ISSN 1023-1072

Pak. J. Agri., Agril. Engg., Vet. Sci., 2010, 26 (2): 1-10

EFFECT OF WATERLOGGING ON FATTY ACID COMPOSITION OF COTTON (*GOSSIPIUM HIRSUTUM* L.) SEEDLING ROOTS

S. A. Sheikh, M. Shahnawaz and S. K. Baloch

Sindh Agriculture University Tandojam, Pakistan.

ABSTRACT

A classical research work was done to evaluate the effect of waterlogging on fatty acid composition of cotton seedling roots. The experimental work was carried out at the School of Biological Sciences, University of Wales, Bangor, U.K. Cottonseeds were delinted and germinated for 48 or 72 hour at 25^{o}C. The longest 25 seedlings were selected and their roots were extracted using the water-saturated butanol. They were purified by Sephadex G-25 and finally fatty acid in ethyl esters were prepared and analyzed by Gas-Liquid chromatography. The results revealed that waterlogging brings changes in both the levels and relative compositions of fatty acid. The research findings suggested that the waterlogging treatment enhances the synthesis of low molecular weight compounds and obscures the synthesis of all the normal fatty acids.

Keyword: Cotton, waterlogging, seedlings, fatty acids.

INTRODUCTION

It is well established fact that membrane lipids and storage lipids of higher plants are altered by environmental factors, such as temperature, water stress, nutritional deprivation and salt-stress (Harwood, 1984). In case of water stress, presence of excess water around seedlings creates an anaerobiosis atmosphere for the roots, which in turn alters biochemical processes within the seedlings. The metabolism of lipids, like other components are adversely and strongly affected when rice coleoptiles are grown under anaerobic conditions (Brown and Beevers, 1987). Philips and Chilton (1996) reported that molecular oxygen is essential for membrane integrity. Biosynthesis of unsaturated fatty acids requires molecular oxygen and is therefore essential for membrane to maintain their structure and functions (Quinn and Chapman, 1980). Brown and Beevers (1987) reported that the synthesis of unsaturated fatty acids is inhibited in rice coleoptiles when grown under anaerobic conditions. In contrast, Vartapetian *et al.* (1978a) found that rice coleoptiles grown under anaerobic conditions had higher amounts of fatty acids. They further added that anaerobiosis induced higher proportion of oleic and linoleic acids than in coleoptiles grown in the air. Kuiper (1984) reported that the

Corresponding author: sashaikhsau@gmail.com

changes in membrane lipid composition may be attributed to stress-induced degradation processes. Enzymes such as lipoxygenase and phospholipase may be involved in these processes.

Very little work has been done on the effect of waterlogging on fatty acid composition. However, more work has been done on the effects of drought on fatty acid composition. Liljenberg and Kates (1982) reported that water stress causes reduction in membrane acyl lipids, as measured by the total fatty acid content in oat seedling roots.

Keeping in view the above facts and the results of our earlier experiments (Sheikh *et al.,* 2002), the present experiment was carried out to determine the effect of waterlogging on the fatty acid composition of cotton seedlings.

MATERIALS AND METHODS

In this experiment, seed of S-12 cultivar obtained from Pakistan Central Cotton Research Institute, Multan was used. Cotton seedlings that had been germinated for 48 hours at 25^{o}C were waterlogged for 6 hours. After waterlogging treatment, they were drained and returned to the incubator at 25^{o}C for recovery up to 72 hours. Control treatment consisted of seeds germinated for 48 or 72 hours at 25^{o}C. At the end of the experiment, the 25 longest seedlings were selected and their roots were isolated. The root lipids were then extracted using hot water–saturated butanol (Colborne, 1974). After extraction, the crude lipid was purified by chromatography on columns of sephadex G-25. Free fatty acid and esterified fatty acid fractions were prepared from the purified lipids fraction and the esterified fatty acids were further separated into neutral lipids, glycolipid and phospholipids fractions. Finally, fatty acid methyl esters were prepared from the various fractions and analyzed by gas-liquid chromatography.

The statistical analysis (Student's T-test) was done using a personal computer with the Systat/Sygraph software (Systat, Inc. Evanston, IL. USA). A probability value of 0.05 or less was considered to be significant and any value above that was considered to be insignificant.

RESULTS AND DISCUSSION

In control (non-waterlogged) roots, the major fatty acids present in all the lipid fractions were palmitic (C16:0), stearic acid (C18:0), oleic acid (C18:0), linoleic acid (C18:2) and linolenic acid (C18:3). The two most abundant fatty acids were palmitic and linoleic acids, together comprising over 60% of the total fatty acids in each lipid fraction, except in the glycolipid fraction at some stages of germination. In the control, seeds germinated for 72 hours, for example, linoleic acid was the major fatty acid. In addition to the major fatty acid methyl esters, a few low molecular weight peaks were found in the chromatograms from some tissues, especially in the neutral lipid and glycolipid fractions. They ran ahead of palmitic acid methyl ester in the chromatograms. The appearance of some of these peaks was spasmodic, however they were therefore not included in the results. On the other hand, some of the peaks appeared frequently. They are therefore included in the results and mentioned as unknowns. When the logarithms of retention time of these unknowns, together with the corresponding values for the methyl esters of 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 and appeared to correspond to lauric acid (C12:0) and myristic acid (C14:0) and were tentatively identified as these fatty acids.

In addition to the low molecular-weight of unknowns, a high molecular weight was observed as a large peak in chromatograms from waterlogged root tissues. Its appearance was regular and occurred approximately 60 minutes later than the linolenic acid methyl ester peak. The peak disappeared again during recovery. It was difficult to quantify as the peak was very broad and such data was not included in these results.

The total level (TL) of all fatty acids (esterified fatty acids plus free fatty acids) in the various fractions are presented in Fig. 1. The total amount of fatty acids at 48 hours germination was 1970 μg (25 roots)$^{-1}$ which increased slightly but non-significantly in 72 hour control to 2110 μg (25 roots)$^{-1}$ (P=0.10). In the seedlings waterlogged for 6 hours there was a marked and significant increase (P<0.001) of 46% to 2870 μg (25 roots)$^{-1}$. During recovery, the level fell again but it remained significantly above the 72 hours control (P=0.012).

A marked increase in neutral lipid (NL) fatty acids was found during germination from 48 to 72 hours. The increase was from 348 to 439 μg (25 roots)$^{-1}$. Because the values had large standard deviations, however, the significance in the change could not be proved (P=0.12).

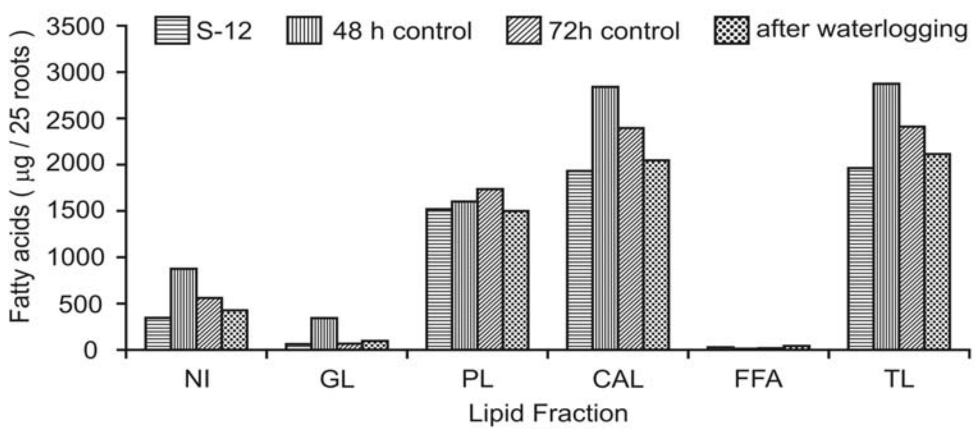

Figure 1. Effect of waterlogging on total fatty acids in various lipid fractions in cultivar after recovery, NL, neutral lipid; GL, glycolipd PL, phospholipids, CAL, combined acyl lipids, FFA, free fatty acids, TL, total lipids.

Seedlings waterlogged for 6 hours showed a significant increase (P<0.001) in this value to 875 μg (25 roots)$^{-1}$. At the end of the recovery period, following waterlogging, the level had declined again to 570 μg (25 roots)$^{-1}$. However, due to large standard deviations the decrease was not significant (P=0.09).

During germination between 48 and 72 hours, the glycolipid (GL) fatty acids increased from 75 to 107 μg (25 roots)$^{-1}$, yet that increase was not significant. The seedlings which were waterlogged for 6 hours showed a significant increase in this fraction. The increase was from 75 to 359 μg (25 roots)$^{-1}$. At the end of the recovery following waterlogging, the glycolipid fatty acids had decreased markedly down to 82 μg (25 roots)$^{-1}$, a value not significantly different from the 72 hour control.

The amount of phospholipids (PL) fatty acids remained unchanged between 48 and 72 hours germination at a value of about 1500 μg (25 roots)$^{-1}$. Seedlings waterlogged for 6 hours showed a small increase of 1610 μg (25 roots)$^{-1}$. At the end of the recovery period, the value had further increased (by about 16%) to 1740 μg (25 roots)$^{-1}$. All of these changes were statistically non-significant.

Fig. 1 shows the data for total confined acyl lipids (CAL) (neutral lipid + glycolipid + phospholipids). The combined fatty acids in these fractions changed under various treatments. The value for 48 hour control was 1940 μg (25 roots)$^{-1}$, and that for 72 hour control was 2050 μg (25 roots)$^{-1}$. The difference between these two control values was not significant. The level following waterlogging increased significantly to 2840 μg (25 roots)$^{-1}$ (P<0.001), however, at recovery period it was reduced again to increase (46%) in the total acyl lipids which decreased in the subsequent recovery period.

In the free fatty acid fraction, the level significantly increased during germination from 27 μg (25 roots)$^{-1}$ at 48 hours to 62 μg (25 roots)$^{-1}$ at 72 hours (P<0.001). Waterlogging for 6 hours did not show any significant effect on the level compared with the 48 hour (control), but it prevented any increase in the level during the recovery period.

The ratios of the individual fatty acids in the neutral lipid fraction are shown in Fig. 2. In roots germinated for 48 hours, the fatty acid composition of this fraction was 24.4% palmitic acid, 8.8% stearic acid, 15.8% oleic acid, 37.7% linolenic acid, 10.3% linolenic acid and 2.9 unknowns. During the next 24 hours of germination there were only limited changes in these proportions. In particular, the proportion of linoleic acid increased to 12.2% at the expense of linoleic acid. The change in linoleic acid was significant (P=0.04). In seedlings waterlogged for 6 hours there was a large increase in the set of unknown low molecular weight compounds (including lauric and myristic acids) from 2.9 to 25.0 % (P<0.001) and similar decreases in the proportions of the known fatty acids. Thus, waterlogging caused significant changes in stearic, oleic and linoleic acids (P<0.02). At the end of the recovery period following waterlogging, the proportion of these compounds had decreased again from 25.0 to 9.2%. The proportions of palmitic, oleic, linoleic and linolenic acids had increased to the level for 72 hours (control).

The results for the glycolipid fraction are shown in Fig. 3. In the roots of seeds germinated for 48 hours, the proportions of the fatty acids were 24.9% palmitic acid, 7.9% stearic acid, 15.1% oleic acid, 23.1% linoleic acid 27.7 % linolenic acid and 1.3% unknowns. During the next 24 hours germination, considerable changes in these proportion of linolenic acid from 27.7% to 37.0% at the expenses of stearic and oleic acids. The increase in the proportion of linolenic acid was not significant, however, due to large standard deviation in the later value. The relative amount of palmitic and linoleic acids remained constant. Seedlings waterlogged for 6 hours showed a very large increase in the unknowns from 1.3 to 63.3% and marked decrease in all other fatty acids (P<0.001).

The greatest decrease was in the proportion of linoleic acid from 27.7 to 6.4%. At this stage a small amount (2.0%) of palmitolic acid (C16:1) was also found (tentatively identified and data not shown). At the end of recovery period, increases in the proportions of all the known fatty acids occurred. The largest increases were in those of palmitic acid from 12.9 to 32.0% and linolenic acid from 6.4 to 35.1% which occurred at the expense of the unknown low molecular weight compounds; the later declined from 63.6 to 1.2%.

The proportions of fatty acids in the phospholipids fraction presented in Fig. 4 did not show any significant changes between 48 and 72 hours. In seedlings waterlogged for 6 hours, there was a large and significant increase (P<0.001) in the set of unknowns at the expense of all the known fatty acids. This increase ranged from 0.6 to 7.3. At the end of the recovery period, the amount of palmitic, oleic, linoleic and linolenic acids increased again at the expense of the unknowns. The percentage of the later felt back to about 0.6%.

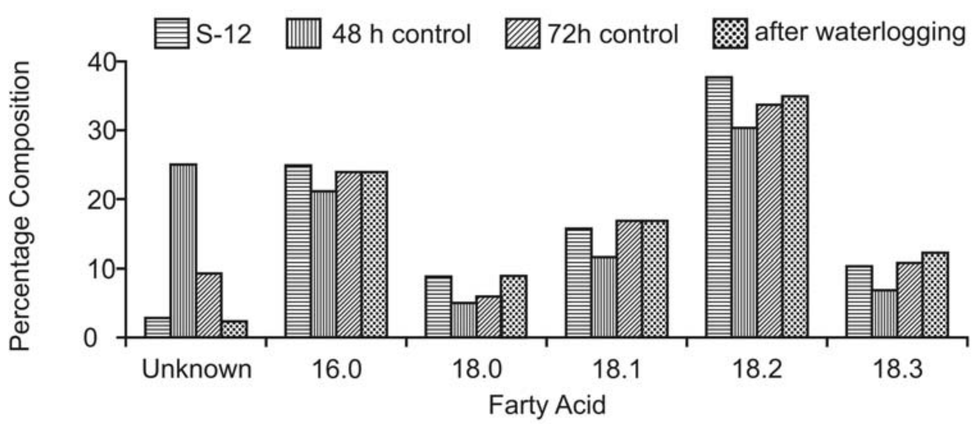

Figure 2. Effect of waterlogging on fatty acid composition in neutral lipid fractions in cultivar after recovery.

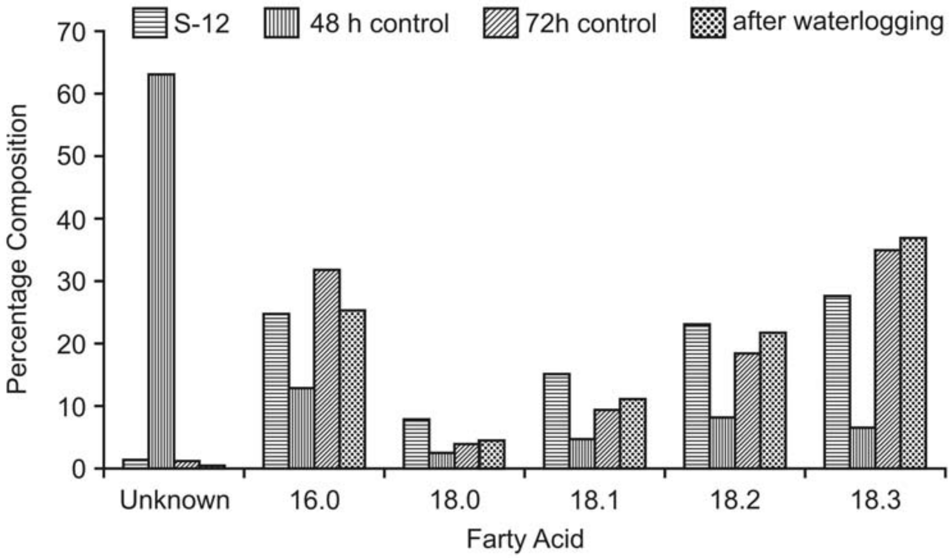

Figure 3. Effect of waterlogging on fatty acid composition in the glycolipid fractions in cultivar after recovery.

In the roots of seeds germinated for 48 hours, the proportions of the free fatty acids (Figure 5) were 36.7% palmitic acid, 13.1% stearic aid, 11.5 oleic acid, 25.2% linoleic acid, 10.7 linolenic and 2.8 unknowns. During the next 24 hours germination, the most significant increases were in the proportions of linoleic acid, from 25.5 to 33.0% and linolenic acid, from 10.7 to 17.8%. These increases were mainly at the expenses of palmitic and stearic acids. These changes were significant statistically ($P<0.03$). Seedlings waterlogged for 6 hours showed sharp increase in the percentages of the unsaturated fatty acids and decrease in palmitic and stearic acids. However, all of these changes were non-significant, ($P>0.12$). At the end of recovery period, insignificant increase was observed in the percentage of oleic acid ($P=0.17$) and there was a further increase in linolenic acid ($P=0.30$) at the expenses of palmitic and linolenic acids ($P>0.08$).

Fatty acids level in cotton roots during germination and following waterlogging showed changes in the compositions of both the free and esterified fatty acid fractions. The present results indicated that the total amount of esterified plus free fatty acids increased with increase in germination time. The increase was presumably due, in part at least, to the synthesis of lipids for new membrane formation. The fatty acid levels in individual fractions also increased during germination with the exception of the phospholipids.

Seedlings waterlogged for 6 hours showed a marked increase in the amount of total esterified fatty acids. Vartapetian *et al.* (1978a) reported similar results for rice coleoptiles during anaerobic growth. They found that the rate of increase in total lipids under anaerobiosis was almost twice that in aerobiosis. They further added that this increase was due to increased coleoptile growth. Similarly, Misra *et al.* (1986), working on mangrove *Avicennia officinalis* and *Acanthus illicifolius,* reported that total lipids were higher in the submerged plants. Waterlogging also caused increases in the levels of neutral lipids and glycolipids.

The greater increases in the levels of these lipids is probably due to increased synthesis of these 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. Similarly, Vartapetian *et al.* (1978a) found higher amounts in the rice coleoptiles growing under anaerobic conditions.

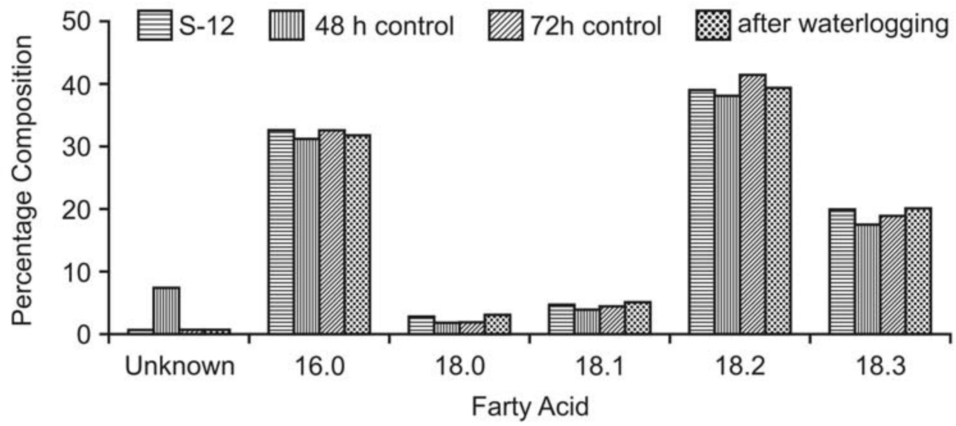

Figure 4 Effect of waterlogging on fatty acid composition in the phospholipid fractions in cultivar after recovery.

The amounts 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 phospholipids increased slightly. These increases in phospholipids were very small, however, and they were not significant statistically.

In earlier results, Sheikh *et al*. (2002) working on cotton seedling roots in cultivar MNH-93 reported that waterlogging increased free fatty acids, which were not found in cultivars S-12. Increased levels of free fatty acids might cause the disruption of membrane structures and decreased the membrane stability. Weiss (1980) reported that the formation of free fatty acids by endogenous lipases decreased the stability of membrane structures within plant cells. In this context, the present results when compared with the earlier results, this would suggest that cultivar S-12 should be more tolerant to waterlogging than cultivar MNH-93.

During germination between 48 and 72 hours at 25^{o}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 phospholipids fraction. John and Christiansen (1976) reported that the fatty acid composition of polar lipids in cotton seeds 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.

It is noteworthy that the changes in the fatty acid compositions of the various lipid fractions 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 characterized fatty acids and greatly enhanced the

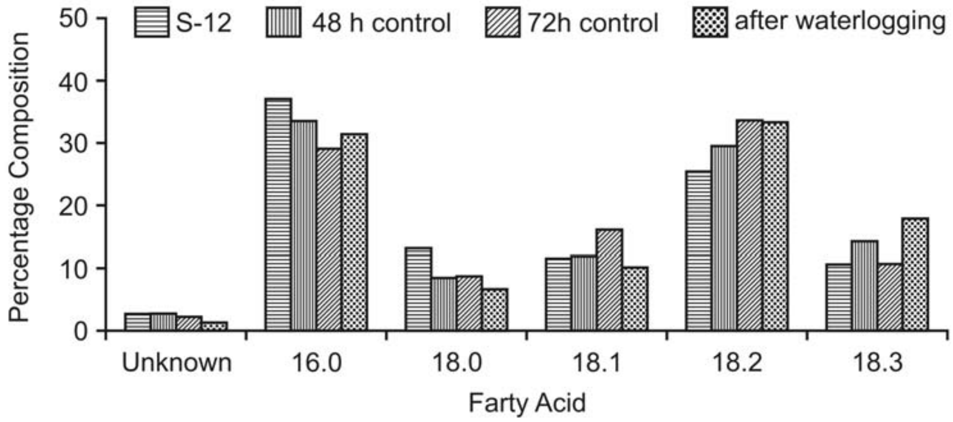

Figure 5. Effect of waterlogging on fatty acid composition in the free fatty acids in cultivar after recovery.

synthesis of a set of low molecular weight compounds. The largest amount of these compounds was found in the glycolipid fraction. Waterlogging stimulated the formation of these low molecular weight compounds in the phospholipid fraction and reduced the proportions of almost all the characterized fatty acids accordingly. Vartapetian *et al.* (1978b) reported a greater proportion of short chain fatty acids (C12:0 and C14:0) in the lipids of coleoptiles from seedlings grown in a media deprived of oxygen. Misra *et al.* (1986) observed that submerged mangrove plants synthesize proportionately more short carbon chain hydrocarbons and n-alcohols. 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.

In addition to the synthesis of the low molecular weight compounds (fatty acids), water logging also caused the accumulation of a relatively large amount of 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, our earlier results suggested that long-term waterlogging (12 hour or more) damaged the cotton roots. It might be that this high molecular weight compound (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.

It is obvious from the present results that, at the end of the recovery period following waterlogging, the amount of combined acyl lipids remained significantly higher than its 72 hour control. The patterns for neutral lipids and phospholipids were similar. Nevertheless, from these results, we might, anticipate that S-12 is a relatively waterlogging-tolerant cultivar because it can maintain higher membrane lipid levels under stress conditions. Similar results were also reported by Sheikh *et al.* (1996) where it was found that S-12 is relatively better cultivar in root growth under short term waterlogging conditions.

REFERENCES

Brown, D. J. and H. Beevers. 1987. Fatty acids of rice coleoptiles in air and anoxia. Plant Physiol., 84: 555-559.

Colborne, A. J. 1974. Development processes in the aleurone cells of the germinating wheat grains, Ph.D Thesis University of Wales, U.K.

Harwood, J. L. 1980. Plant acyl lipids: structure, distribution and analysis. In, Biochemistry of Plants (ed. P.K. Stumpf and E.E. Conn), Vol. 4, pp. 1-55. Acad. Press New York.

John, S.J. B and M.N. Christiansen. 1976. Inhibition of linolenic acid synthesis and modification of chilling resistance in cotton seedlings. Plant Physiol., 57: 257-259.

Kuiper, P. J. C. 1984. Lipid metabolism of higher plants as a factor in environmental adaptation. In, Developments in Plant Biology, structure, function and metabolism of plant lipids. Vol. 9 (ed. P.A. Siegenthaler and W. Eichenberger) pp. 525-530. Elsevier, Amsterdam.

Liljenberg, C. and M. Kates. 1982. An effect of water stress on lipid composition of oat seedling root cell membranes. In: Developments in Plant Biology, Biochemistry and Metabolism of Plant Lipids, Vol. 8, (ed. J.F.G.W. Wintermans and P.J.C. Kuiper), pp. 441-444. Elsevier Biomedial, Press B.V.

Misra, S., A. Choudhury, P. K. Pal and G. Amitabha. 1986. Effect on the leaf lipids of thre sp[ecies of mangrove of periodic submergence in tidal water. Phytochem. 25: 1083-1087.

Phillips, W. D. and T. J. Chilton. 1996. Biology, Revised Edition, Oxford University Press, pp. 30-46.

Quinn, P. J. and D. Chapman. 1980. The dynamics of membrane structure. C.R.C. Critical Rev. Biochem., 8: 1-117.

Sheikh, S. A., D. L. Laidman, R. B. Mirbahar, G. H. Jamro and M. I. Keerio.1996. Effect of waterlogging on cotton seedlings root development. Scientific Sindh, Annual J. Res., 3: 83-92.

Sheikh, S. A., M. I. Keerio, M. A. Rustamani, and M. A. Mirjat. 2002. Effect of water logging on fatty acid composition in cotton seedling roots. Pak. J. App. Sci., 2 (1): 48-50.

Vartapetian, B. B., R. Bazier and C. Costes. 1978a. Lipids in rice seedlings grown under anaerobic and aerobic conditions. In, Plant life in Anaerobic Environ, (ed. D. Hook and R.M.M. Crawford), pp. 539-548. Ann. Arbor, Sci., Michigan.

Vartapetian, B. B., P. Mazlik, and C. Lance. 1978b. Lipids biosynthesis in rice coleoptiles grown in the presence or in the absence of oxygen. Plant Sci. Lett., 13:321-328.

Weiss, S. 1980. Cited membrane lipids in heat injury of spinach chloroplast, Santarins, K.A, 1980.

(Received 12 November, 2008; Revised 03 August, 2011)