Abstract

Cotton plants (Gossypium hirsutum L. cv. S4) were grown and irrigated with increasing salt concentrations: 0, 50, 100 and 150 mM NaCl. Lipids from developing seeds, at stages of 3, 4, 5 and 6 weeks after anthesis, were analysed. High salt dose (150 mM NaCl) affected triacylglycerol levels drastically at the end of ripening. HPLC analysis of triacylglycerols in control seeds showed ten molecular species. Palmitodilinolein (PLL) is the major fraction, representing about 25% of total molecular species. The levels of trilinolein (LLL), palmitolinooleo-olein (PLO) and oleodilinolein (OLL) molecules varied between 13 and 16% of the total. The other molecular species: triolein (OOO), dioleopalmitin (POO) and dipalmito-olein (POP) are minor and do not exceed 5%. Moreover, the composition of triacylglycerol molecular species was almost constant during developing stages of control seeds. However, amounts of triacylglycerol species LLL, OLL and palmitodilinolein (PLL) as expressed on a dry-matter basis (mg/g), decreased severely under the highest NaCl concentration, while contents of triacylglycerol species OOO, POO, POP were unchanged. These findings confirm our previous results, which indicated that the amount of linoleic acid in cotton seeds was reduced by salt stress and that the amount of oleic acid remained unchanged.

Introduction

The effects of environmental factors, such as light or temperature, on developing seeds have been studied extensively. Cautison et al. [1] investigated maturation changes and temperature effects on fatty acid composition in developing sunflower seeds with highly saturated fatty acids. Slack and Rougan [2] showed that temperature modifies triacylglycerol (TAG) composition of developing linseed and soya bean cotyledons. Trémolieres [3] indicated that temperature changes affect fatty acid composition of sunflower oil and rape during maturation. On the other hand, sunflower plants subjected to various NaCl concentrations showed some changes of fatty acid composition in seeds [4].

Cotton seed is a highly important byproduct, it contains 18-20% edible oil [5]. In some agricultural areas, cotton plants are irrigated with

References

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The aim of the present study was to investigate the effects of salt on TAG molecular species in developing cotton seeds.

Experimental

Plant material

Cotton plants of *Gossypium hirsutum* L. cv. S4 were grown in pots filled with farming soil. Culture was conducted in a greenhouse under controlled conditions. Young seedlings were displayed in four sets of 15 seedlings. The first set (control) was irrigated with tap water, the other sets were supplied with tap water with 50, 100 or 150 mM NaCl added. Seeds were harvested weekly for 6 weeks after anthesis (WAA). The sixth WAA stage was considered to be the last stage of ripening before dehiscence of the capsule.

Lipid analysis

Total lipids were separated by TLC on silica gel plates (Merck G 60). TAGs were separated, according to the method of Mangold [10]. TAG spots were visualized by spraying on water, scraped off and eluted into a solvent mixture of chloroform/methanol (9:1, v/v).

The solvent mixture, containing TAG separated by TLC, was evaporated and 0.5 ml of acetone added. TAG molecular species were analysed by HPLC with a Waters 600 Chromatograph (Milford, MA, U.S.A.). Molecular species were detected by a refractometric detector (Waters 400). The column used was a reversed-phase C18 (Lichrospher 100 RP-18) with apolar stationary phase. The column was maintained at 15 °C in a thermostat-controlled water bath. The elution was performed with the solvent mixture acetone/acetoneitrile (7:3, v/v). The flow rate was 1.4 ml/min. Molecular species were identified by reference to TAG standards.

Results and discussion

Evolution of TAG molecular species in ripening control seeds

HPLC analysis of TAG isolated from control 3-WAA-old seeds showed ten main molecular species (Table 1). PLL, the major TAG fraction, represented about 22% of the total TAGs. Levels of TAG molecules containing linoleic acid: trilinolein (LLL), palmitolinoleo-olein (PLO), oleodilinolein (OLL), dipalmitolinolein (PLP) and dioleolinolein (OOL), varied between 8 and 15%. The other TAG molecular species, dioleopalmitin (POO), dipalmitolein (POP), triolein (000) and dilinoleopalmitolein (POLL), were relatively minor and each represented less than 6%.

During ripening and until 6 WAA, levels of TAG species were practically unchanged (Table 1). This finding is in good agreement with that observed by Boukhchina et al. [11], who reported constant levels of TAG molecular species during sunflower seed maturation.

Evolution of TAG molecular species in salt-stressed ripening seeds

As shown by Table 2, TAG molecular species composition was changed by salt concentrations and especially by a high NaCl dose (150 mM), as compared with the control, for different ripening stages.

In fact, levels of linoleic acid-rich TAG fractions, especially palmitodilinolein (PLL) and LLL, were drastically reduced by NaCl treatments, while levels of oleic acid-rich species such as POO, POP and OOO were enhanced during ripening of cotton seeds. These results confirm our previous findings [12,13] related to changes in total fatty acid composition induced by salt stress. Indeed, we have observed a depressed level of linoleic acid paralleled with increased levels of oleic acid.

Figure 1 showed changes in the amounts of TAG species expressed in mg/g of dry matter. At the sixth WAA and for the high NaCl concentration (150 mM), amounts of all molecular species containing linoleic acid were severely depressed by salt stress (PLL, LLL, PLO, OLL, PLP, PLO). In contrast, contents of TAG molecular species containing oleic acid (POO, POP and OOO) remained unchanged by a high dose of NaCl.

Effects of salt stress has been studied on olive oil composition by Zarrouk [14], who reported depressed levels mainly of triolein, the major TAG fraction in olive oil. However, in cotton seeds (our
Table 1
Changes in TAG fractions (%) of cotton seeds during stages of maturation

SLL, dlinoleostearin

| WAA | NaCl (mM) | PLL | LLL | PLO | OLL | PLP | OOL+SSL | POO | POP | OOL | P|LL |
|-----|-----------|-----|-----|-----|-----|-----|---------|-----|-----|-----|-----|
| 3   | 0         | 22.1| 14.3| 14.9| 12.1| 10.7| 8.3     | 5.8 | 4.3 | 3.9 | 1.7 |
| 4   | 50        | 21.8| 15.4| 15.3| 12.9| 9.6 | 7.8     | 5.9 | 4.9 | 2.6 | 1.6 |
| 5   | 100       | 24.7| 18.0| 15.3| 13.9| 9.4 | 6.4     | 4.4 | 4.0 | 1.4 | 0.9 |
| 6   | 150       | 23.3| 16.3| 15.2| 13.4| 9.6 | 7.0     | 5.0 | 4.2 | 2.8 | 0.8 |

Table 2
Changes in TAG molecular species (%) with various NaCl concentrations in developing cotton seeds

| WAA | NaCl (mM) | PLL | LLL | PLO | OLL | PLP | OOL+SSL | POO | POP | OOL | P|LL |
|-----|-----------|-----|-----|-----|-----|-----|---------|-----|-----|-----|-----|
| 3   | 0         | 22.1| 14.3| 14.9| 12.1| 10.7| 8.3     | 5.8 | 4.3 | 3.9 | 1.7 |
| 50  |           | 20.0| 13.4| 14.4| 11.7| 9.7 | 9.1     | 7.0 | 4.6 | 5.5 | 1.5 |
| 100 |           | 17.2| 13.2| 15.4| 13.5| 8.1 | 8.6     | 7.2 | 5.5 | 3.0 | 1.5 |
| 150 |           | 14.5| 7.4 | 14.5| 15.7| 5.8 | 5.8     | 11.8| 9.3 | 9.7 | 1.7 |
| 4   | 0         | 21.8| 15.4| 15.3| 12.9| 9.6 | 7.8     | 5.9 | 4.9 | 2.6 | 1.6 |
| 50  |           | 26.0| 18.1| 15.0| 13.4| 11.1| 5.8     | 3.6 | 4.1 | 0.9 | 1.5 |
| 100 |           | 25.1| 16.5| 15.4| 13.0| 11.7| 6.1     | 3.9 | 4.4 | 1.2 | 1.1 |
| 150 |           | 15.4| 7.5 | 16.0| 10.1| 7.2 | 8.9     | 13.2| 8.2 | 4.7 | 8.2 |
| 5   | 0         | 24.7| 18.0| 15.3| 13.9| 9.4 | 6.4     | 4.4 | 4.0 | 1.4 | 0.9 |
| 50  |           | 26.2| 16.8| 15.4| 12.6| 11.2| 5.9     | 4.1 | 4.5 | 1.0 | 1.3 |
| 100 |           | 25.0| 17.4| 15.7| 13.4| 10.5| 6.3     | 4.3 | 4.0 | 1.5 | 1.2 |
| 150 |           | 12.8| 7.5 | 17.7| 11.6| 7.3 | 10.9    | 13.1| 7.2 | 5.8 | 0.3 |
| 6   | 0         | 23.3| 16.3| 15.2| 13.4| 9.6 | 7.0     | 5.0 | 4.2 | 2.8 | 0.8 |
| 50  |           | 24.5| 14.8| 16.6| 12.7| 11.6| 6.3     | 4.6 | 5.2 | 1.1 | 1.0 |
| 100 |           | 23.0| 13.5| 17.5| 12.9| 10.8| 7.3     | 5.7 | 5.3 | 1.6 | 1.1 |
| 150 |           | 13.1| 11.8| 16.4| 11.8| 6.5 | 11.2    | 12.8| 6.7 | 6.9 | 0.3 |

Figure 1
Changes in TAG amounts of cotton seeds from control and 150 mM NaCl-treated plants

DM, dry matter.
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There were reduced levels of linoleic acid-rich TAGs (PLL and LLL), which together form more than 36 % of total TAG species. These conflicting results could be explained by the fact that salt stress especially affects the biosynthesis of the major fatty acid: oleic acid in olives or linoleic acid in cotton seed.

The drop in TAG synthesis may be due to inhibition of photosynthetic activity in leaves induced by high NaCl concentrations, leading to less transport of photosynthetates from leaves to seeds.

References


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Changes in plasma-membrane lipid composition: a strategy for acclimation to copper stress

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Abstract

Wheat seedlings were grown hydroponically in the presence of 50 μM Cu²⁺. The copper stress resulted in plasma-membrane (PM) changes of the root cells as altered lipid composition, a decreased phosphatidylcholine (PC)/phosphatidylethanolamine (PE) ratio from 0.7 to 0.3, a decreased fatty acyl unsaturation and a decrease in the lipid/protein ratio. Membrane vesicles made from total lipid extracts of isolated PMs of wheat grown under copper excess showed a remarkably low permeability to polar molecules like glucose, as compared with the control, and no difference in proton permeability. Permeability studies of vesicles of plasma-membrane lipids, which were selectively modified by addition of specific lipids such as PC and PE, were also performed. The results are discussed with emphasis on the role of the increased PE proportion.

Introduction

Copper is an essential micronutrient for higher plants, but is extremely toxic when its concentration in the soil exceeds trace levels. Excess copper causes severe damage to plant organelles and inhibits metabolic activity, which leads to suppressed growth [1]. The root-cell plasma membrane (PM) is the first functional barrier that comes into contact with metal ions present at toxic concentrations. Maintenance of the structural integrity of the PM is a prerequisite for survival. When wheat is grown in the presence of excess copper there are remarkable changes in the lipid composition of the PMs of the wheat root cells, a decrease in fatty acyl unsaturation and a decrease in the lipid-to-protein ratio [2].

Experimental

Wheat (Triticum durum Desf. cv. Creso) was grown for 11 days using solutions containing...