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EFFECTS OF DROUGHT-STRESS ON LIPIDS IN RAPE LEAVES

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Abstract—One-month-old plants of rape (*Brassica napus* cv. Drakkar) were subjected to water-deficit stress by withholding irrigation. Leaf lipid composition from control plants (well-hydrated) and water-stressed plants was analysed. Drought-stress induced a sharp decrease in galactolipid and phospholipid contents, whereas the content of neutral lipids increased. The fatty acid composition of leaf lipids was also affected under water-deficit stress. The percentage of linolenic acid (18:3) decreased mainly in monogalactosyldiacylglycerol and that of linoleic acid (18:2) in phospholipids.

INTRODUCTION

Drought has profound effects on the lipid metabolism of higher plants. Various studies showed that water-stress resulted in alteration of phospholipid and galactolipid contents in plant tissues [1–5]. Other work showed an increase of triacylglycerol contents in the leaves of plants subjected to water-deficits [6, 7]. It has also been shown [8] that water-stress induced a decline in the amounts of sterols in rape roots; similar results have been found in maize leaves [9]. Increased saturation of fatty acids in the roots of cotton under water-stress was also observed [10]. In cotton leaves, drought inhibits the biosynthesis of polyunsaturated fatty acids [3] and desaturase activities [11].

In a previous study, it was reported [8] that water-deficit leads to a large decline in phospholipids and to a slight decrease in esterified and free sterols in rape roots. The aim of the present investigation was to study the effects of drought on the lipid and fatty acid composition of rape leaves.

RESULTS

Brassica napus belongs to the so-called 16:3 plants, therefore the fatty acid composition of the leaf lipids was characterized by an appreciable content of hexadecatrienoic acid (16:3) which represented 15% of the total fatty acids. The other important fatty acids were 18:3 (the major component), 16:0, 16:1, 18:0, 18:1 and 18:2 (Table 1).

The total fatty acid composition of rape leaves was altered by drought treatment. There was a decrease in the percentages of 18:3 and 16:3 mainly in severely stressed leaves ($\Psi = -2.7$ MPa) (Table 1). This decrease was paralleled by an increase in 16:0 and 16:1 percentages.

The content of total lipids of rape leaves, as measured by total fatty acid content (mg g^{-1} dry wt), was observed to decline significantly with increasing degree of water-stress (Table 2). In severely desiccated plants, the amount of total lipids represented only 46% of the control. The drop in lipid content was due to a large decrease in the amounts of galactolipids and phospholipids (Table 2). Therefore, in highly-stressed leaves the amounts of these compounds were, respectively, 25.5% and 30% of controls. On the other hand, neutral lipids increased under water-stress and reached 172% of control levels in severely stressed leaves (Table 2 and Fig. 1).

Changes in the contents of main lipid components produced by water-stress are shown in Fig. 1. There was a pronounced decrease in the amounts of monogalactosyl diacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphaditylcholine (PC), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), while amount of phosphatidic acid (PA) increased and that of phosphatidylinositol (PI) varied slightly.

These results are similar to those obtained by Svenningsson and Liljenberg [8], who reported a significant decrease in total lipids, galactolipid and phospholipid contents and an increase of neutral lipids of rape roots submitted to drought treatment. In the same way, it has been shown [12] that water-deficit resulted in a large decrease in various lipid classes (MGDG, PC and PE), while the triacylglycerol amounts increased considerably in leaves of two *Lupinus albus* genotypes.

The changes in fatty acid composition due to stress differed somewhat between the individual lipids (Tables 3 and 4). Within MGDG, the 18:3 content varied from 56% in control to 42% in severely stressed leaves. The

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Table 1. Effect of water-deficit on total fatty acid composition of rape leaves*

Treatment†	% of total fatty acids						
	16:0	16:1	16:3	18:0	18:1	18:2	18:3
C	14.0 ± 1.3	2.6 ± 0.3	14.7 ± 0.6	2.1 ± 0	5.3 ± 1.0	13.8 ± 1.5	47.3 ± 1.7
MS	12.1 ± 0.9	6.4 ± 2.1	14.9 ± 1.2	2.5 ± 0.2	5.7 ± 0.7	12.9 ± 0.8	45.5 ± 1.4
SS	19.7 ± 2.2	10.6 ± 1.6	10.5 ± 1.2	2.8 ± 0.4	4.0 ± 0.9	11.5 ± 0.7	40.8 ± 2.0

*Results are given as % of total fatty acids. Values are means of 10 plants ± s.d.
†C, control; MS, mild stress; SS, severe stress.

Table 2. Effect of water deficit upon the lipid content (mg g^{-1} dry wt) of rape leaves*

	Control	Mild stress	Severe stress
Total lipids	28.0 ± 2.0	20.4 ± 2.2	12.9 ± 1.6
Phospholipids	10.9 ± 0.7	7.5 ± 0.8	3.3 ± 0.3
Galactolipids	13.6 ± 0.6	6.6 ± 0.4	3.5 ± 0.5
Neutral lipids	3.6 ± 0.2	6.4 ± 0.5	6.1 ± 0.3

*Values are means of 10 plants ± s.d.

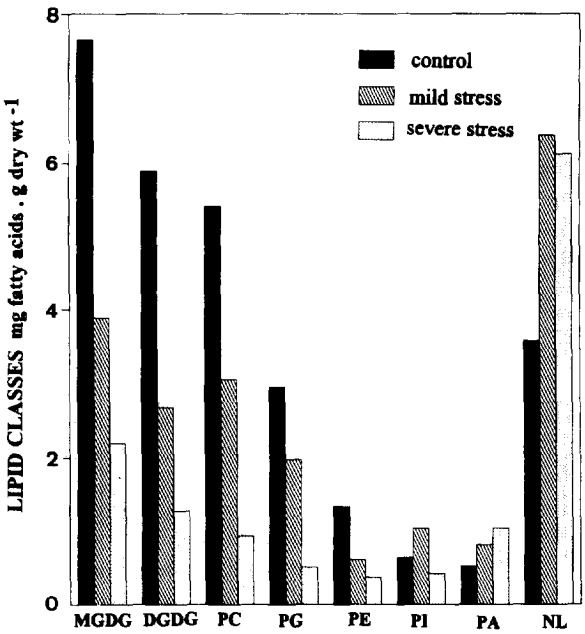

Fig. 1. Lipid classes of rape leaves subjected to water-stress.

16:3 content decreased slightly only in severely stressed leaves and that of 16:0 increased. The fatty acid composition of DGDG was not affected. Similar changes in fatty acid composition of phospholipids were observed. Therefore, in all phospholipids, the 18:2 percentage decreased with an increase of 16:0. In neutral lipids, 18:3 increased at the expense of 16:1. These results showed that water-stress tends to reduce the degree of unsaturation of rape leaf lipids. Svenningsson and Liljenberg [8] have also reported decreased unsaturation in the phospholipids in rape roots under water stress. Zuily-Fodil *et al.* [13] also showed a decrease in the degree of unsaturation of MGDG, PC and PG in three cultivars of *Cajanus cajan* submitted to water deficit. This decrease was due to decreased levels of 18:2.

DISCUSSION

Water stress induced a large decline in the amount of total lipids in the leaves of rape. The galactolipids (MGDG and DGDG) and phospholipids (PC, PG and PE), the major lipid classes present, decreased significantly to very low levels in response to water deficit. Such findings are in agreement with previous studies [5, 8, 12]. The decrease in galactolipids (main chloroplastic lipids) and in phospholipids (PC and PE) indicates that drought affects the chloroplast as well as the extrachloroplastic compartment.

Fatty acid composition of the main lipids of rape leaves was also affected under water stress. The major changes concerned the decrease in levels of 18:3 and 16:3 of MGDG and that of 18:2 in phospholipids. These results are in agreement with those obtained by Pham Thi [14] on cotton leaves submitted to drought.

In parallel with the decrease in the amounts of polar lipids, an increase in the neutral lipids was observed in rape leaves subjected to drought conditions. Such neutral lipid content and mainly triacylglycerol accumulation has been previously observed in other plants submitted to water stress [7, 12]. Some authors [8] suggested that

Table 3. Fatty acid composition of neutral and galactolipids from rape leaves after water-deficit stress*

Lipid	Treatment†	% of total fatty acids						
		16:0	16:1	16:3	18:0	18:1	18:2	18:3
MGDG	C	3.6 ± 0.8		34.3 ± 2.3	1.1 ± 0.1	0.6 ± 0.2	3.7 ± 0.4	56.6 ± 1.6
	MS	11.0 ± 0.6	—	33.2 ± 1.1	1.5 ± 0.0	0.9 ± 0.2	3.4 ± 0.5	50.0 ± 2.4
	SS	15.4 ± 0.9	1.4 ± 0.5	29.1 ± 1.6	3.3 ± 0.4	2.6 ± 0.2	5.3 ± 0.4	42.8 ± 3.1
DGDG	C	21.4 ± 1.0	0.7 ± 0.2	2.9 ± 0.8	2.3 ± 1.4	2.2 ± 0.4	9.1 ± 0.7	61.2 ± 1.9
	MS	19.7 ± 1.7	0.8 ± 0.5	5.3 ± 0.4	2.6 ± 0.3	1.8 ± 0.0	8.2 ± 0.5	61.5 ± 1.4
	SS	22.2 ± 0.6	0.9 ± 0.3	5.7 ± 0.6	3.6 ± 1.0	2.9 ± 0.7	7.9 ± 0.7	56.7 ± 3.3
Neutral lipids	C	20.1 ± 2.0	30.0 ± 3.3	13.7 ± 1.4	5.5 ± 0.4	2.6 ± 0.3	9.6 ± 0.7	18.4 ± 0.9
	MS	16.1 ± 1.7	10.9 ± 1.0	30.7 ± 3.6	5.5 ± 0.2	2.7 ± 0.1	7.0 ± 1.0	26.7 ± 2.1
	SS	29.8 ± 3.1	10.0 ± 0.5	13.3 ± 1.5	10.3 ± 0.8	2.0 ± 0.1	6.9 ± 0.6	27.6 ± 1.8

*Values are means of 10 plants ± s.d.
†C, control; MS, mild stress; SS, severe stress.

Table 4. Fatty acid composition of phospholipids from rape leaves after water-deficit stress*

Lipid	Treatment†	% of total fatty acids						
		16:0	16:1	16:3	18:0	18:1	18:2	18:3
PC	C	35.0 ± 2.4	1.5 ± 0.5	0.5 ± 0.6	4.1 ± 1.4	8.7 ± 0.5	22.2 ± 1.3	27.9 ± 2.2
	MS	40.8 ± 1.6	2.2 ± 0.3	2.4 ± 0.4	3.7 ± 0.9	6.5 ± 0.4	17.8 ± 0.8	26.6 ± 2.6
	SS	51.4 ± 2.9	1.3 ± 0.5	2.5 ± 0.2	3.4 ± 0.5	3.5 ± 0.2	12.9 ± 1.1	24.8 ± 4.5
PG	C	33.0 ± 2.0	24.3 ± 1.2	3.1 ± 0.4	1.6 ± 0.5	3.4 ± 0.3	15.7 ± 1.3	18.7 ± 0.6
	MS	37.1 ± 1.4	27.8 ± 3.6	2.6 ± 0.6	1.9 ± 0.2	2.3 ± 0.1	12.7 ± 0.7	15.4 ± 1.4
	SS	44.4 ± 4.1	22.7 ± 1.6	—	7.0 ± 5.0	4.1 ± 0.4	8.7 ± 0.8	12.8 ± 0.5
PE	C	31.3 ± 1.5	3.1 ± 0.9	2.0 ± 0.4	5.3 ± 0.7	6.5 ± 1.3	32.9 ± 1.8	18.8 ± 0.7
	MS	40.7 ± 1.9	4.5 ± 1.1	1.5 ± 0.5	5.3 ± 1.0	4.4 ± 0.2	26.7 ± 0.8	16.7 ± 2.3
	SS	40.6 ± 1.7	9.1 ± 2.0	5.6 ± 1.3	5.1 ± 1.0	4.6 ± 0.2	14.4 ± 0.5	20.5 ± 0.4

*Values are means of 10 plants ± s.d.
†C, control; MS, mild stress; SS, severe stress.

the adaptation significance of this accumulation resides in the properties of these molecules, their high calorific value and their hydrophobic character. Alternatively, it could be supposed that free fatty acids, produced during water stress by action of lipases on polar lipids, could be stored in triacylglycerols, in order to avoid oxidation by free radicals and activated oxygen forms. Neutral lipid production can be considered as a defence mechanism of the plant against drought [12]. However, the decreased contents of polar lipids in rape leaves upon exposure to

water stress indicates disorganization of cellular membranes, particularly those of chloroplast. A reduction in amounts of membrane lipids was observed in senescent plants [15]. It could be supposed that drought induced an anticipated senescence in rape.

EXPERIMENTAL

Plant material and drought treatments. Rape (*Brassica napus* L. cv. Drakkar) plants were cultivated in clay pots (20 cm diameter) filled with sand and kept in a greenhouse under controlled conditions: 14 hr day at 25°, 10 hr night at 20°, relative humidity 50%. Plants were irrigated × 3 per week using a nutrient soln [16] and used for experimentation when they were 4 weeks old. At this stage, plants had four well-developed leaves. 4-week-old plants were then subjected to 2 levels of water deficit by withholding irrigation. Watering was suspended for 5 days for mildly stressed plants (MS, leaf water potential $\Psi = -1.3$ MPa) and 10 days for severely stressed ones (SS, leaf water potential $\Psi = -2.7$ MPa). Control plants (C, well hydrated) were irrigated every day and maintained a leaf water potential ($\Psi = -0.5$ MPa). Leaf water potentials were measured in a pressure chamber [17]. Ten plants were used for sampling and the third and fourth leaves starting from the apex were harvested.

Lipid analysis. Total lipids were extracted using $CHCl_3$–MeOH–H_2O (1:1:1) [18]. Polar lipids were separated by TLC on silica gel plates 60 (Merck) in $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (1:4:2:2:1) [19]. Lipid spots were detected by brief exposure to I_2 vapour. Identifications were by comparison with reference lipids and by specific stains for phospholipids and galactolipids. Fatty acids from total lipids and lipid classes were transmethylated with MeOH–BF_3 [20]. Fatty acid Me esters were analysed by FID–GC on a metal column (1.8 m × 3 mm i.d.) packed with GP 3% SP-2310/2% SP-2300. Carrier gas N_2, flow rate 20 ml min^{-1}. The column was maintained isothermally at 190°. Injector and detector temps were held, respectively, at 210° and 240°. For measuring the amounts of fatty acids, 17:0 was added as int. standard before methylation. Calculation of fatty acid quantities was obtained using an integrator.

REFERENCES

1. Chetal, S., Wagle, D. S. and Nainawatee, H. S. (1981) *Plant Sci. Lett.* **20**, 225.
2. Chetal, S., Wagle, D. S. and Nainawatee, H. S. (1983) *Plant Sci. Lett.* **29**, 273.
3. Pham Thi, A. T., Borrel-Flood, C., Veira Da Silva, J., Justin, A. M. and Mazliak, P. (1985) *Phytochemistry* **24**, 723.
4. Navarri-Izzo, F., Quartacci, M. F. and Izzo, R. (1989) *J. Exper. Bot.* **40**, 675.
5. Monteiro De Paula, F., Pham Thi, A. T., Zuily-Fodil, Y., Ferrari-Iliou, R., Veira Da Silva, J. and Mazliak, P. (1993) *Plant Physiol. Biochem.* **31**, 707.
6. Douglas, T. J. and Paleg, G. (1981) *J. Exper. Bot.* **32**, 499.
7. Martin, B. A., Schoper, J. B. and Rinne, R. W. (1986) *Plant Physiol.* **81**, 789.
8. Svenningsson, H. and Liljenberg, C. (1986) *Physiol. Plant.* **68**, 53.
9. Navarri-Izzo, F., Izzo, R., Bottazzi, F. and Ranieri, A. (1988) *Phytochemistry* **27**, 3109.
10. Ouedraogo, M., Trémolières, A. and Hubac, C. (1984) *Z. Pflanzenphysiol.* **114**, 239.
11. Pham Thi, A. T., Borrel-Flood, C., Veira Da Silva, J., Justin, A. M. and Mazliak, P. (1987) *Physiol. Plant.* **69**, 147.
12. Hubac, C., Guerrier, D., Ferran, J. and Trémolières, A. (1989) *Plant Physiol. Biochem.* **27**, 737.
13. Zuily-Fodil, Y., Pham Thi, A. T., Tashakkorie, A. and Veira Da Silva, J. (1992) in *Metabolism, Structure and Utilization of Plant Lipids* (Chérif, A., Ben Miled Daoud, D., Marzouk, B., Smaoui, A. and Zarroük, M., eds), pp. 352–355. CNP Tunisia.
15. Fergusson, C. H. R. and Simon, E. W. (1973) *J. Exper. Bot.* **24**, 307.
16. Keldey, L. S. and Bowling, D. J. F. (1975) *Ann. Bot.* **39**, 1032.
17. Scholander, P. F., Hammel, H. T., Hemmingsen, E. A. and Bradsteet, E. D. (1965) *Science* **148**, 339.
18. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
19. Trémolières, A. and Lepage, M. (1971) *Plant Physiol.* **47**, 329.
20. Metcalfe, D., Schmitz, A. A. and Pelka, J. R. (1966) *Anal. Chem.* **38**, 524.