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FATTY ACID COMPOSITION IN WATER- AND OXYGEN-STRESSED LEAVES OF MAIZE AND WHEAT STRAINS

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Key Word Index—*Zea. mays* 4; *Triticum aestivum*; Gramineae; fatty acids; oxygen-stress; water-stress.

Abstract—Leaves of maize and wheat strains with different sensitivity to water-stress were subjected to 0.5 M mannitol and 100% O_2 for 48 hr. A higher ratio of unsaturated to saturated fatty acids was found in the resistant strains. These results suggest a close relationship between fatty acid composition and plant stress-resistance.

INTRODUCTION

Higher plants respond to water shortage in many ways which may enable the plant to adjust to or counter this form of stress [1]. Drought, apart from other things, generated reduced forms of O_2 in chloroplasts through a drought-impaired electron transport system [2]. These forms of active O_2 are highly destructive to all biological systems [3] and can be also generated by high O_2 pressures [4, 5].

Cellular membranes are particularly vulnerable to drought damage and are probably the primary site of cellular injury. After exposure of plants to freezing temperatures, dessication or other stresses, a common symptom of injury is disruption of membrane function and integrity as shown by leakage of cytoplasmic solutes and loss of osmotic responsiveness [1]. Several environmental stresses promote the formation of O_2-free radicals which mediate in phospholipid degradation, leading to the irreversible formation of gel phase domains and loss of membrane function [6]. It has been suggested that an increase of fatty acid unsaturation would tend to maintain the liquid-crystalline phase and prevent lipid peroxidation [6].

Increases in unsaturated fatty acids were found in wheat roots subjected to frost-hardening [7], salt-stressed peanut seedlings [8], roots and leaves of winter rape plants and the microalga, *Porphyridium cruentum*, subjected to low temperatures [9, 10] and in salt- and water-stressed maize roots [11]. It was demonstrated that the unsaturation of glycerolipids stabilizes the photosynthetic apparatus against low-temperature phytoinhibition, as was proposed as a mechanism of chilling tolerance [12]. However, other authors have found that water-stress decreases unsaturated fatty acid biosynthesis in bean, cotton and cowpea leaves. This effect was less pronounced in drought-tolerant varieties of cotton and cowpea, which indicates greater stability of the cell membrane [13–15]. Increases in the saturation of fatty acids were found in oat roots subjected to water-stress and could result in changed physical behaviour and permeability properties of plasma membranes leading to increased water retention [16].

The aim of the present work was to analyse the effects of water-stress and 100% O_2 on fatty acid composition and its relation to stress-resistance in maize and wheat leaves, with different degrees of sensitivity to water-stress.

RESULTS AND DISCUSSION

Either water-stress or 100% O_2 caused damage as was observed previously in leaves of the same wheat and maize strains [5, 17]. In both species, there were clear symptoms of stress damage, with a decrease in chlorophyll content and an increase of lipid peroxidation and conductivity, these changes being more marked in the sensitive strains of maize (LG11) and wheat (Leones).

When wheat and maize strains were subjected either to water-stress of to 100% O_2, fatty acids with shorter chain-length, like 16:0 and 16:1, tended to decrease and also to disappear, whereas fatty acids with longer chain-lengths such as 20:1, 20:2, 22:0 and 24:0 increased in the water-stress resistant strains, LIZA and Cruz Alta (Tables 1 and 2). Saturated fatty acids, such as 22:0 and 24:0, were detected only in resistant LIZA maize leaves, following stress treatments. In wheat leaves, 22:0 was induced by the treatments in both strains but especially in the resistant one. The presence of saturated fatty acids

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Table 1. Effect of water-stress and 100% O_2 on fatty acid composition of leaves from maize strains, resistant (LIZA) and sensitive (LG11) to water-stress

	LIZA				LG11			
	I	C	M	O	I	C	M	O
16:0	15.1 ± 0.8	9.2 ± 0.5	—	—	11.8 ± 0.9	12.7 ± 0.8	7.6 ± 0.4	11.0 ± 0.5
16:1	4.4 ± 0.2	3.0 ± 0.1	—	—	5.6 ± 0.4	4.7 ± 0.4	5.1 ± 0.3	3.9 ± 0.3
18:0	3.9 ± 0.6	3.0 ± 0.3	—	—	15.1 ± 0.9	15.9 ± 0.9	8.2 ± 0.3	6.1 ± 0.4
18:1	4.8 ± 0.1	4.2 ± 0.3	8.7 ± 0.4	8.7 ± 0.3	6.3 ± 0.3	4.3 ± 0.2.	31.2 ± 2.3	22.7 ± 1.4
18:2	3.9 ± 0.2	12.5 ± 1.0	20.0 ± 0.6	23.5 ± 1.4	28.7 ± 1.8	30.7 ± 1.9	32.8 ± 1.6	36.6 ± 1.3
20:0	8.7 ± 0.4	7.3 ± 0.4	1.7 ± 0.1	2.0 ± 0.2	26.6 ± 1.2	25.7 ± 1.9	7.9 ± 0.2	10.5 ± 0.5
18:3	59.2 ± 3.5	60.7 ± 2.5	64.0 ± 3.8	64.4 ± 3.2	5.9 ± 0.5	5.9 ± 0.5	7.2 ± 0.3	9.2 ± 0.7
22:0	—		3.4 ± 0.3	0.5 ± 0.02	—	—	—	—
24:0	—		2.2 ± 0.1	0.9 ± 0.04	—	—	—	—
Uns/Sat	2.6	4.1	12.7	28.4	0.9	0.8	3.2	2.6

Values are expressed as percentages of total lipid in the tissue determined after leaves were exposed to 0.5 M mannitol or 100% O_2 for 48 hr.

I, Initial; C, control; M, 0.5 M mannitol; O, 100% O_2.

Unsaturation/saturation = (16:1 + 18:1 + 18:2 + 18:3)/(16:0 + 18:0 + 22:0 + 24:0).

Values are means of three different experiments ±SD.

Table 2. Effect of water-stress and 100% O_2 on fatty acid composition of leaves from wheat strains, resistant (Cruz Alta) and sensitive (Leones) to water-stress

	Cruz Alta				Leones			
	I	C	M	O	I	C	M	O
16:0	17.5 ± 1.0	14.5 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	18.2 ± 0.7	15.2 ± 0.9	8.3 ± 0.4	6.1 ± 0.2
16:1	6.2 ± 0.4	6.0 ± 0.3	0.3 ± 0.1	0.6 ± 0.1	4.2 ± 0.1	2.5 ± 0.1	0.3 ± 0.1	—
18:0	5.1 ± 0.2	8.1 ± 0.6	3.8 ± 0.3	3.7 ± 0.2	4.9 ± 0.6	6.4 ± 0.2	10.1 ± 0.8	8.5 ± 0.6
18:1	9.4 ± 0.3	15.5 ± 0.9	12.8 ± 0.7	13.4 ± 0.8	8.8 ± 1.0	13.4 ± 0.9	10.7 ± 0.6	4.9 ± 0.9
18:2	27.5 ± 1.5	18.6 ± 1.3	32.8 ± 2.0	23.1 ± 1.8	12.3 ± 1.1	16.4 ± 1.8	12.5 ± 2.0	12.2 ± 1.4
20:0	7.6 ± 0.5	7.8 ± 0.5	4.0 ± 0.5	4.1 ± 0.3	9.2 ± 0.8	9.9 ± 0.8	12.9 ± 1.2	9.6 ± 1.0
18:3	21.9 ± 1.1	24.5 ± 1.5	28.1 ± 1.8	34.3 ± 1.2	36.8 ± 1.8	31.7 ± 1.1	32.2 ± 1.2	46.5 ± 2.2
20:1	4.8 ± 0.5	5.0 ± 0.4	7.7 ± 0.6	11.2 ± 0.8	5.6 ± 0.3	4.5 ± 0.3	8.8 ± 0.8	10.1 ± 0.9
20:2	—	—	5.6 ± 0.2	5.0 ± 0.1	—	—	3.1 ± 0.1	1.2 ± 0.3
22:0	—	—	4.4 ± 0.3	4.3 ± 0.2	—	—	1.1 ± 0.1	0.9 ± 0.2
Uns/Sat	2.3	2.3	4.5	4.7	2.1	2.5	1.8	2.8

Values are expressed as percentages of total lipid in the tissue determined after leaves were exposed to 0.5 M mannitol or 100% O_2 for 48 hr.

I, Initial; C, control; M, 0.5 M mannitol; O, 100% O_2.

Unsaturation/saturation = (16:1 + 18:1 + 18:2 + 18.3)/(16:0 + 18:0 + 20:0 + 22:0 + 24:0).

Values are means of three different experiments ±SD.

with longer chain-lengths under stress conditions could be a protective response against the damage caused by the treatments, and could be a mechanism for water retention, as has been suggested previously [16]. This mechanism of protection seemed to be more relevant in the water-stress resistant strains LIZA and Cruz Alta.

Unsaturated fatty acids (18:1, 18:2 and 18:3) were increased by both stress treatments in the wheat and maize strains (Tables 1 and 2). A higher ratio of unsaturated to saturated fatty acids was found in the water-stress resistant strains LIZA and Cruz Alta. The increase of fatty acid unsaturation could be another mechanism of protection against the oxidative damage provoked by water-stress and 100% O_2, since unsaturated fatty acids would tend to maintain the liquid-crystalline phase and prevent lipid peroxidation [6]. This mechanism of protection seemed to be more efficient in the resistant strains than in the sensitive ones. Under low temperature-stress, an increase in the content of unsaturated fatty acids was also found in the microalga

Porphyridium cruentum [10], and was suggested as a mechanism for the cell to maintain its membrane permeability. The sharp decrease in galactolipid content of cotton leaves subjected to water-stress is the result of a degradation process rather than inhibition of synthesis [14]. It seems probable that the mechanism of water-stress resistance of our maize and wheat strains could be, among others, to prevent this degradation process by increasing the biosynthesis/degradation rate of fatty acids. Such a mechanism would tend to maintain the content of total fatty acids. In the resistant strains of maize (LIZA) and wheat (Cruz Alta), total fatty acid content was decreased by the stress treatments, this effect being significantly greater in the sensitive ones, LG11 and Leones (Tables 3 and 4). This is consistent with previous results with the same maize and wheat strains, in which lipid peroxidation was significantly increased in the sensitive strains, LG11 and Leones, by the effect of water-stress and 100% O_2 [5, 17]. The larger increase of lipid peroxidation in the sensitive strains of maize and wheat could be accelerating fatty acid degradation.

In previous work, we suggested that the antioxidative potential was closely related to stress-resistance to paraquat, H_2O_2, water-stress and 100% O_2 [5, 18, 19]. The protection provided by antioxidant enzymes at the cellular level could be supporting another mechanism of protection against oxidative damage, but at the membrane level. It seems probable that the higher resistance of the LIZA and Cruz Alta strains may be supported by two lines of protection, the first one at the membrane level, by increasing the unsaturation/saturation ratio and increasing saturated fatty acids with longer chain-lengths, the second consisting of overproduction of antioxidant enzymes, such as superoxide dismutase and glutathione reductase [5, 18–20]. Results presented in this work suggest that a close relationship between fatty acid composition and stress-resistance could exist in maize and wheat leaves.

Table 3. Effect of water-stress and 100% O_2 on total fatty acid contents of maize strains, resistant (LIZA) and sensitive (LG11) to water-stress

	LIZA	LG11
Initial	13.88 ± 0.73	13.57 ± 0.63
Control	13.61 ± 0.31	13.18 ± 0.79
0.5 M mannitol	9.91 ± 0.26	6.16 ± 0.53
100% O_2	9.16 ± 0.48	6.99 ± 0.51

Values are expressed as mg total fatty acids g^{-1} fr. wt. determined in the leaves after exposure to 0.5 M mannitol or 100% O_2 for 48 hr.

Values are means of three different experiments ±SD.

Table 4. Effect of water-stress and 100% O_2 on total fatty acid contacts wheat strains, resistant (Cruz Alta) and sensitive (Leones) to water-stress

	Cruz Alta	Leones
Initial	9.68 ± 0.51	10.78 ± 0.83
Control	9.28 ± 0.44	10.96 ± 0.67
0.5 M mannitol	8.15 ± 0.56	5.09 ± 0.11
100% O_2	8.89 ± 0.32	5.75 ± 0.49

Values are expressed as mg total fatty acids g^{-1} fr. wt. determined in the levels after exposure to 0.5 M mannitol or 100% O_2 for 48 hr.

Values are means of three different experiments ±SD.

EXPERIMENTAL

Seeds of *Zea mays* L. and *Triticum aestivum* L. strains were sown in vermiculite. Seeds of maize were provided by the Station d'Agronomie Clermont–Ferrant (INRA, France), one resistant (LIZA) and one sensitive (LG11) to water-stress. Wheat seeds were provided by Instituto Nacional de Tecnología Agropercuaria (INTA Marcos Juárez, Córdoba, Argentina), one resistant (Cruz Alta) and one sensitive (Leones) to water-stress. Strains were selected by their water-stress resistance and their recovery capacity after stress [5, 18].

Water-stress treatment was done by placing 15 g of maize and wheat leaves in trays containing 500 ml of 0.5 M mannitol or dist. H_2O (control) for 48 hr in an ambient air (21% O_2) [21]. High O_2 pressure treatment was carried out for 48 hr by floating 15 g of leaves in trays containing 500 ml of dist. H_2O which were placed in glass containers [22]. A mixt. of 99.95% O_2 in N_2 (v/v) was passed continuously through the glass containers at a flow rate of 150 ml min^{-1}.

After stress treatments, 10 g of leaves were used for total lipid extraction according to the method of ref. [23]. Me esters were prepd according to ref. [24]. Leaf oil (0.5 ml) was added to 15 ml of a soln containing 1 N KOH in MeOH and the mixt. boiled for 15 min. The soln was then washed twice with 30 ml of hexane and the lower phase sepd. The upper phase was added to 30 ml of a soln containing H_2SO_4 in MeOH and boiled for 15 min. The two phases were sepd by adding hexane to the mixt. (× 2) and shaking vigorously. The upper phase was sepd, dried (Na_2SO_4), filtered and evapd. Me esters were analysed by GC at 150° on a 2 m glass column packed with 6% diethylene glycol succinate on Diatoport S with N_2 as carrier. Peaks were identified by comparison with known standards. Percentages of individual fatty acids were calculated by triangulation.

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