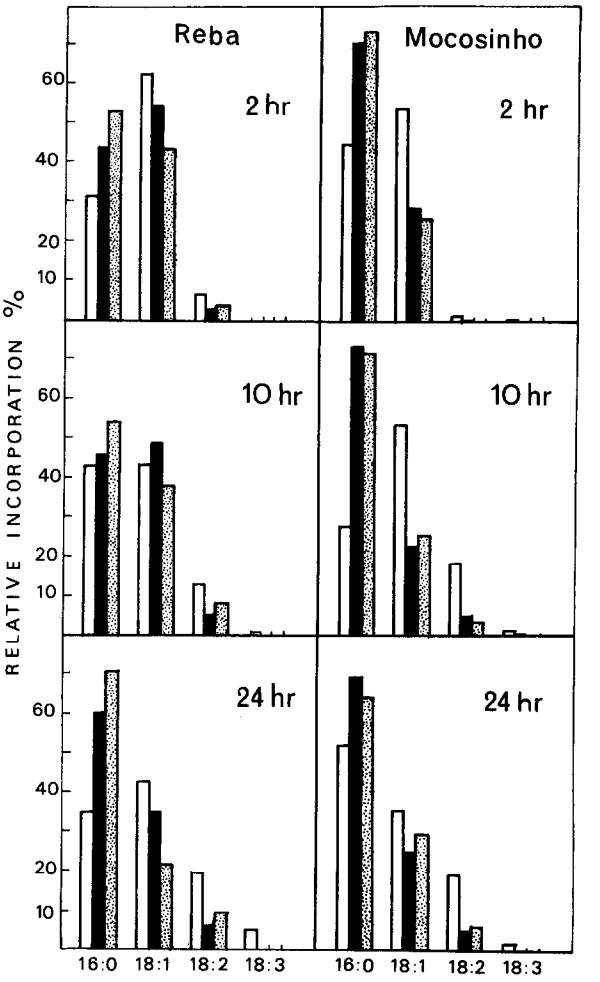

Fig. 4. Effect of water stress on the relative radioactivity of fatty acids from total lipids of cotton leaves after 2, 10 and 24 hr labeling with [1-^{14}C]acetate. Symbols as in Fig. 2.

was still observable in leaves subjected to very low water potentials whereas it was entirely inhibited in Reba.

DISCUSSION

Water stress provokes striking changes in the lipid metabolism of cotton leaves, especially a marked reduction in galactolipid and PC biosynthesis, and a relative increase in NL formation. Concerning PC and galactolipids, these results are in agreement with the variations in amounts of these lipids [5]. Nevertheless, due to the very slow rate of galactolipid biosynthesis, one can state that the sharp decrease in galactolipid content of leaves [5] is the result of a degradation process rather than an inhibition of the synthesis.

In barley leaves, a rapid turnover of PC was observed in conditions of water deficits [9]. We have not found such an increase in PC labeling in cotton leaves. However, it is to be noticed that, under water stress, barley accumulates betaines whereas cotton which accumulates proline and amides, probably does not synthesize much betaine [10]. As PC is a precursor of glycinebetaine biosynthesis [11], the different lipid metabolisms in barley and cotton leaves

can be understood in the light of different mechanisms of drought resistance in these two species.

Fatty-acid desaturation is markedly inhibited by drought, resulting in a sharp decrease in linoleic and linolenic acid biosynthesis. A net reduction in linolenic acid percentage in water-stressed cotton leaves has been observed previously [5]. In the drought resistant variety, variations in lipid and fatty acid metabolism under dehydration are less pronounced than in the drought sensitive variety, and this can be related to a greater stability of the membrane system, as attested by electron microscope observations [12], hydrolytic enzyme measurements [13] and leakage of inorganic phosphorus [14].

EXPERIMENTAL

Plant material and drought treatment. Cotton plants (*Gossypium hirsutum* L., cv Reba, drought-sensitive, and cv Mocosinho, drought resistant) were grown in conditions previously described [15]. When plants were 5 weeks old, drought was induced by withholding irrigation. Labeling experiments were made on leaves from well hydrated plants (control C), from plants submitted to mild water stress (S1) and plants submitted to severe water stress (S2).

Leaf water potentials, measured in a pressure chamber [16] were as follows: Reba C: −40 MPa, Reba S1: −160 MPa, Reba S2: −230 MPa, Mocosinho C: −35 MPa, Mocosinho S1: −165 MPa, Mocosinho S2: −275 MPa.

[1-^{14}C]*Acetate incorporation.* Microdroplets of sodium [1-^{14}C]acetate (5 μmoles, specific activity 134.7×10^{10} *Bq.* mole^{-1}) were deposited on the abaxial surface of the 3rd leaf from the top [6]. Labeling experiments started at the beginning of a light period and lasted 2, 10 and 24 hr. Leaves were then harvested, rinsed with distilled water and lipids were extracted.

Lipid extraction and analysis. Lipids were extracted in $CHCl_3$–MeOH (1:2) [17]. For total fatty acid analysis samples of lipid extracts were saponified in NaOH (0.5 N in MeOH), methylated with BF_3 [18] and analysed by radio-GC [8]. Lipid classes were separated by TLC on silica gel plates (Merck G-60) [19]. After visualisation with Rhodamine 6-G, the bands were scraped off, saponified and methylated. Fatty acid methyl esters were analysed by 2 methods: For fractions having more than 10000 dpm, radio GC was used. For fractions having less than 10000 dpm, fatty acids were separated by TLC on activated silica gel containing $AgNO_3$ [20], and detected by radioautography. Radioactivity of the samples was measured by liquid scintillation with a Packard spectrometer having a 84% counting yield.

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