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# Effect of Water logging on Fatty Acid Composition in Cotton Seedling Roots

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**Abstract:** Fatty acid analyses in cotton seedlings following waterlogging showed changes in both their levels and relative composition. This treatment caused a marked increase in the amount of esterified fatty acids. Waterlogging also enhanced the synthesis of a set of low molecular weight compounds and suppressed the synthesis of all the "normal" fatty acids. A significant increase in free fatty acids was also observed.

**Keywords:** Cotton, Fatty Acids, Seedlings, Waterlogging

## Introduction

Fatty acids present in the structures of both storage lipids and structural membrane lipids play an important role in the fluidity of the membranes. Membrane lipids contain high levels of unsaturated fatty acids that create the proper environment for the proteins of the membrane (Lem *et al.*, 1980). It is a well established fact that the membrane lipids and the storage lipids of higher plants are altered by environmental factors, such as temperature, water, stress, nutritional deprivation and salt stress (Harwood, 1984). In the case of water stress, the presence of excess water around seedlings creates anaerobic atmosphere for roots, which in turn will alter the biochemical processes. The metabolism of lipids, like other component which are adversely and strongly affected when rice coleoptiles are grown under anaerobic conditions (Brown and Beevers, 1987). It indicated that molecular oxygen is essential for maintaining membrane integrity. Biosynthesis of unsaturated fatty acids requires molecular oxygen and is therefore essential for membranes to maintain their structures and functions (Brown and Beevers, 1987). However, the synthesis of unsaturated fatty acids is inhibited in rice coleoptiles when grown under anaerobic conditions. In contrast, Vartapetian *et al.*, (1978) found that rice coleoptiles grown under anaerobic conditions showed higher amount of fatty acid. They further added that anaerobiosis induces higher proportions of oleic and linoleic acids than in coleoptiles grown in the air. Kuiper, (1984) reported that the changes in membrane lipid composition may be attributed to stress-induced degradation processes. Enzyme such as lipoxygenase and phospholipase may be involved in these processes.Little or no work has been done on the effect of waterlogging on lipid composition of cotton seedlings. Present study was carried out to observe the effect of waterlogging treatment on the fatty acids composition of cotton seedlings.

## Material and Methods

Seed of cotton cultivar MNH-93 was used. Cotton seedlings that had been germinated in rolled filter paper for 48 hours at 25°C (Sheikh, *et al.*, 1996). The rolled filter papers alongwith their contents were taken out from the plastic bags and placed in glass jars (30 cm high 8 cm diameter) filled with water upto 26cm. This was enough to completely cover the rolled filter paper and its seedlings. All these treatments were carriedout under controlled conditions at 25°C. The jar was covered with a glass lid and a piece of cling film was wrapped over it to prevent air exchange between the inside of the jar and the outside atmosphare. The jar and its contents were then incubated at 25°C for 6 hours. After the waterlogging 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 longest 25 seedlings (average root length was about 93 mm) were harvested and their roots were isolated. 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 analyzed by gas-liquid chromatography. All means, standard deviation and standard error values were calculated using a pocket scientific calculator. The statistical analysis (Student's T-test) was done and probability value of 0.05 or less was considered to be significant and any value above that was considered to be non-significant.

## 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.

The results presented in Table 1 suggests that fatty acids levels in cotton roots during germination and following waterlogging show changes in the compositions of both the free and esterified fatty acid fractions. The present results indicate that the total amount of esterified and free fatty acids increased with increasing germination time. The increases were presumably due, in part of the synthesis of lipids for new membrane formation. The fatty acid levels in individual fractions also increased during germination. The greater increase of fatty acids in the phospholipid fraction was presumably due to the formation of new membranes. The fresh weight values for the roots used in this experiment confirms this; the fresh weight of the roots increased from 1.8g at 48 hours to 3.3g at 72 hours. Seedling waterlogged for 6 hours showed a marked increase in the amount of total esterified fatty acids. Vartapetian *et al.*, (1978) have reported similar results for rice coleoptiles during anaerobic growth. They found that the rate of increase in total lipids under anaerobiosis was almost twice than in aerobiosis. They further added that this increase was due to increase coleoptile growth. The fresh weight of the roots in the present experiment showed no further increase in growth during waterlogging. This indicates that the increase in total esterified lipids due to waterlogging may be due to synthesis of lipids not associated with extra growth. Misra *et al.*, (1986),

working on Mangrove Avicennia officinalis and Acanthus illicifolius, similarly reported that total lipids were higher in the submerged plants. Waterlogging also caused increase in the levels of fatty acids in the neutral lipid and glycolipid fractions (Table 1). The increase in the levels of the lipids is probably due to increased synthesis of the lipids rather than reduced breakdown, because the fatty acid composition of these fraction also changed Misra *et al.*, (1986) reported a higher proportion of triacylglycerols in the submerged mangrove plants. Vartapetian *et al.*, (1978) similarly found higher amounts in the rice coleoptiles growing under anaerobic conditions. The amount of phospholipids remained unchanged during this period. During recovery from waterlogging, the levels of fatty acids in the neutral lipid and glycolipid fractions decreased, while the levels of phospholipid increased slightly. The increase in phopholipids was very small, however, and they were not significant statistically. A significant increase in free fatty acid was also observed. Increased levels of free fatty acids might cause the disruption of membrane structures and decrease the stability of membranes. Wolf *et al.*, (1982) reported that the formation of free fatty acids by endogenous lipases decreases the stability of membrane structures within plant cells. 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 were found in the phospholipid fraction (Table-2). El-Nockrshy *et al.*, (1974) and St. John and Christiansen, (1976) have reported that the fatty acid composition of polar lipids in cotton seed changed markedly during germination. Increasing germination time was associated with increased accumulation of unsaturated fatty acids, especially linolenic acid, and decreased accumulation of palmitic acid. This is similar to the present observations. It is noteworthy that the changes in the fatty acid compositions of the various lipid fraction and the gross changes in the amounts of these lipids are two processes occurring in parallel.The results for neutral lipids and glycolipids suggest that waterlogging inhibits the synthesis of almost all the characterised fatty acids and greatly enhanced the synthesis of a set of low molecular weight compounds. The largest amount of these compounds was found in the glycolipid fraction.The results for the phospholipid fraction showed no significant changes in the relative proportions of saturated and unsaturated fatty acid and no formation of the low-molecular-weight compounds. Vartapetian *et al.*, (1978) reported a greater proportion of short chain fatty acids (C12:0 and C14:0) in the lipid of coleoptiles from seedlings grown in a media deprived of oxygen. Misra *et al.*, (1986) observed that submerged mangrove plants synthesize proportionately mor short carbon chain hydrocarbons and n-alcohol. They further suggested that this is probably necessary for the leaves to maintain proper flexibility under water. It may therefore be a general response of plant tissues to waterlogging and anaerobic conditions. It should be noted that the hydrocarbons and alcohols reported by Misra *et al.*, (1986) are likely to be located in the cell wall and not in cellular membranes. It is not known whether the appearance of the low molecular weight compounds in the present study is due to their de novo synthesis, but it is hard to imagine how such large accumulations could occur by other means, Young and Anderson, (1974) reported that short-chain fatty acyl-CoA synthetase activity, present in dry ungerminated seeds of Pinus radiata, remained at approximately the same level for 11 days after imbibition. We can expect a similar activity to be present in cotton seeds and be stimulated under waterlogging conditions.In addition to the synthesis of the low molecular weight compounds (fatty acids?), waterlogging also caused the accumulation of a relatively large amount of a high molecular weight compound in the neutral lipid, glycolipid and phospholipid fractions (data not presented). The greatest amount was again found in the glycolipid fraction. In this context, earlier studies by Sheikh *et al.*, (1996) suggested that long-term waterlogging (12 hour or more) damaged the cotton roots. It might be due to high molecular weight compounds(s), together with the unknown low molecular weight compounds, play a role in membrane disruption and finally tissue damage. Alternatively, they may have a protective role. The present experiments are unable to choose between these two possibilities.

Table 1: Effect of Waterlogging on the Esterified and Free Fatty Acids in Cotton Seedlings

| Lipid fraction | Total germ. time (h) | Fatty acid content µg (25 roots)$^{-1}$ |
|---|---|---|
| Neutral lipids | 48h control | 537 ±218 |
| | 72h control | 718 ±144 |
| | After waterlogging | 809 ±257 |
| | After recovery | 677 ±96 |
| Glycolipids | 48h control | 52 ±34 |
| | 72h control | 123 ±16 |
| | After waterlogging | 195 ±80 |
| | After recovery | 144 ±12 |
| Phospholipids | 48h control | 1655 ±320 |
| | 72h control | 2453 ±212 |
| | After waterlogging | 1648 ±80 |
| | After recovery | 1776 ±116 |
| Free fatty acids | 48h control | 25 ±8 |
| | 72h control | 32 ±8 |
| | After waterlogging | 35 ±7 |
| | After recovery | 78 ±23 |
| Combined acyl-Lipids | 48h control | 2240 ±290 |
| | 72h control | 3200 ±180 |
| | After waterlogging | 2650 ±290 |
| | After recovery | 2600 ±260 |
| Total lipids | 48h control | 2270 ±290 |
| | 72h control | 3330 ±180 |
| | After waterlogging | 2690 ±300 |
| | After recovery | 2680 ±280 |

Each value is the mean content ±sd from four determinations.

Table 2: Effect of Waterlogging on the Percentage Fatty Acid Compositions in Cotton Seedlings

| 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 flooding | 15.9±0.7 | 24.8±2.3 | 6.0±0.9 | 12.2±0.5 | 32.4±1.2 | 6.8±1.2 |
| | After recovery | 2.3±0.7 | 26.2±1.6 | 5.9±0.4 | 18.5±0.9 | 36.2±1.0 | 10.9±2.2 |
| 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 flooding | 45.5±6.6 | 19.2±3.2 | 3.7±0.4 | 6.9±0.7 | 11.4±2.4 | 10.7±3.0 |
| | After recovery | 0.7±0.1 | 29.2±2.5 | 5.1±0.8 | 13.3±1.0 | 27.8±8.4 | 24.8±3.2 |
| 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 flooding | 0.8±0.2 | 33.9±0.2 | 3.1±0.2 | 4.3±0.3 | 41.6±0.8 | 15.9±1.4 |
| | After recovery | 0.5±tr | 32.8±1.0 | 3.8±0.9 | 5.3±1.0 | 40.8±0.9 | 15.7±0.9 |
| Free fatty acids | 48h control | 1.8±0.0 | 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 flooding | 2.4±(tr) | 34.5±1.3 | 6.2±1.5 | 11.2±1.1 | 36.8±2.0 | 8.0±1.3 |
| | After recovery | 31.4±5.8 | 22.2±3.0 | 4.6±0.8 | 13.1±6.7 | 19.3±6.6 | 4.7±1.8 |

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

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