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Fatty Acids Composition in Germinating Cotton Seedlings Affected by High Temperature Stress

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Abstract: The results of fatty acids analyses suggested that greater unsaturation occurred with increasing germination time. High temperature had a marked effect on fatty acid composition and caused significant changes especially in neutral lipid and glycolipid fatty acids. Small changes were also observed in phospholipid fatty acids. A large increase in free fatty acids was also found, since free fatty acids are known to be damaging to cells.

Keywords: Temperature, Cotton Seedlings, Fatty Acids, Lipids

Introduction

Under normal conditions, the polar part of the lipid molecule is localised at the outer faces of biological membranes, with the fatty acid moieties embedded within the membrane structure. Both the polar head and the hydrocarbon tails of phospholipids play an important role in determining the structure and physico-chemical properties of membrane lipid bilayers. Thompson and Zalik, (1973) suggested that both the unsaturation levels of the fatty acid moieties and the polar end of the lipids influence the properties of the membranes. Membrane lipids contain high levels of unsaturated fatty acids which create the proper environment for the proteins of the membranes (Lem *et al.*, 1980).

Temperature has been identified as an important environmental factor affecting the fatty acid composition in seeds (Tremolieres *et al.*, 1978), roots (Simolenska and Kuiper, 1977) and leaves (St. John and Christiansen, 1976). Several workers have observed that the proportions of these fatty acids are strongly influenced by high temperatures (Matsuzaki *et al.*, 1988). They further reported that the molar proportion of oleic acid increased and that of linoleic and linolenic acid decreased as temperature increased. This was confirmed by St. John and Christiansen, (1976) studying cotton seed germinated at 15, 20, 25 and 30°C. They showed that, as the temperature increased, the linoleic acid content of the polar lipid fraction decreased. Sheikh *et al.*, (1996) have reported that 2 hours heat-shock at 40°C reduced total phospholipid levels. Apparently, this temperature did not visibly damage the root tissues, although above this temperature damage was evident. In the present experiment, therefore, a temperature of 40°C was used to study the effect of heat-shock on the fatty acid composition of germinating cotton seedlings.

Materials and Methods

In this experiment, seeds of cultivar MNH-93 was used. Cotton seedlings that had been germinated for 48 hours at 25°C were heat-shocked for 2 hours at 40°C in a water bath (Sheikh *et al.*, 1996). After the heat-shock treatment, they were returned to the incubator at 25°C for recovery up to 72 hours. Controls consisted of seeds germinated for 48 and 72 hours at 25°C. At the end of the experimental treatments, the seedlings of the first quartile (average root length = 93 mm) were harvested and their roots were isolated from the cotyledons using a scalpel. The root lipids were then extracted using hot water-saturated butanol. After extraction, the crude lipid was purified by chromatography on columns of Sephadex G-25. Free fatty acids and esterified fatty acids were separated from the purified lipid fraction and the esterified fatty acids were further separated into neutral lipid, glycolipid and phospholipid fractions by chromatography on columns of silicic acid. Finally, fatty acid methyl esters were prepared from the various fractions and analysed by gas-liquid chromatography.

Results and Discussion

In addition to the expected fatty acid methyl esters, a few low molecular weight peaks were found in the chromatograms from some tissues, some of these peaks appeared frequently. They are therefore included in the results and mentioned as unknowns. When the log of retention times of these unknowns together with the corresponding values for three authentic fatty acids, palmitic (C16:0), margaric (C17:0) and stearic acid (C18:0), were plotted against the number of carbon atoms, two of the unknowns fell onto the straight line obtained and they appeared to correspond to lauric acid (C12:0) and myristic acid (C14:0). They were tentatively identified as these fatty acids.

The major fatty acids present in all the lipid fractions were palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. The two major fatty acids were palmitic and linoleic acids, together comprising over 60% of the total fatty acids in each lipid fractions except in the glycolipid fraction at some stages of germination. In the control seeds germinated for 72 hours, for example, linolenic acid was the major fatty acid in this fraction.

Analysis of the fatty acids from cotton roots following heat shock showed changes in the compositions of both the free and esterified fatty acid fractions. Similar observations have been reported by several workers who have shown that temperature change alters fatty acid composition (Slack and Roughan, 1978; Wolf *et al.*, 1982; Matsuzaki *et al.*, 1988).

Total amount of esterified and free fatty acids increased with increasing germination time. The increase was presumably associated with the synthesis of phospholipids for new membrane formation. The fatty acid levels in individual fractions also increased with increasing germination time between 48 and 72 hours. The greater increase of fatty acids in the phospholipid fraction may be due to the formation of more

Table 1: Effects of High Temperature Stress on the Esterified and Free Fatty Acids

Lipid fraction	Total germ time (h)	Fatty acid content μg (25 roots)$^{-1}$
	48h control	537 ±218
Neutral lipids	72h control	718 ±144
	After heat shock	749 ±324
	After recovery	581 ±220
	48h control	52 ±34
Glycolipids	72h control	123 ±16
	After heat shock	139 ±19
	After recovery	154 ±44
	48h control	1655 ±320
Phospholipids	72h control	2453 ±212
	After heat shock	1580 ±79
	After recovery	2254 ±180
	48h control	25 ±8
Free fatty	72h control	32 ±8
acids	After heat shock	40 ±6
	After recovery	78 ±4
	48h control	2240 ±290
Combined acyl-	72h control	3200 ±180
Lipids	After heat shock	2470 ±480
	After recovery	2990 ±500
	48h control	2270 ±290
Total lipids	72h control	3330 ±180
	After heat shock	2510 ±490
	After recovery	3070 ±500

Each value is the mean content ±sd from four determinations

Table 2: Effect of High Temperature Stress on the Percentage Fatty Acid Compositions

Lipid fraction	Germination time (hours)	Fatty acids					
		Unknown	C16:0	C18:0	C18:1	C18:2	C18:3
Neutral lipids	48h control	2.3 ± 0.5	27.5 ± 2.4	6.2 ± 2.0	20.3 ± 2.2	40.4 ± 2.7	3.9±1.3
	72h control	2.7 ± 0.7	23.8 ± 1.9	4.5 ± 1.2	18.6 ± 3.0	41.0±3.3	9.1±1.9
	After heat-shock	2.7 ± 1.0	29.9 ± 1.2	5.3 ± 0.9	16.8 ± 2.5	42.9±4.1	2.5±0.2
	After recovery	13.7 ± 7.1	24.4 ± 3.3	5.0 ± 0.5	17.5 ± 2.3	35.7±3.0	3.8±0.8
Glycolipids	48h control	--	36.8 ± 1.5	3.9 ± 0.7	18.2 ± 3.2	25.3±1.6	15.7±3.7
	72h control	--	26.7 ± 5.4	6.7 ± 2.8	10.4 ± 3.9	21.2±7.0	35.0±9.3
	After heat-shock	2.6 ± 1.6	42.3 ± 6.0	4.3 ± 0.8	11.7 ± 4.2	23.4±3.0	15.8±2.6
	After recovery	3.6 ± 2.0	39.2 ± 2.7	3.6 ± 0.8	13.4 ± 2.2	18.1±0.8	22.1±1.6
Phospholipids	48h control	0.5 ± (tr)	34.1 ± 2.8	3.0 ± 0.6	5.8 ± 0.8	40.1±1.6	16.6±1.2
	72h control	0.7 ± 0.1	30.4 ± 0.8	2.2 ± 1.4	4.7 ± 1.6	41.3±1.1	20.8±2.9
	After heat-shock	1.4 ± 0.4	35.1 ± 3.6	2.5 ± 0.8	5.2 ± 1.1	39.2±1.5	14.3±0.5
	After recovery	0.9 ± 0.4	32.6 ± 2.1	2.7 ± 0.4	6.8 ± 1.1	37.2±4.0	18.3±2.7
Free fatty acids	48h contro	I.8 ± (tr)	48.7 ± 5.5	5.3 ± 2.8	14.5 ± 4.0	24.8±2.7	5.9±0.3
	72h control	--	28.8 ± 1.1	6.1 ± 0.9	10.2 ± 3.4	43.8±3.5	10.9±0.7
	After heat-shock	1.2 ± 0.2	40.2 ± 2.0	3.9 ± 0.6	8.9 ± 1.0	40.2±0.9	5.5±2.7
	After recovery	1.1 ± 0.2	45.1 ± 2.6	5.5 ± 2.2	5.6 ± 1.1	32.7±0.3	10.0±1.8

Each value is the mean ratio ±sd from four determinations. tr=sd<0.05

Heat shock caused increases in the levels of the neutral lipid fatty acids and decreases in the levels of phospholipid fatty acids. The increases in neutral lipid fatty acids must be due to increased synthesis of neutral lipids such as triacylglycerol at the raised temperature. Bowman and Mumma (1967) working on the oomycete *Pythium ultimum* reported that there is a tendency for more triacylglycerol to be synthesized at higher temperatures. Skogqvist (1974) working on wheat reported that soon after heat treatment, the triacylglycerol content of the root tips was higher than in control roots. She further added that high amounts of triacylglycerol might be derived from the cell membranes, because the permeability of the membranes were changed by the heat treatment. This would presumably involve the converting of phospholipids to triacylglycerols and/or diacylglycerols. The decrease in the levels of phospholipid fatty acids observed in the present study reflects a similar associated reduction of total phospholipid from cell membranes associated with neutral lipid formation in both cultivars. According to Skogqvist, (1974), the higher content of triacylglycerols found in wheat roots subjected to heat treatment was probably derived from membrane lipids such as PC, PE, PS and perhaps galactosyl diacylglycerols. These polar lipids were hydrolysed to phosphatidic acid, which was then dephosphorylated and esterified to form triacylglycerols. In the present study, heat shock also caused a significant increase in the levels of the glycolipids. The increases in glycolipid fatty acids might

be due to increased synthesis of glycolipids at this temperature. When the ratio of phospholipids to glycolipids are calculated the result indicated a decrease in the proportion of phospholipids and a corresponding increase in glycolipids. Quinn, (1988) reported that growth at low (unspecified) temperature caused a small increase in the proportion of phospholipids to galactolipids. However, the levels of neutral lipid fatty acids and phospholipid fatty acids remained below the 72 hour control values while the level of glycolipid fatty acids showed a small increase. A significant increase in free fatty acids was observed. Such increased levels of free fatty acids might cause the disruption of membrane structures and decrease the thermostability of membranes. Santarius, (1980) has reported that the formation of free fatty acids by endogenous lipases decreases the thermostability of membrane structures within plant cells. Santarius, (1980) also suggested that small amounts of unsaturated free fatty acids liberated by heat-stimulated hydrolysis of lipids may contribute to changes in membrane lipid interactions.

The results of phospholipid fraction are in agreement with the results reported by Sheikh *et al.*, (1996). These results suggest that heat shock at 40°C had no significant effect on phospholipid fatty acid composition or totalphospholipid levels.

During germination between 48 and 72 hours at 25°C, significant changes were observed in the proportions of fatty acids in the neutral lipid, glycolipid and free fatty acid fractions. Only small changes in the phospholipid fraction were found however. El-Nockrashy *et al.*, (1974) and St. John and Chritiansen, (1976) have reported that the fatty acid composition of polar lipids in cotton seeds changes markedly during germination. Increasing germination time caused increased synthesis of unsaturated fatty acids especially linolenic acid, and decreased synthesis of palmitic acid similar to the present observations.

The results for the glycolipid and phospholipid fatty acids suggest that high temperature inhibits the synthesis of unsaturated fatty acids and enhances the synthesis of saturated fatty acids in these polar lipid fractions. The greater saturation occurs in the glycolipids fraction while, in phospholipid fraction, the changes were small. The situation for both cultivars was similar. Raison *et al.*, (1983) reported that increased temperature causes changes in fatty acids but the changes in fatty acids associated with the phospholipid fraction were considerably less than the glycolipids. These results confirm reports by Wolf *et al.*, (1982) and Matsuzaki *et al.*, (1988) working on soybean. They showed that the proportions of linoleic and linolenic acids decreased as growing temperature increased. At the other extreme, Quinn, (1988) reported that growth at low temperature caused decrease in lipid saturation. Although roots contain only small amounts of glycolipids, these glycolipid fatty acids are very susceptible to high temperature. The levels of the unsaturated fatty acids decreased markedly in this fraction suggesting that, in cotton roots, the changes in the proportion of the unsaturated fatty acids are an important response to high temperature. This is presumably due to effects on the compositions of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), which are known to be highly susceptible to temperature. Raison *et al.*, (1983) reported that changes in temperature from high to low provoke greater changes in the composition of glycolipid fatty acids than in other fractions.

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