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Effects of temperature and waterlogging on cotton seedlings roots

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EFFECTS OF TEMPERATURE AND WATERLOGGING ON COTTON SEEDLING ROOTS

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by

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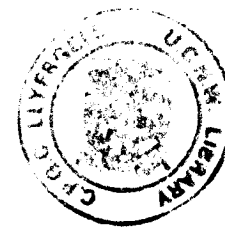

Dedicated to

my Parents, to my Wife Fahmida Saghir

and to my Son Arslan Saghir

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ABSTRACT

Experiments were carried out to study changes in the physiological and biochemical properties of cotton seedling roots with respect to high temperature and waterlogging treatments. It is clear from these studies that both high temperature and waterlogging significantly damaged the seedling roots and affected their physiological and biochemical activities.

Cultivars showed no effect on their root growth for heat stresses up to 30 °C but were adversely affected when stressed above this temperature. Cultivar MNH-93 performed well up to about 38 °C, while Qalandari showed an inhibition at temperatures of 35 °C and above. At a temperature of 45 °C, roots of both cultivars were irreparably damaged. When careful statistical analysis of the data was carried out, however, no significant differences were found between the cultivars studied. Fresh weight and dry weight values were only determined for MNH-93, but they confirmed the findings of the root growth measurements for that cultivar.

Stress temperatures above 40 °C caused a loss of phospholipids. The results of (Me-^{14}C) choline incorporation studies revealed that each of four cultivars showed different rates of incorporation into PC under normal germinating conditions at 25 °C. Longer roots incorporated more (Me-^{14}C) choline than shorter roots. At temperatures of 40 °C and above, (Me-^{14}C) choline incorporation decreased with increasing temperature. At 50 °C, more than 80% of the activity was lost in all the four cultivars studied. In general, no significant differences were found between the cultivars studied.

The results of fatty acid 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 of free fatty acids was found in MNH-93 but not in S-12 at the end of recovery following heat shock. In this respect, the cultivar S-12 appeared to be a relatively better cultivar than MNH-93, since free fatty acids are known to be damaging to cells.

Waterlogging had significant effects on root growth in all the four cultivars studied. Increasing waterlogging time caused greater damage to the root system and waterlogging for 24 hours stopped further root growth completely. In terms of both root growth and visible damage, S-12 was clearly a more tolerant cultivar than MNH-93, Rehmani or Qalandari. Aerated waterlogging caused smaller reductions in root growth in two cultivars studied (Qalandari and S-12). In this treatment, no visible damage to the roots was seen.

Waterlogging also caused large reductions in respiratory activities. During recovery following waterlogging treatments, Qalandari recovered its respiratory activity much faster than the cultivar S-12, however. In this respect, Qalandari appeared to be a more tolerant cultivar than S-12.

Fatty acid analyses following waterlogging showed changes in both their levels and their 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 observed in MNH-93 but not in S-12. This particular set of results suggests that S-12 was a relatively better cultivar than the MNH-93.

When taken altogether, the results suggested that there is very little genetic variability between the cultivars of cotton commonly grown in Pakistan with respect to heat tolerance and waterlogging tolerance.

LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
°C	centigrade
Me-^{14}C	methyl-radiolabelled carbon
PC	phosphatidyl choline
PE	phosphatidyl ethanolamine
PS	phosphatidyl serine
PI	phosphatidyl inositol
PG	phosphatidyl glycerol
PA	phosphatidic acid
LPA	lyso-phosphatidic acid
G.3.P	glycerol-3-phosphate
CDP, CTP	cytosine di-, triphosphate
CCTase	CTP:phosphorylcholine- cytidyltransferase
CGTase	CDP-choline: diacylglycerophosphotransferase
MGDG	monogalactosyldiacylglycerol
DGDG	digalactosyldiacylglycerol
SL	sulphoquinovosyldiacylglycerol (sulpholipid)
O_2	oxygen
CO_2	carbon-di-oxide
Ca^{+2}	calcium ion
Mg^{+2}	magnessium ion
K	potassium
P	phosphorus
N	nitrogen
Zn	zinc
Fe	iron
Mn	manganese
cm	centimeter

mm	millimeter
cv	cultivar
g	gram
mg	milligram
nmol	nanomole
µmol	micromole
mmol	millimole
DNA	deoxy ribonucleic acid
mRNA	messenger ribonucleic acid
rRNA	ribosomal ribonucleic acid
µCi	microcurie
mCi	millicurie
MPa	megapascal
NAD	nicotinamide adenine dinucleotide (oxidized)
NADH	nicotinamide adenine dinucleotide (reduced)
NADP	nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
PS-I	photosystem 1
PS-II	photosystem 2
h	hour
se	standard error
sd	standard deviation
UV	ultraviolet
%	percent

CONTENTS

CHAPTER ONE

Chapter One

General Introduction

This chapter gives a general description of the cotton plant, its production and importance in daily use. The chapter also explains the germination process and the various changes which takes place during germination. A general account of lipids, especially phospholipids and the metabolism of phospholipids, are also explained. The effects of environmental factors on the germination and growth of the cotton plant are then discussed.

1.1 General description of cotton crop

Cotton is one of the most important crops grown commercially in about 60 developing countries between 47°N and 35°S (Bielorai *et al.*, 1983). These countries include Pakistan, India, China, Egypt, Mexico and Peru. The United States of America is also a major producer. According to the United Nations Food and Agriculture Organization's statistics, the world's planted area in cotton is about 33 x 10^6 ha, about 23% of the worldwide cultivated arable land (FAO, 1978). It has been reported that cotton has evolved in several different locations, although its exact origin is unknown. The earliest written records indicate that cotton was known in Mexico as early as 5000 B.C., Pakistan in 3000 B.C., Peru in 2500 B.C. and in India at least by 2000 B.C.

Cotton belongs to the genus *Gossypium* of the Family *Malvaceae*. The modern cultivated cottons fall into two different groups consisting of two diploid (2n=26) "Old world" species (*G. arboreum* L. and *G. herbaceum* L.) and two tetraploid or amphidiploid (2n=52) "New world" species (*G. hirsutum* L. and *G. barbadense* L.). In addition to

the modern cultivated species, the genus also includes 46 wild and lint-less species, that are widely scattered geographically and which can grow at high temperatures (Fryxell *et al.*, 1992). During the twentieth century, however, the New World species *G. hirsutum*, has become the most popular species and it supplies over 85% of the world's supply of raw cotton for factory use. The remaining 15% of production is covered by the remaining three cultivated species, *G.barbadense*, *G. arboreum* and *G. herbaceum*.

Cotton is a warm-season crop growing in subtropical and tropical countries and requiring a temperature range of 20-30°C from germination to harvest (Bhatti, 1975; Abdel Magid and Osman, 1977). It is not grown in regions in which the mean annual temperature is below 16°C (Chapman and Carter, 1976). Cotton is a long-season annual crop requiring about 180-200 frost-free days. It grows to a height of 2-5 feet (60-120 cm) and develops a deep tap root and a widely branching secondary root system. It requires adequate moisture (Arnon, 1972) and continuously warm days with relatively warm nights for optimum growth and development (Gipson and Joham, 1968).

Cotton requires high light intensity during most of the growing season for efficient growth and good economic yields. According to Chapman and Carter (1976), plenty of sunshine is vital for cotton and areas with more than 50% cloudiness are not suitable for the crop.

Cotton requires moderate to fairly large amounts of water. The minimum is about 20 inches (50 cm) of rainfall per year. Heavy rain can injure the young seedlings, however, as well as the fully grown plant. The optimum for economic yield is about 60 inches (150 cm) distributed throughout the season. Irrigation requirements can vary from 24 to 72 inches (60-180 cm). Excess moisture, whether from irrigation or rainfall, delays maturation and interrupts harvesting.

Cotton can be produced on a variety of soils. Deep, friable soils with good humus content and favourable moisture-holding capacity are preferred. Generally, those that are high in organic matter produce higher cotton yields. Loamy soils (clay and sand mixtures) and alluvial soils are ideal for cotton. Very sandy soils can produce poor cotton yields, however. Cotton can be grown over a wide range of soil pH, but the optimum lies between 5.0 and 8.0 (Arnon, 1972; Chapman and Carter, 1976). In Pakistan, the cotton crop is mainly grown in the Indus valley in Sindh and Punjab provinces, which have a continental type of climate characterized by extreme variations of temperature, both seasonally and daily. The summer growing season starts from the month of April and ends in September. In summer the temperature often exceeds 45°C and the diurnal variations may be as much as 20°C. The soils of the Indus valley are mainly alluvial. The average annual rainfall is about 7 inches (180mm), coming mainly in July and August. The type of soil and especially the availability of irrigation water are therefore the main factors which determine the choice of cotton as a crop.

Basically, Pakistan is an agricultural society and cotton plays an important role in the country's economy. The country obtains about one-third of its agricultural export earnings and one-tenth of its total export earnings from cotton. Due to environmental factors such as high temperature and waterlogging, poor quality of seed supplied to the farmers, and poor farming practices, low productivity remains a severe problem.

The parental backgrounds of the cultivars used in this study are as follows:

Qalandari: G. anomalum x (wild species) x M4 (G. hirsutum)

Rehmani: G. hirsutum 21 x McNaire TH-14920

S-12: MNH-93 x 7203-14-4 Arizona

MNH-93: C-158 x MS-39 x MEX12

1.2 Importance of cotton

The cotton plant produces a wide range of products and they can be used in several ways. Although it is mainly grown for its fibre, it also plays an important role in producing edible oil. Several workers have reported that cotton seed production and processing ranks second among the five major oilseed crops, which are soybean, cotton, sunflower, peanut and rape seed (Anon, 1982; Van Waal wijkn van Doorn, 1982).

The seed produces two kinds of hairs. One is long, thick and white or creamy in colour and it is called lint or fibre. The other is very short and attached more strongly to the seed coat; it is called fuzz or linters. The cotton lint, after ginning is graded according to fibre length, strength and fineness. The colour of the fibre and presence of trash also affect grade. Continuous exposure in the field to weathering and the action of microorganisms can cause white cotton to lose its brightness and become darker.

Cotton lint is a raw material for the textile industry. It is stronger than the fibre of other crops (eg. flax, hemp *etc.*) and it has other qualities of primary importance in many textile products. Lint strength, length, smoothness and maturity are the main of these properties. The longer lint is finer and more expensive, while short staple is relatively less expensive. Long staple is mainly used in high quality clothes. Short staple is used for making carpets, rugs, blankets, ropes and other rough material.

After removing the lint, the cotton seeds is further processed to yield about 16% crude oil, 9% linters, 20% hull and 45% meal (Chapman and Carter, 1976). The oil is the most valuable among the above products, accounting for about 50% of the value of the crop. It is mainly used for human consumption. The cottonseed cake or meal is rich in protein and is mainly used as a livestock feed supplement. The linters are a source of cellulose used for making rayons, explosives, film, shatter proof glass, plastics *etc.* (Berger, 1969). The hull is less expensive and is mainly used as roughage in livestock feed.

1.3 Structure of the cotton seed

The structure of the cotton seed is shown in Figure 1.1. It is simple in structure and about 6 X 10 mm in size. It consists of an embryo covered by a seed coat or testa. The embryo consists of two large but very thin cotyledons, an epicotyl or plumule, hypocotyl and a radicle. Structurally, the cotyledons are analogous to leaves. They are attached at the upper end of the short, thick stem-like axis of the embryo called the hypocotyl. Most of the storage materials (mainly lipid and protein) are present in the cotyledons. The plumule is located just above the point at which the cotyledons are attached to the hypocotyl. The primary root of the plant develops from the lower end of the hypocotyl. The embryo is surrounded by a thin endosperm, nuclear fragments and the hard seed coat consisting of more than five cell layers (Christiansen and Moore, 1959). The colour of seed coat is dark brown or black.

Several workers have studied the ultrastructure of the cotyledons and reported that the cells have many clear-cut structural characteristics including nuclei, spherosomes (fat storage bodies) protein bodies (protein storage bodies), globoids (phytin particles

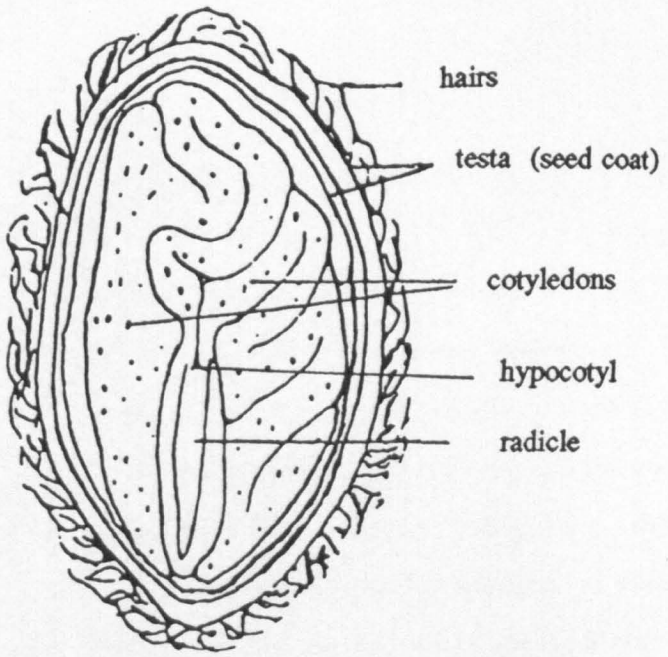


Figure 1.1 Structure of the cotton seed.

within the protein bodies), and cellular cytoplasm in which the nucleus, spherosomes and protein bodies are embedded (Figure 1.2). Gossypol glands containing toxic polyphenols and gossypols can be seen in the cell walls of the seed coat (Yatsu, 1965; Dieckert and Dieckert, 1972, 1976). These cellular structures, mainly those of the cotyledons, are strongly reflected in the chemical composition of the seed.

1.4 Chemical composition of seed

According to their storage materials, the seeds of different species can be divided into two groups.

1. Those with lipids as their main storage material.
2. Those with carbohydrates as their main storage material.

Cotton belongs to the first group. The chemical composition of the cotton seed can vary among cultivars, location and according to environmental factors (Copeland, 1976; Cherry, 1983). The seed is an excellent source of lipids and proteins. Its oil content is about 20%. Brohult and Sandegren (1954) determined the total protein content of *G.herbaceum*, and reported that it was approximately 20-22% of the dry seed weight with 90% of the proteins being globulin and the remaining 10% glutelin. Cherry *et al.* (1981); and Cherry (1983) reported that phytin, a major phosphorus-containing compound, is also present in the cotyledons of cotton seed and is located within the protein bodies. The seeds also contain a small amount of mineral material, which tends to accumulate in the hull and structural tissues. The typical structural and chemical composition of cotton seeds (Cherry *et al.*, 1981; Cherry, 1983) is given in Table 1.1.

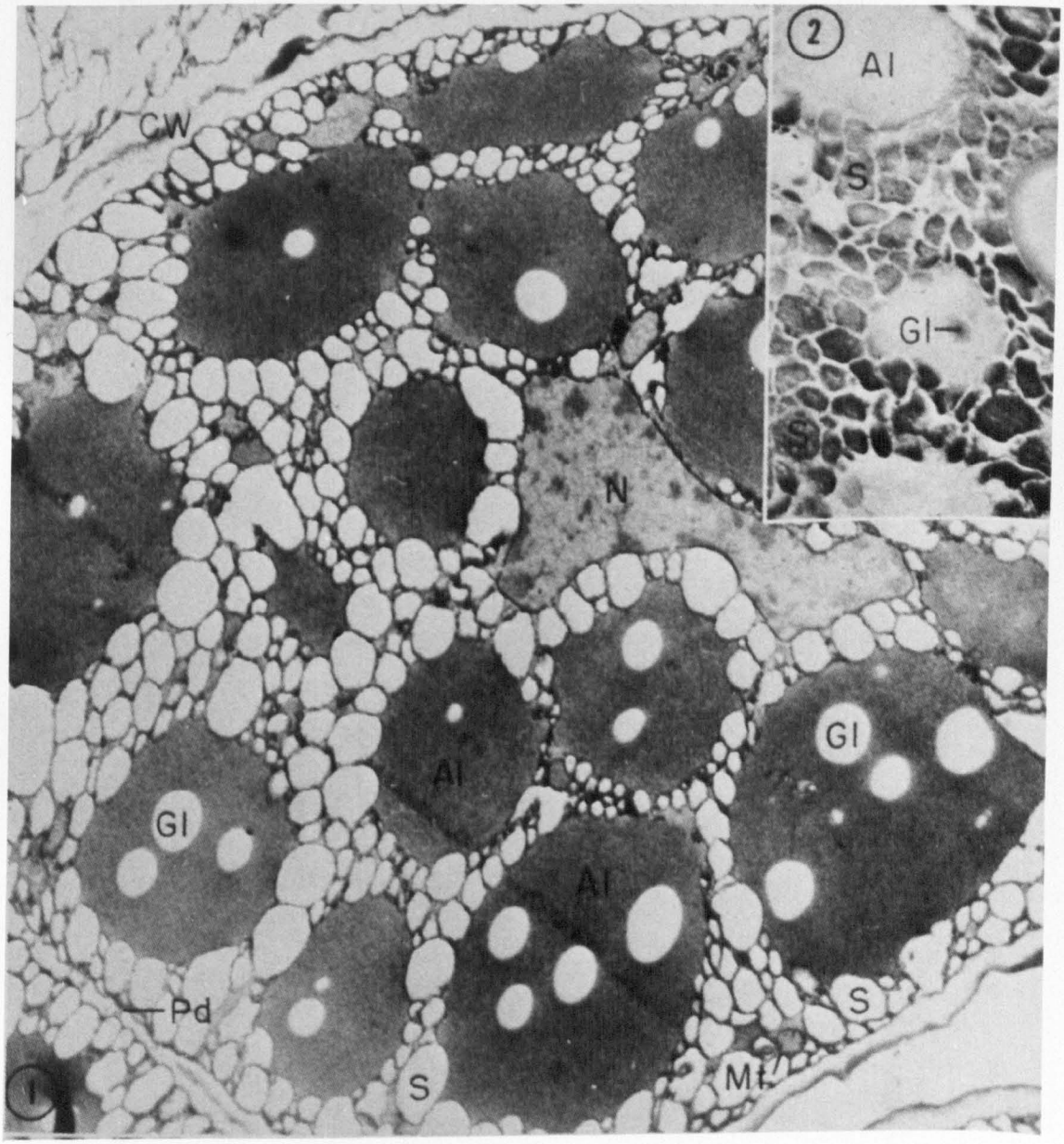


Figure 1.2 Ultrastructure of cotyledonary tissue (From Yatsu, 1965). (1) A typical spongy parenchymal cell from the cotyledon of a dry cotton seed fixed with lithium permanganate, x 7000. (2) osium tetroxide fixation, X 7000. AL, aleurone grain; CW, cell wall;
S, spherosome; GL, globoid; MT, mitochondria; N, nucleus; Pd, plasmodesmata.

Table 1.1 Structural and chemical composition of cotton seeds *G. hirsutum*

Component	Content %
Structural composition:	
Hull	41.6
Kernel	45.4
Lint	14.0
Chemical composition:	
Oil	19.0
Protein	22.3
Free fatty acids	0.2
Free gossypol	1.0
Total gossypol	1.1
Phosphorus	0.9

The hull, kernel and lint values are percentage of whole seed.

The oil, free fatty acid, protein, free gossypol, total gossypol and phosphorus values are percentage of delinted seeds.

1.5 The Germination Process

Under suitable conditions of water availability, oxygen tension and temperature, the seed rapidly absorbs water, increases its metabolic activity and then germinates (Abdel Baki, 1969). The entire metabolic apparatus of the dry seed becomes operative in a sequential fashion. The term germination can be defined as those activities which begin with water uptake and which terminate with the emergence of the radicle or hypocotyl (Bewley and Black, 1978; Roberts, 1988).

The cotton seed has an epigeal germination system in which the cotyledons emerge above the ground. In the first stages of germination, the radicle or primary root develops from the cotton seed and elongates downward (Figure 1.3). It is a tap root system and when the root reaches a length of 20-25 cm, the cotyledons begin to emerge above the ground. Lateral roots start to develop after the emergence and unfolding of the cotyledons (Arnon, 1972). In the later stages of germination, the hypocotyl extends upward pulling the attached cotyledons towards the soil surface. The seedling emerges from the soil 5-12 days after sowing (Bielorai *et al.*, 1983). After opening of the cotyledons, they quickly become green and photosynthesis begins. Plumule growth proceeds and later the exhausted cotyledons wither and fall to the ground in a few days time.

Leffler and Williams (1983) have reported that during the first few hours, the water content of delinted seeds increased sigmoidly. After an initial rapid increase in the rate of uptake, further absorption occurs but at a reduced rate (Krieg and Bartee, 1975). When the water enters the seed, it first reaches the interior of the seed coat. There is a two-fold increase in size of the seed coat, which becomes turgid and the chalazal pore opens. The seed coat then becomes permeable to oxygen and carbon dioxide which are necessary for

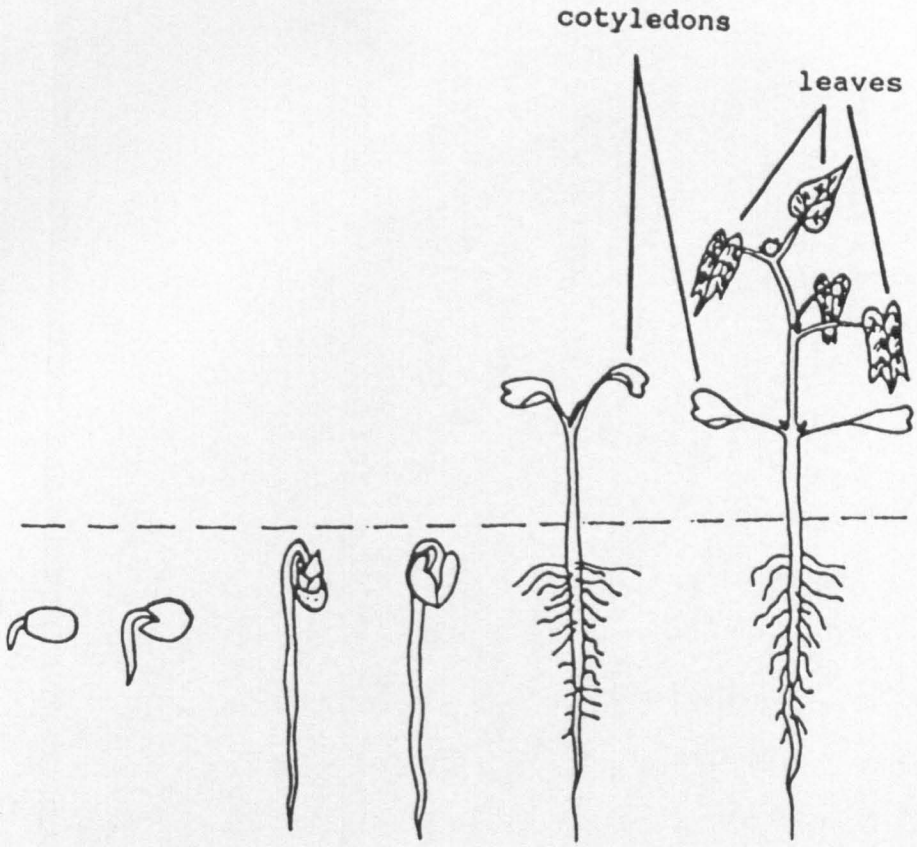


Figure 1.3 Epigeal germination in cotton.

respiration. The further uptake of large amounts of water causes expansion and the rupture of seed coat. Cotton seeds are normally fully hydrated in approximately 4 to 5 hours at 30°C at a soil moisture level of 55% (Benedict, 1984).

The hydration of the seed initiates metabolic activity by activating enzymes, polysome formation, transcription and translation, which are required for growth and cell division.

As mentioned above, the hydration process activates various enzymes available in the seed and which then support metabolism and ATP production (Christiansen and Moore, 1961). The early phase of germinative metabolism is dominated by the mobilization of the lipid reserves. Lipase enzymes are reported to be present in the cotton seed and are activated during germination (Yuldashev and Rakhimov, 1970). Lipase acts on glycerides to produces free fatty acids, although only low amounts of free fatty acids are reported during germination (Christiansen and Moore, 1961; St. Angelo and Altschul, 1964). The free fatty acids are converted into carbohydrate via the glyoxylate pathway. Reserve carbohydrates are broken down by various enzymes (eg. starch by amylase). These carbohydrates serve as substrates for respiration and energy production. Proteinases (endopeptidases, carboxypeptidases and aminopeptidases) are responsible for breaking the peptide bonds of the storage proteins and releasing amino acids (Ryan, 1973). Phytin is hydrolysed by phytase. Rakhimov *et al.* (1986) reported that, under dry conditions, cotton seeds possess low phytase activity, but it increases gradually during germination, reaching a peak on the fifth day when its activity is nearly seven times higher than the original value. Many other enzymes required for germination (eg. enzymes of intermediary metabolism such as glycolysis, tri-carboxylic acid cycle and oxidative phosphorylation) are already

synthesized during embryogenesis (Trelease, 1984). As proteins break down during seed germination, there is a concomitant rise in amino acids in the cotyledons followed by protein synthesis in the growing parts of the embryo (Copeland, 1976). The dry seeds also store pre-existing mRNA. The early protein synthesis following imbibition is fully dependent on this pre-existing messenger. Later events, however, associated with cell division and differentiation, require protein synthesized using newly synthesized mRNA. In cotton seeds, some preformed mRNAs are immature and require polyadenylation during early imbibition before being translated (Copeland, 1976). It has been reported that, in cotton seed cotyledons, glyoxysomes increased 7-fold in volume within 36 hours after imbibition (Kunce *et al.*, 1984) presumably reflecting membrane synthetic activities in the imbibed seeds.

Several important factors which affect the germination process are as follows:

Temperature: Each crop has its own temperature requirement for optimum germination. Ong and Baker (1985) reported that changes in temperature can affect germination and seedling growth. Christiansen and Moore (1961) reported that the mobilization of cotton seed reserves are adversely affected by low (10-15°C) temperatures. Arndt (1945) concluded that each stage of cotton seed germination has its own optimum temperature so that the temperature response may change through the germination period. The overall optimum temperature range is between 20-30°C (Abdel Magid and Osman, 1977; Kreig and Carrol, 1978; Sethar, 1993).

Water: All seeds require sufficient moisture for imbibition and germination. Different seeds have different water requirements for their germination however. For example, in seeds such as beetroot

(*Beta vulgaris*) and spinach, an excess of water can reduce the permeability of the seed coat to oxygen and inhibit germination (Gulliver and Heydecker, 1973). Low soil water potentials also reduce the germination rate of cotton (Jordan, 1982) and, *once* germinated, hypocotyl elongation is reduced more than radical extension (Wanjura and Buxton, 1972). At optimum temperatures, cotton seedlings emerge in about 5 days at -0.03 MPa, 7 days at -0.3 MPa and no emergence was evident after 13 days at -1.0 MPa water potential (Wanjura and Buxton, 1972).

Gases: Oxygen plays an important role in oxidative metabolic processes. Germination in most species may therefore be retarded if the oxygen concentration is reduced substantially below that of air (21%). The influence of carbon dioxide on seed germination is usually the reverse of oxygen and most species fail to germinate if the carbon dioxide partial pressure is increased beyond the atmospheric level (0.03%). Cotton seeds can tolerate a slightly increased concentration of carbon dioxide however (Patterson *et al.*, 1988). A reduction in the level of carbon dioxide does not affect germination.

Seed viability and vigour: Seed quality is one of the major factors that determines the success or failure of germination and crop establishment. All seeds can retain their viability to germinate for considerable periods if they are stored under suitable conditions. Damage during harvesting, processing and handling is a major problem in many seed industries. Injury symptoms such as damage to the seed coat, internal physiological damage (visible only after germination), sensitivity to microorganisms and hidden injuries of a physiological nature all reduce germination vigour, subsequent plant growth rate and ultimately reduce the yield. Kreig and Bartee (1975) and Leffler (1981) reported that good quality seed is therefore one of the first

pre-requisites of cotton production.

Presoaking: Pre-sowing soaking of seeds in water is a way to stimulate germination in several species, especially seeds such as cotton with hard seed coats. Imbibition and metabolic processes begin during the presoaking, resulting in the early availability of energy and metabolic precursors to be utilized for biosynthesis and germination.

1.6 Cell Membranes

Biological membranes contain a diversity of lipids and proteins. The lipids are amphipathic (possessing hydrophobic and hydrophilic residues). A small amount of carbohydrate is also present. The composition of membranes, however, varies from plant to plant, organ to organ and membrane to membrane. Furthermore, their composition may also alter with age or environment (Parsons, 1975). The ratio of lipids to proteins reflects the different functions of the membranes (Finean *et al.*, 1978). For example, myelin membranes in mammals contain 75% of lipids, whereas mitochondrial and chloroplast inner membranes contain more than 70% protein (Houslay and Stanley, 1982).

The protein content of a membrane undoubtedly reflects the metabolic activity of that membrane. There are two types of proteins present in the membranes, which can be classified on the basis of dissociability. One type consists of intrinsic or integral proteins. The other type consists of extrinsic or peripheral proteins. Integral proteins interact extensively with the hydrocarbon chains of the membrane lipids and extend deep into the membrane structure. Peripheral proteins are bound to membranes by electrostatic and hydrogen bond interaction near to the surface of the membrane. For a detail account of membrane proteins, the reader is referred to texts (eg. Metzler, 1977; Finean *et al.*, 1978; Houslay and Stanley,

1982).

Plant lipids: In this thesis we are mainly concerned with membrane lipids. The lipid content of membranes ranges from 25-70% by weight and the nature of the lipid varies with each membrane (Parsons 1975). They are mainly composed of polar lipids with smaller amounts of neutral lipids. The polar lipids are often peculiar to certain types of membranes and as such are specific markers for membranes (Getz, 1970). They include phospholipids and glycolipids. In animal tissues, glycolipids are rarely detectable. In contrast, plant tissues often have high glycolipid levels of which significant proportions are galactolipids (Douce and Joyard, 1980). Two types of galactolipids are mainly present, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). In photosynthetic membranes, these galactolipids comprises more than 70% of the total lipid present (Roughan and Batt, 1969). The presence of galactolipids in other membranes is still in doubt (Douce and Joyard, 1980). Small amounts of sulpholipids (sulphoquinovosyldiacylglycerol) are also present in chloroplasts, especially in the lamellae (Douce *et al.*, 1973).

Phospholipids are the most important lipids in all other biological membranes (Tevini and Lichtenthaler, 1977). All biological membranes are believed to consist of a bilayer of polar lipids, which serve as a matrix into which the various intrinsic and extrinsic proteins are integrated. The phospholipid molecule contains a polar head group and two hydrophobic fatty acyl tails. The fatty acyl chains vary in length (normally from 14-24 carbon atoms) and usually contain one or more cis double bonds (Figure 1.4). Each double bond creates a bend in the tail. The difference in tail length and saturation are important because they influence the fluidity of the membrane.

Among all the phospholipids, the simplest phospholipid is

Lauric acid (C12:0)	$CH_3(CH_2)_{10}.COOH$
Myristic acid (C14:0)	$CH_3(CH_2)_{12}.COOH$
Palmitic acid (C16:0)	$CH_3(CH_2)_{14}.COOH$
Stearic acid (C18:0)	$CH_3(CH_2)_{16}.COOH$
Oleic acid (C18:1)	$CH_3(CH_2)_7\ CH{=}CH(CH_2)_7.COOH$
Linoleic acid (C18:2)	$CH_3(CH_2)_3(CH_2.CH{=}CH)_2(CH_2)_7.COOH$
Linolenic acid (C18:3)	$CH_3(CH_2.CH{=}CH)_3(CH_2)_7.COOH$

Figure 1.4 Structures of the common plant fatty acids

phosphatidic acid (PA) to which other phospholipids are related. This phospholipid serves as a precursor to the phospholipid constituents of membranes as well as to the di- and - triacylglycerols (Mazliak *et al.*, 1982). Phosphatidyl choline (PC) is the most abundant phospholipid in nature. This phospholipid is thought to be present in all the membranes and comprises more than 45% of the total phospholipid fraction of non-photosynthetic membranes of plants, including cotton (Rakhimov *et al.*, 1986), wheat (Vakharia, 1986), spinach (Wintermans, 1963)) and runner beans (Kates, 1960). The second major phospholipid is phosphatidyl ethanolamine (PE). The proportion of this phospholipid can vary from plant to plant. It is present in sugar beet leaves where it comprises 23% of total phospholipids (Wintermans, 1960), but only 1% in barley leaves (Benson and Maruo, 1958). Dry cotton seeds contain about 25% of this phospholipid. The third major phospholipid in cotton seed is phosphatidyl inositol (PI), which comprises about 15% of the total phospholipids (Babaev *et al.*, 1974). Phosphatidyl glycerol (PG) is a major phospholipid in photosynthetic membranes, constituting as much as 25% of the total phospholipids. Other phospholipids are also present in the membranes, but they are in smaller quantities and do not usually comprise more than 3% of the total phospholipids. For example, phosphatidyl serine (PS) is widely distributed in animal tissues, but also occurs in plant tissue in minor quantities (Mazliak, 1980). Lastly, diphosphatidyl glycerol (DPG) has been identified in plant tissues by Benson and Strickland (1960).

Unlike polar lipids, the neutral lipids contain no ionizable groups. These lipids include sterols and their derivatives including the steryl esters of fatty acids. The sterols of the neutral lipid fraction (frequently called phytosterols) can be divided into two

classes, the free sterols and the steryl esters. Sitosterol, stigmasterol and campesterol are the major sterols found in plants with isofucosterol and cholesterol present in minor quantities. The sterols are present in all subcellular membrane fractions (Kemp and Mercer, 1968; Grunwald, 1975; Janiszowska *et al.*, 1979).

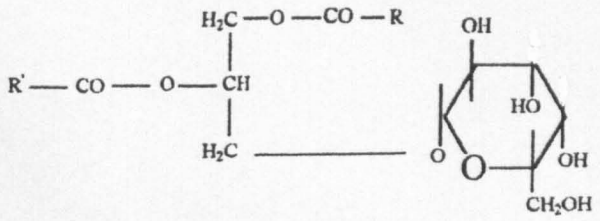
Variation in the relative contents of phosphatides and sterols may change the properties of membranes (Cobon and Haslam, 1973; Feo *et al.*, 1975; Demel and de Kruff, 1976; Grunwald, 1978). Feo *et al.* (1975) reported that sterols affect the permeability of membrane vesicles and liposomes. Cholesterol in particular has an ordering effect upon membranes, increasing the phase-transition temperature and reducing permeability. The importance of this effect in plant membranes is uncertain.

The major class of neutral lipids of plants is the triacylglycerols, however. They are synthesised in very large amounts as energy and carbon storage compounds in seeds and they are the principle components of seed oils (cf. Table 1.1). Smaller amounts of diacylglycerols are also present.

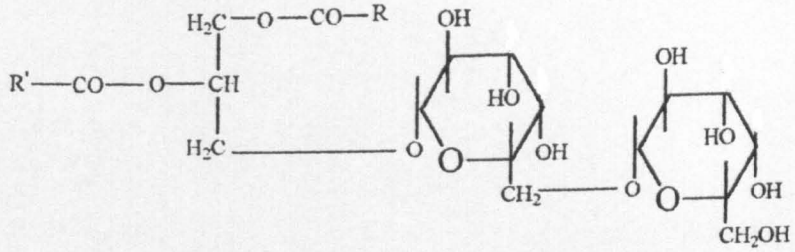
The chemical structures of the major plant lipids are shown in Figures 1.5 to 1.8.

Biosynthesis of phospholipids: The relatively few studies of plant phospholipid biosynthesis have indicated that the processes involved are essentially the same as those which exist in animal tissues (Stumpf, 1975). These studies have been extensively reviewed (Metzler, 1977; Mazliak, 1980; Mudd, 1980). Because PC is the major membrane phospholipid, the present account will mainly concentrate on the synthesis of this lipid.

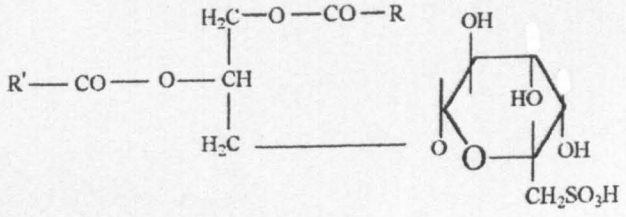
The principal biosynthetic pathways to the phospholipids are shown in Figure 1.9. Lord *et al.* (1973) reported that the endoplasmic



Monogalactosyl diacylglycerol (MGDG)

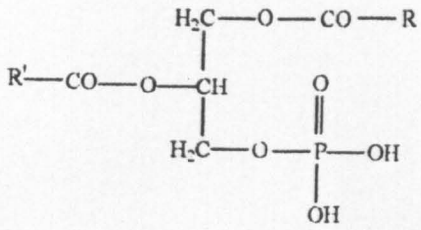


Digalactosyl diacylglycerol (DGDG)

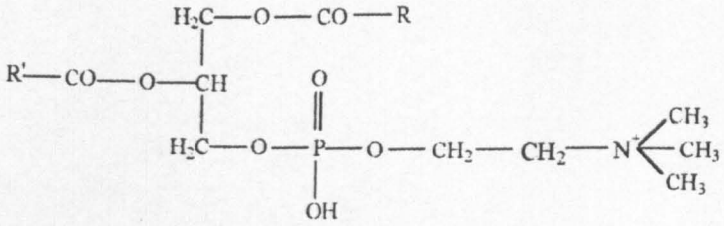


Sulphoquinovosyl diacylglycerol
(sulpholipid)

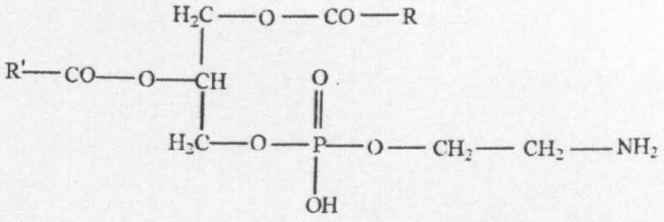
Figure 1.5 Structures of the common glycolipids



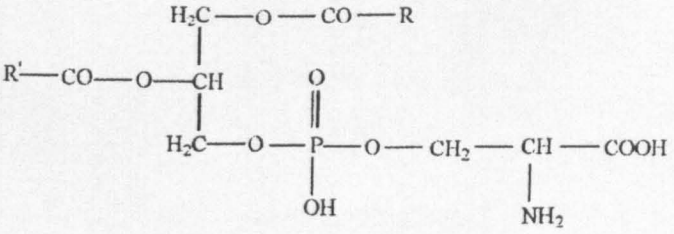
Phosphatidic acid



Phosphatidyl choline

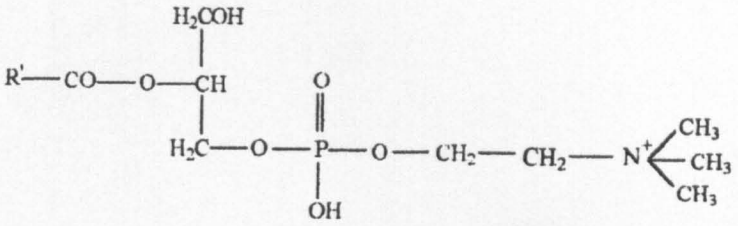


Phosphatidyl ethanolamine

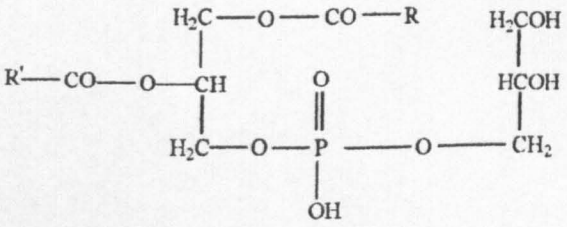


Phosphatidyl serine

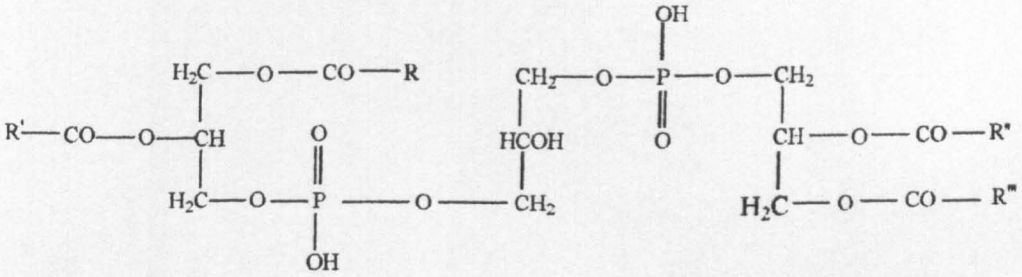
Figure 1.6. Structures of the common phospholipids



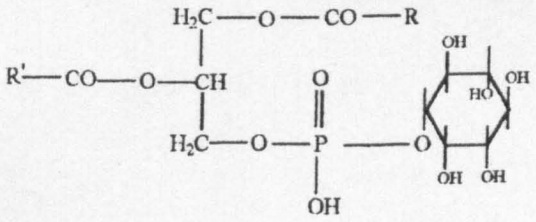
Lysophosphatidyl choline



Phosphatidyl glycerol

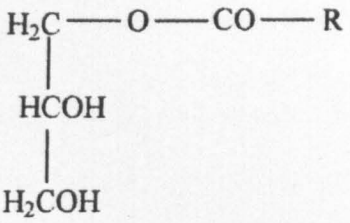


Diphosphatidyl glycerol

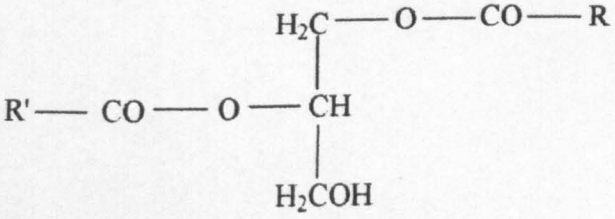


Phosphatidyl inositol

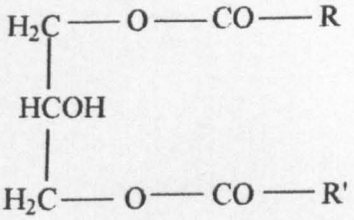
Figure 1.6 (continued)



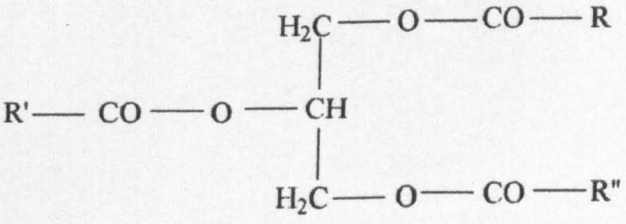
1-Monoacyl glycerol



1,2-Diacyl glycerol

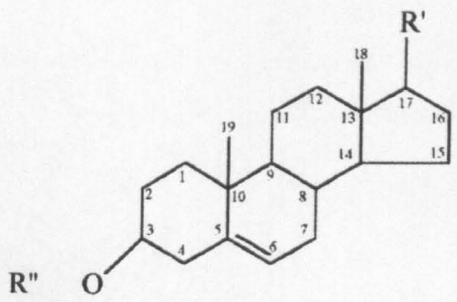


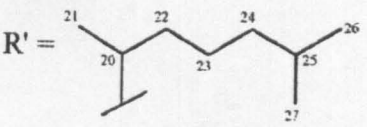
1,3-Diacyl glycerol



Triacyl glycerol

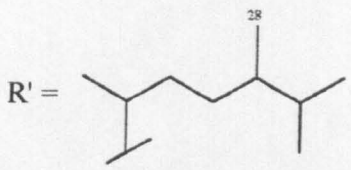
Figure 1.7. Structures of the common neutral lipids.





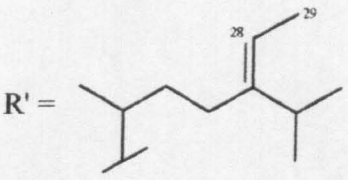
Cholesterol

$C_{27}\ \Delta^5$



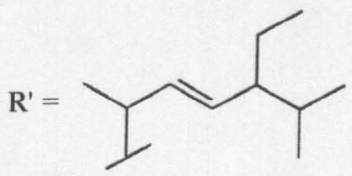
Campesterol

$C_{28}\Delta^5$



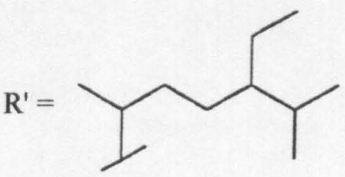
Isofucosterol

$C_{29}\Delta^{5,24(24')}$



Stigmasterol

$C_{29}\Delta^{5,22}$



Sitosterol

$C_{29}\Delta^5$

R" = - H — Free Sterol

R" = - Fatty acid — Sterol ester

R" = - Sugar — Sterol glycoside

R" = - Sugar - Fatty acid — Acylsterol glycosid

Figure 1.8. Structures of the common plant sterols and their derivatives.

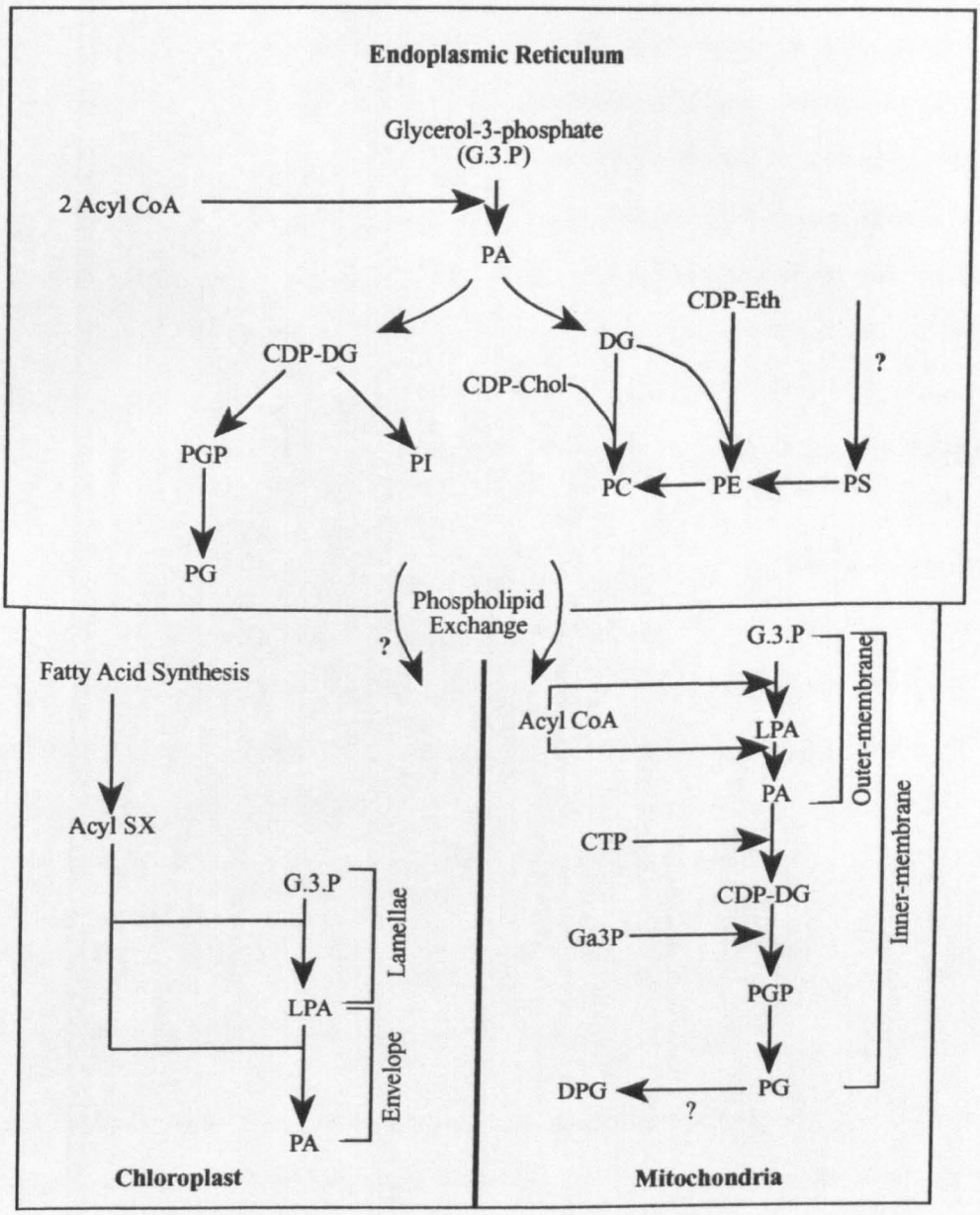


Figure 1.9. Compartmentation of phospholipid synthesis in plants (From Vakharia, 1986). (CoA, Co-enzyme A; Eth, ethanolamine; Chol, Choline; SX, acyl carrier protein; LPA, lyso-PA; DG, diacylglycerol; PGP, phosphatidyl glycerol phosphate).

reticulum is a site of PC formation where it plays a major role in the biogenesis of cell membranes generally. The mitochondria also play an important role in the synthesis of phospholipids. Thus, a major portion of enzyme activity for the pathway from PA to PG and also some synthesis of PC and PE occur in the mitochondria. The mitochondrial enzymes of the PG synthetic pathway are all associated with the inner mitochondrial membrane, as is PC synthesis by methylation of PE (Sparace and Moore, 1979). However, PC and PE biosynthesis by head-group transferase activity occurs in mitochondrial membrane fractions (Sparace and Moore, 1979).

There are three alternative routes in both animal and plant tissue for the synthesis of PC, the nucleotide pathway, the methylation pathway and the base-exchange pathway. Among these three, the nucleotide pathway is quantitatively the most important. It consists of three reactions:-

1. Choline + ATP $\xrightarrow[\text{(choline kinase)}]{}$ phosphoryl choline + ADP

2. Phosphoryl choline + CTP $\xrightarrow[\text{(CCTase)}]{}$ CDP choline + PPi

3. CDP choline + DAG $\xrightarrow[\text{(CGTase)}]{}$ PC + CMP

This pathway was first established in animals by Kennedy and Weiss (1956) and called the Kennedy pathway. The enzymes responsible are reported to be present also in plant tissues (Mudd, 1980; Moore *et al.*, 1983).

The second pathway of PC synthesis is by the methylation of the head group of phosphatidyl ethanolamine. This pathway proceeds by three sequential methylations of the ethanolamine of phosphatidyl ethanolamine. Some evidence indicates the presence of this pathway in *Chlorella* (Sastry and Kates, 1965), tomato root (Willemot and Boll, 1967) and spinach leaf (Marshall and Kates, 1973). Moore (1976)

reported that it accounts for only 4-5% of total PC synthesis in plant tissues however.

The third process, producing PC via a base-exchange reaction between PC and PE, may be present in plant tissues (Marshall and Kates, 1974). The reaction occurs at very slow rates in plant extracts and it is considered unlikely that it has much significance *in vivo*.

Phospholipid Catabolism: The methods by which lipids can be degraded in cells are as follows:

Lipolytic acyl hydrolases can be involved, including phospholipases A_1, A_2, B. They have low specificity and they catalyse the hydrolysis of fatty acyl ester bonds in the phospholipid molecule to release free fatty acids (Figure 1.10). Free fatty acid is required for full activity in crude extracts, probably acting to disrupt the membrane bilayer structures.

Phospholipase C hydrolyses the glycero-phosphate bond of phospholipids (see Figure 1.10). Two enzymes have been identified in plant extracts. The first enzyme, which hydrolyse PC, was identified in spinach chloroplasts by Kates (1955). Recently, a PI phospholipase C (phospho-diesterase) has been identified in celery stem (Irvine *et al.*, 1980). It hydrolases PI to DAG, orthophosphate and inositol.

Phospholipase D is widely distributed in higher plants (Kates, 1960; Quarles and Dawson, 1969) and catalyses the production of PA from PC, PE, PG and PS in decreasing order of activity (Rodionov and Zakharova, 1978). This activity leads to the release of the corresponding head-group moiety, choline, ethanolamine, *etc.* (Kates, 1960).

Lipoxygenase may catalyse the peroxidation of linoleic and linolenic acid to produce hydroperoxide products (Figure 1.11), which

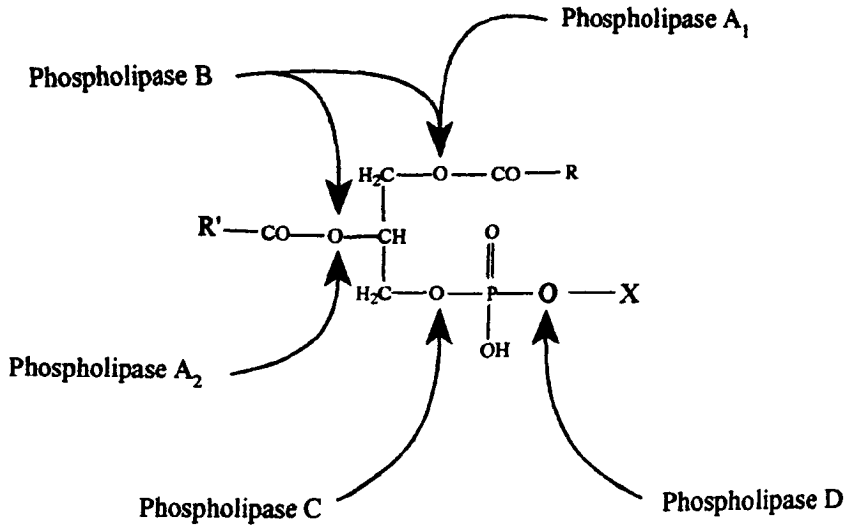


Figure 1.10. Sites of attack of phospholipases on phospholipids.
X, base.

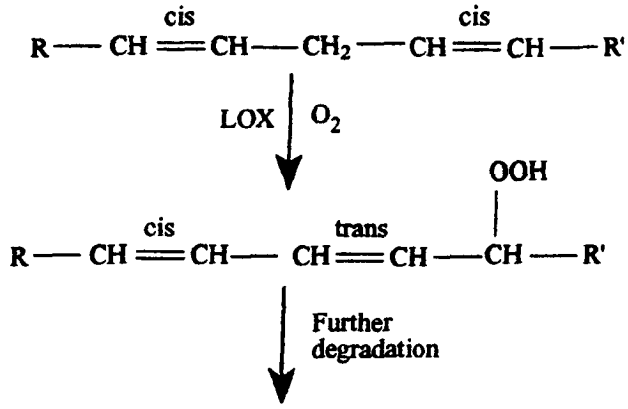


Figure 1.11. Action of Lipoxygenase on unsaturated fatty acids.

can be further metabolised by cleavage enzymes. The hydroperoxide products are highly reactive compounds which are known to damage membrane structure (Tappel, 1973; Mead, 1976) as well as Proteins (Gardner, 1979). The lipoxygenase are most active against free fatty acids, but the fatty acids of glycerolipids and phospholipids can be attacked also (Kuhn *et al.*, 1990).

1.7 Environmental stresses on plants

Environment has a profound effect on the growth and development of plants. Higher plants are subjected to a large number of environmental and biological stresses and these adverse conditions may interfere with their normal growth and development. There are two types of stresses, biotic and abiotic, which the plant may experience during its life cycle. Biotic stress pertains to living organisms, such as aerial or soil-borne pathogenic organisms, insects, pests and other plants for example weeds. Abiotic stresses involve all physical and non-living chemical factors which includes temperature, air pollution, salinity, deficiency or excess of nutrients, high solar irradiation, water stress and anaerobiosis caused by waterlogging. They usually cause damage to crop plants and severely reduce yield and food quality. Therefore, the importance of producing stress-tolerant plants is beyond question.

High temperature: Temperature is a primary environmental factor controlling plant growth and development (Christiansen, 1986; Ehling, 1986). It can determine the rate of seed germination, seedling establishment, time of flowering, fruit formation and growth throughout the life cycle of the plant. There is presently concern that temperature may rise world-wide because of increasing concentrations of carbon dioxide and methane in the atmosphere due to

the excessive burning of fossil fuels. Furthermore, the solar UV radiation reaching the earth may intensify because of the depletion of ozone in the atmosphere caused by man-made fluorocarbon and nitric oxides (Sisson and Caldwall, 1977).

Exposure of plants to high temperature causes a variety of changes in physiological and metabolic processes (Berry and Bjorkman, 1980; Quinn and Williams, 1985). Generally, heat stress in summer crops in tropical and sub-tropical regions appears within the range of 35-42°C, depending upon the species and cultivar. Nightingale (1935) revealed that changes in above-ground temperature can even affect the growth, anatomy and metabolism of roots. At temperatures between 45 and 54°C permanent or lethal injury may occur which can be irreversible even after returning to normal temperatures. Haugen and Smith (1992) reported that a temperature of 38°C caused marked differences in the morphology and growth of the root system of mungbean. In wheat leaf meristems, a temperature of approximately 45°C caused inhibition of mitosis and cell division (Kharlamov and Mousienko, 1992). This finding has been confirmed by Francis and Barlow (1988), Grace (1988) and Bowen (1991), who stated that the rates of cell division and cell extension are especially sensitive to unfavourable temperatures. An increase in temperature to 47°C resulted in a complete cessation of auxin transport from the apical buds in pea, sunflower and cotton plants (Morris, 1979). High temperature also affects other physiological and biochemical processes, such as photosynthesis, respiration, translocation and membrane lipid metabolism. High temperature increases the respiration and disrupts normal cell metabolism including the control of glycolysis and the TCA cycle. The general release of vacuolar enzymes into the cytoplasm due to loss of integrity of the tonoplast membrane may cause an imbalance

in respiration by causing an increased oxidation of phenolic compounds released from the vacuoles into the cytoplasm.

Temperature is one of the most critical environmental factors known to affect membrane structure and function. The degree of fluidity of the cell membrane greatly affects the ability of cells to survive and function. Quinn (1988) reported that changes in growth temperature are associated with changes in membrane lipids. Chloroplast membrane lipids contain high levels of unsaturated fatty acids, which form the proper environment for the attached proteins (Lem *et al.*, 1980). Both MGDG and DGDG are particularly rich in linoleic and linolenic acid which cause thylakoid membranes to be unusually fluid, so that compounds within them are relatively mobile, including the proteins and lipids (Wolf *et al.*, 1982). Slack and Roughan (1978) working on linseed and soybean cotyledons showed that the molar proportion of oleic acid increased and linoleic and linolenic acid decreased as the growth temperature increased. This was confirmed by Wolf *et al.* (1982), who showed that, in soybean, the linoleic and linolenic acid contents decreased markedly whereas the proportion of oleic acid increased as the temperature increased. St. John and Christiansen (1976) studied cotton seed germinated at 15, 20, 25 and 30°C and also showed that, as the temperature increased, the linoleic acid content of the polar lipid fraction decreased.

At normal temperatures, the membranes are permeable to and actively pump specific ions and molecules. As the temperature is increased, the membranes lose their selective permeability and become more permeable to all species of ions and small molecules. Benzioni and Itai (1973) reported that 2 minutes of heat shock at 47.5°C in tobacco leaves increased the leakiness of all membranes.

This was confirmed by Pagano and Arens (1977) who showed that leakage was greater at 30°C than at 10°C. High temperature is also damaging to the membrane structure (Quinn, 1988). Skogqvist (1974) working on wheat seedlings, showed that 2 minutes of heat shock at 45°C caused an expansion of the endoplasmic reticulum cisternae giving rise to small irregular vacuoles. She further reported that mitochondria were also irregular with fewer tubules and adhering membrane curls.

Chloroplasts like mitochondria are energy-producing organelles. The chloroplast is surrounded by a double membrane which controls the molecular traffic into and out of them. Within the chloroplast there is the thylakoid membrane which contains the photosynthetic pigments. There are two main kinds of pigments, chlorophyll (a and b) and the carotenoids (carotenes and xanthophylls). One half of the total lipid content of the thylakoid membrane consists of pigments, while the other half is composed of galactolipids (glycolipids) with smaller amounts of phospholipids. Several workers have studied the effects of high temperature on chloroplasts. Adelusi and Lawanson (1978) found that chloroplast formation in maize and cowpea is retarded when seedlings were heat stressed for 8 hours at 45°C. Gounaris *et al.* (1983 a and b) found that incubation of bean chloroplasts between 35-45°C, caused complete destacking of the grana, but no alteration in the distribution of the membrane- associated particles between the exoplasmic and protoplasmic fracture faces in freeze-fructure studies. Heating above 45°C lead to an extensive separation of membrane lipids into aggregates of cylindrical inverted micelles. They further reported that 5 minutes incubation at 50°C lead to 75% of the chloroplasts containing non-bilayer

structures.

The photosynthetic process is highly temperature sensitive. Chloroplasts held at 40°C for 10 minutes show greatly reduced rates of photosynthesis and (^{14}C) carbon dioxide incorporation into amino acids, organic acids, sugars and organic phosphorus compounds (Chernov *et al.*, 1971). According to Iordanov and Vasil'eva (1976), exposure of spinach leaves for 10 minutes at 45°C strongly inhibited the rate of photosynthesis and 5 minutes at 50°C resulted in complete loss of photosynthetic activity. In the temperature range 5-30°C, photosystem II donates electron to P700 with maximum efficiency (Tikhonov and Ruuge, 1978). At temperatures above 35°C, however, there is a marked reduction in photosystem II activity (Gounaris *et al.*, 1983 a and b), whereas the photosystem I-mediated photo-oxidation of cytochromes did not become inactivated until 48-52°C (Smillie, 1979). Smillie also reported that heat injury to chloroplast thylakoid membranes begins above 35°C and causes abnormal chlorophyll fluorescence and absorbance leading to inactivation of chloroplast membrane activities.

A major response to high temperature is the synthesis of heat-shock proteins which takes place at the expense of the synthesis of normal proteins. This is known as the heat-shock response. The heat-shock phenomenon is a ubiquitous response of all living organisms, including plants, to high-temperature stress (Schlesinger *et al.*, 1980; Lindquest, 1986). Sachs and Ho (1986) indicated that the optimal conditions for heat-shock protein induction in higher plants is a sharp temperature upshift to 39-41°C. It has been shown, however, that the pattern of synthesis of heat-shock proteins may differ between cultivars and between species. It is believed that accumulation of heat-shock proteins is essential for the plants to cope with the

stress conditions experienced and for the induction of thermal tolerance (Schelsinger *et al.*, 1980; Burke *et al.*, 1985; Key *et al.*, 1985). Besides heat shock, many other stress conditions such as waterlogging, high salt concentrations, intense UV light exposure (Sachs and Ho, 1986), and osmotic stress (Czarnecka *et al.*, 1984; Key *et al.*, 1985) can induce the synthesis of at least some heat-shock proteins.

Low temperature: Similarly to high-temperature stress, the effect of suboptimal temperatures may also result in failed or retarded germination, death or injury of seedlings, slow emergence of cotyledons or the appearance of chlorosis and necrosis in young photosynthetic tissues (Obendroff and Hobb, 1970; Van Hasselt, 1972; Cal and Obendroff, 1972; Nishiyama, 1975; Simon *et al.*, 1976). Low or suboptimal temperatures similarly adversely affect membrane lipid composition. Levitt (1980) reported that low temperature causes phase transitions in the membrane lipids, which cause contraction of the membrane. In addition to this, freezing temperatures lead to strong dehydration of the protoplasm. Such dehydration stress may alter the physical properties of the cellular membranes (Pearce and Williams, 1985) and damage thylakoid membrane structure (Hetherington *et al.*, 1988). Low temperature also affects the fatty acid composition of the membranes. In plants grown at low temperature there was an increased desaturation of palmitic acid to hexadecadienoic acid and oleic acid to linoleic acid (Williams *et al.*, 1988). Similar reports have also been made by St. John and Christiansen (1976) for cotton seedlings. They found that, as the temperature decreased from 30 to 15°C, the linolenic acid content of the polar lipid fraction increased. Lyons *et al.* (1964) suggested that the increase in linolenic acid content is associated with increased membrane fluidity.

Waterlogging: The effects of waterlogging on the yield of many crops is often under-estimated (Drew, 1983 and Hocking *et al.*, 1987). Nevertheless, the need to increase production into regions suffering from waterlogging points to an urgent need for the selection and breeding of plants with increased flooding tolerance. Exposure of plants to excess water or water deficits disrupts the biochemical processes occurring in the plants and may lead to damaging effects. Crop plants are especially vulnerable during the seedling stage because of their small size and the soft and delicate nature of their tissues. In the case of cotton, waterlogging during the early growth phase is an important yield-limiting factor in countries like Pakistan, where waterlogging is not uncommon at the time of seedling emergence.

Temporary or continuous waterlogging is common throughout the world, often large areas of land being covered (Ponnamperuma, 1972). Much waterlogging occurs as a result of overflowing of river banks. For example, countries like Pakistan and India are traversed by numerous river systems that overflow their banks, mainly during the monsoon season in July and August. Waterlogging also occurs in other countries including Australia (Beadle, 1962), New Zealand (Holloway, 1962) and the U.S.A. (Briggs, 1973; Broadfoot and Williston, 1973).

The submergence of plants in compacted soils following waterlogging results in poor yields, because higher plant roots have an absolute requirement of oxygen to sustain metabolism and growth. Most plant tissues will tolerate anoxia for only short periods of time before irreversible changes occur (Hook and Crawford, 1978). It is a well-known fact that waterlogging causes injury through oxygen starvation (anaerobiosis), because it fills the air spaces in the soil through which oxygen diffuses. Cotton appears to be very sensitive to

waterlogging (Hodgson, 1982; Hocking *et al.*, 1987; Woodstock *et al.*, 1985). Similar findings have been reported for other species such as barley, wheat and soybean (Wignarajah and Greenway, 1976; Drew, 1983; 1992; Salam and Scott, 1987). A wide variety of toxic compounds accumulate in waterlogged soils (Ponnamperuma, 1972), including ethylene, organic acids and manganese *etc.*, and there is a decline in nitrate in the soil solution (Drew and Sisworo, 1979). Waterlogging always imbalances the plant mineral nutrition (Kozlowski and Pallardy, 1984). It reduces the concentrations of N, K, P, Ca, Mg and increases the concentrations of Mn and Fe in barley (Leyshon and Sheard, 1974), in *Zea mays* (Lal and Taylor, 1970), in wheat (Trought and Drew, 1980 b; c) and in cotton (Hocking *et al.*, 1987).

Even short-term anaerobiosis caused by waterlogging can injure roots and results in a variety of stress symptoms including decreased root and shoot dry weights, leaf epinasty and wilting (Drew, 1983). Williamson (1968) reported that under anaerobic conditions (nitrogen atmosphere) the proportion of root tip cells apparently undergoing mitosis in *Vicia faba* was reduced in 15-30 minutes after the start of anoxia and ceased completely after 24 hours, by which time the root apices were dead. Reduction of the concentration of oxygen from 0.2 to 0.02 atm. reduced root extension rates from 21.3 to 8.8 mm per day (Lopez-Saez *et al.*, 1969). Root extension in soil is slowed down even before dissolved oxygen is totally depleted (Drew, 1992). Thus, waterlogging slows the production of new cells due to low levels of oxygen (Kozlowski and Pallardy, 1984).

Moldau (1973) reported that the waterlogging of soil caused a sharp decrease in stomatal conductance in *Phaselous vulgaris.* Seedlings of *Quercus marocarpa* exposed to waterlogging closed their stomata within a day or two and remained closed for at least 30 days

(Tang and Kozlowski, 1982 a and b). Hiron and Wright (1973) reported that waterlogging of root systems results in rapid accumulation of abscisic acid which also causes stomatal closure, inhibition of shoot growth and early leaf senescence (Milborrow, 1974). Reduction in leaf extension or shoot elongation has been associated with interference in the synthesis and transport of cytokinin (Railton and Reid, 1973) and gibberellins (Reid and Crozier, 1971).

Anaerobiosis caused by waterlogging brings about changes in respiratory metabolism from aerobic to anaerobic pathways (Gupta, 1977a). The mitochondrial cytochrome chain in root cells ceases to function in the absence of molecular oxygen, leading to the accumulation of $NADH_2$ and suppression the Kreb's cycle (Fitter and Hay, 1987). This in turn produces lactate and ethanol and synthesizes only 2 ATPs per hexose molecule metabolised compared to 36 ATPs under aerobic respiration (Roberts *et al.*, 1984 a and b). It has been reported that the end-products produced by anaerobic respiration (such as ethanol) are potentially harmful to plants (Drew, 1992). Vantoi *et al.* (1987) reported that anaerobiosis in maize roots increased the activities of four enzymes, alcohol dehydrogenase, pyruvate decarboxylase, lactate dehydrogenase and the malic enzyme. Mukherjee *et al.* (1986) specified that reduction in energy charge (Saglio *et al.*, 1980) associated with changes in the plasma membrane and/or tonoplast are responsible for gradual loss of ATPase activity.

As mentioned above the root apical meristem is particularly sensitive to oxygen deprvation (Roberts *et al.*, 1984 a and b). The latter workers further reported that, in maize root tips, the ATP concentration drops steeply during the first minutes of anoxia and then stabilizes at a low level. Roberts and co-workers found that, during extended periods of exposure of root tips to anoxia, the

cytoplasm became more acidic, until cell death occurred after 15-20 hours.

Exposure of plants to excess water also caused reduction in the rate of photosynthesis. Photosynthesis in *Pseudotsuga menziesii* seedlings began to decrease within 4-5 hours after flooding (Zaerr, 1983). This caused changes in carbon dioxide fixing activity, reduced chlorophyll content of leaves, early leaf senescence and abscission. Moldau (1973) and Bradford and Hsiao (1982) deduced that inhibition of ribulose bisphosphate carboxylase/oxygenase production is partly responsible for these changes together with stomatal closure. Sojka and Stolzy (1980) demonstrated that reduction in soil oxygen may cause stomatal closure even at optimum soil matric potentials in several species such as cotton, wheat, sunflower, tomatoes and jojoba.

Waterlogging also reduces the rate of translocation of photosynthetic products from sources to sinks (Sij and Swanson, 1973; Zvareva and Bartkov, 1976). Sachs *et al.* (1980) found that anaerobiosis also modifies the synthesis of proteins, with only 10 major proteins and a similar number of less prominent ones being synthesised. The production of these proteins is in someways analogous to the heat-shock proteins, but their roles appear to be different.

Under normal conditions, oxygen plays an essential role in the biosynthesis of unsaturated fatty acids. As mentioned above, waterlogging causes elimination of oxygen and affects membrane structure and functions. The creation of double bonds by desaturase activity depends on oxygen together with a reductant (NADH or NADPH) and ferredoxin or some other electron carrier (Hitchcock and Nichols, 1971; Stumpf, 1980). As a result of waterlogging, therefore, changes in membrane lipid composition (eg. degree of saturating of fatty acid

residues, chain length and presence of sterols) can be expected to modify membrane fluidity and transport processes. Degeneration of mitochondria under anoxia, extracted from yeast cells is closely associated with interference in the synthesis of unsaturated fatty acids, leading to increased permeability of the inner mitochondrial membrane and uncoupling of ADP phosphorylation from respiration.

Little or no research has been done, however, on the effect of waterlogging on membrane lipid composition. The results reported by different workers on anoxia are complex and puzzling. Vartapetian *et al.* (1978b) reported that the existence of anoxia-tolerant and anoxia-intolerant plants suggests that difference between them might originate either in the synthesis of membrane lipids or avoidance of their degradation. They further reported that in anoxia-tolerant coleoptiles of rice germinating anaerobically, the lipid composition, including the unsaturated fatty acids, was similar to that in control tissue. Labelling studies with (^{14}C) acetate revealed that *de novo* synthesis of unsaturated fatty acids occurs under anoxia (Vartapetian *et al.*, 1978b). The authors concluded that these puzzling results suggest either the existence of an unusual pathway for lipid synthesis or transport of lipids from embryo without being converted to carbohydrate via the glyoxylate and gluconeogenesis pathways.

Contradictory results were also obtained by Hetherington *et al.* (1982) working on rhizomes of *Iris pseudacorus* (anoxia-tolerant) and *Iris germanica* (anoxia-intolerant). The lipid composition of the two species was similar in aerated controls. When rhizomes were subjected to 14 days of anaerobic treatment, there was a marked decline in the content of the saturated fatty acids (palmitate and stearate) but not, as might have been expected, the unsaturated ones. In contrast, results reported by Rebeille *et al.* (1980) working on anoxia-

intolerant *Acer pseudoplatanus* cells in suspension culture, showed that the synthesis of polyunsaturated fatty acids was progressively suppressed when the oxygen partial pressure was at or below 0.03 atmospheres. Chirkova *et al.* (1981) found a comparable reduction in total unsaturation in the phospholipids extracted from homogenates or mitochondria of wheat and rice roots which had been anoxic for several days.

Drought: Water deficit or drought has profound effects on growth, yield and plant quality. The first effect of this stress is a loss of turgor that affects the rate of cell expansion and ultimately cell size. Loss of turgor is probably the process most sensitive to drought. This in turn decreases the growth, stem elongation, leaf expansion and stomatal aperture opening. Higher plants subjected to low water stress close their stomata in order to prevent water loss. This in turn slows down carbon dioxide uptake which affects not only photosynthesis but sooner or later primary metabolism in general (Spear *et al.*, 1988). Plants also lose their membrane integrity and become leaky due to low water stress (Gupta, 1977b). Pham-Thi and Vieira-da-Silva (1975 and 1980) reported that drought provokes a disorganization of cell metabolism and damages the membrane structure in the drought-sensitive species. Water stress affects the lipid composition of cotton leaves, decreasing total fatty acids mainly due to a decrease in linolenic acid (Pham-Thi *et al.*, 1982).

Salt stress: Plant growth is also affected by salinity or salt stress. Salt stress consists of two components. The first is the increased external osmotic pressure due to the high salt concentration. The second component involves the physiological and metabolic effects of the salt in the plant cell. Among the metabolic disturbances, the entry of sodium into the cytoplasm is the most important primary event

affecting the cell (Katsuhara and Tazawa, 1986). Water potential, osmotic potential and turgor pressure are quickly perturbed in many species, including maize and sorghum (Erdie and Taleisnik, 1992). Later effects involve enzyme inhibition (Katsuhara and Tazawa, 1986), changes in plasma membrane composition including phospholipid:protein ratios, sterol:protein ratios, and ATPase activity (Zwiazek *et al.*, 1992). Changes in amino acid and mineral ion partitioning between compartments within the cell also occurs (Stassart, 1992).

Muller and Santarius (1978) and Harwood (1984) reported that salinity caused a reduction in total chloroplast lipids accompanied by a decrease in the degree of fatty acid unsaturation. Zarrouk and Cherif (1984) specified that increases in the concentration of sodium chloride greatly affect the fatty acid composition. Other studies revealed that salt stress in sunflower, *Plantago L.* (Ribwort plantain) and olive induces decreased levels of all lipid classes in a total lipid extract (Bettaieb *et al.*, 1980; Erdei *et al.*, 1980; Zarrouk and Cherif, 1981).

Other stresses: In recent years, the harmful effects of atmospheric pollution have been widely publicised. Ozone is one of the gases which may accumulate through the action of ultra-violet radiation and the gases released into the atmosphere from motor exhausts. In plants, the appearance of water-soaked spots on the leaves is one of the recognizable symptoms (Rich, 1964). Later, the leaves become bleached and necrotic (Simon, 1974). Ozone also causes the breakdown of the plasma membrane (Thomson *et al.*, 1966). It has been suggested that it acts directly upon membranes by reacting with the double bonds of unsaturated fatty acids.

1.8 Strategic basis for the present study

It is quite obvious from the brief summary above that crops are very susceptible to several environmental stresses both singly and in combination. It is also very clear that much work remains to be done, especially with respect to the production of stress-tolerant varieties. There is evidence from agronomists and plant breeders that cotton suffers significant damage due to high temperature and waterlogging. The cotton crop is believed to be very susceptible, especially during early growth, flowering and boll formation. Pakistani scientists have produced very little evidence to support these statements, however, possibly due to the lack of research training and facilities. Yet, high temperature and waterlogging are not uncommon in Pakistan during the early development of the cotton plant. These stresses must cause severe damage to early seedling development as well as to the young plants. Because of these problems, the present study was undertaken to measure some basic but important physiological and biochemical parameters in the growth and development of cotton seedlings and to determine the effects of high-temperature stress and waterlogging on their activities.

It has been reported that cellular membranes are the primary site of high temperature injury in plants (Raison *et al.*, 1980). Membrane lipids undergo phase-transition (abrupt reduction in viscosity) at supraoptimal temperature which affects, for example, membrane transport process, photosystem II activity in chloroplast and electron transport systems in chloroplast and mitochondria. Any damage to membrane may also affect the activities of membrane -bound enzymes. The enzymes responsible for membrane lipid synthesis may be on such system affected by high temperatures. It has been reported that high temperature also damages cells via thermal denaturation of proteins

and nucleotides (Pace and Campbell, 1967). Hydrogen bonding becomes weakened (Hochachka and Somero, 1973) and is considered to be responsible for alterations in protein stability at high temperature (Langridge, 1963).

Because of the lack of general research on cotton crop, especially on environmental stress and membrane lipid metabolism, the present research was carried out to answer the following questions:-

1. Does heat shock affect the development and survival of seedling roots?
2. Does heat shock influence the membrane phospholipids in seedling roots?
3. Does heat shock affect the uptake and incorporation of (Me-^{14}C) choline in phosphatidyl choline?
4. Does heat shock affect the fatty acid composition in neutral lipids, glycolipids and phospholipids?
5. Does anaerobic or aerobic waterlogging influence the development and survival of seedling roots?
6. Does waterlogging affect the respiration rates of the seedling roots?
7. Does waterlogging influence the fatty acid composition in neutral lipids, glycolipids and phospholipids?
8. Are there any differences between cultivars or between seedlings of high and low vigour with respect to the above parameters.

CHAPTER TWO

Chapter Two

Material and Methods

2.1 Plant material

Cotton (*G. hirsutum* L.) varieties S-12 and MNH-93 were supplied by the Pakistan Central Cotton Committee's Research Institute at Multan (Punjab), Pakistan. Cultivars Qalandari and Rehmani were supplied by The Cotton Research Institute, Sakrand (Sind), Pakistan. The seeds used in these experiments were from the 1989 to 1992 harvests. The detail for the seeds used in each experiment is given in the individual results chapters.

2.2 Glassware

All glassware was cleaned by soaking overnight in a phosphate-free detergent (Lipsol liquid, LIP Equipment and Servicing Ltd., Shipley, U.K.). After scrubbing with a brush, the glassware was washed with distilled water and placed for one hour in dilute hydrochloric acid (to remove remaining detergent). It was finally washed three times with distilled water and dried in an oven at 110°C.

Glassware contaminated with radioactive material was pre-soaked in Decon detergent (Decon Laboratories Ltd. Hove, U.K.) for 4-5 hours followed by above cleaning procedure.

2.3 Reagents

Unless otherwise stated, reagents were obtained from BDH Ltd. Poole, Dorset. Radiolabelled compounds were supplied by The Radiochemical Centre, Amersham, Bucks, U.K. Sephadex G-25 (coarse) was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweeden. Fatty acid standards were from Sigma Chemical Company, London, U.K.

The Sep-Pak chromatography cartridges for lipid fractionation were obtained from Waters Chromatography Division, Millipore Corporation Milford, Massachusetts, U.S.A.

Buffers were prepared according to standard procedures (Dawson *et al.*, 1969) and their pH was checked before use using a laboratory pH meter.

2.4 Solvents

All solvents used in these experiments were AnalaR grade, except butanol which was laboratory reagent grade. This solvent was re-distilled prior to use.

2.5 Seed treatment

Cotton seeds were delinted by the following procedure. The seeds (20g) were placed in a 200 cm^3 beaker and approximately 4 cm^3 of concentrated sulphuric acid were added (1cm^3 per 5g seeds). The seeds and acids were stirred thoroughly with a glass-rod for 3 minutes and left for 5 minutes. They were then stirred for 2 minutes and transferred to a Buchner funnel where they were washed with running tap water for 3 minutes. The seeds were then placed in 200 cm^3 1% sodium bicarbonate solution for 10 minutes to neutralise remaining acid and washed thoroughly with distilled water. The delinted seeds were soaked for an hour in 1 litre of distilled water. After this time, the floaters were discarded and the sinkers were placed in a 1.5% sodium hypochlorite solution (0.1% available chlorine) for 20 minutes to further sterilize them. Finally, the seeds were washed eight times with sterile distilled water to remove the hypochlorite.

2.6 Germination and selection of quartiles

Fifty delinted and sterilized seeds were placed in 10 rows with 5 seeds in each row, on a sheet of Whatman No. 1 filter paper (23 x 57 cm). The filter paper was moistened with 40 cm^3 sterile distilled water. The filter paper was then rolled up round a wooden stick (32 x 0.5 cm) and placed in a polythene bag (35 x 23 cm). The top of the bag was folded over and tied lightly to maintain sterile conditions and allow air exchange. Finally, the bag and its contents were stood upright in a 500 cm^3 beaker and incubated in an incubator in the dark at 25°C. After 24 and 48 hours, the bag and its contents were rolled to re-moisten the seeds with water that had collected at the bottom of the bag. For each analysis, 100 seeds were germinated in this way in two filter paper rolls.

After 48, 54, 60, 66 and 72 hour germination periods, the seeds were harvested and the best 75 seedlings were selected. The 75 seedlings were divided into three quartiles according to their root lengths as follows:

First quartile: the longest 25 roots (93mm ±12 at 72h).

Second quartile: the medium 25 roots (72mm ±9 at 72h).

Third quartile: the shortest 25 roots (52mm ±6 at 72h).

The root length values are for the cultivar MNH-93. Other cultivars showed a similarly wide range of values. The fourth quartile was of very poor quality and it was discarded.

Roots were detached from the harvested seedlings with a scalpel and the fresh weight of each quartile was determined prior to lipid analysis. Dry weight values were determined from separate experiments in which roots samples were dried at 70°C for 48 hours.

2.7 Heat-shock treatment

Two kinds of heat-shock treatment were given to seedlings. For the determination of root length , seedling samples were heat-shocked at 25, 30, 35, 37.5, 40, 42.5 and 45°C. This was done by transferring batches of seedlings that had germinated for 48 hours and still in their rolled paper and plastic bags from the 25°C incubator to an air incubator set at the heat-shock temperature. After 3 hours at this temperature the seeds were returned to the incubator at 25°C for further germination up to 72 hours. For the fresh weight, dry weight, phospholipid and fatty acid determinations, seedling samples were heat-shocked at 35, 40, 42.5, 45 and 50°C. This was done by transferring batches of seedlings that had germinated for 48 hours and still in their rolled paper and plastic bags from the 25°C incubator to a water bath set at the heat-shock temperature. Care was taken to avoid water entering the plastic bags from the bath. After 2 hours at this temperature, the seeds were returned to the incubator at 25°C for a further germination up to 72 hours. At the end of this time the seedlings were harvested for analysis.

2.8 Waterlogging treatment

Cotton seeds were germinated in rolled filter papers for 48 hours as described in section 2.6. The rolled filter papers along with their contents were taken out from the plastic bags and placed in glass jars (30 cm high x 8 cm diameter) filled with water up to 26 cm. This was enough to completely cover the rolled paper and its seedlings. All these treatments were carried out 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 atmosphere. The jar and its contents

were then incubated at 25°C for 6, 12 or 24 hours. The oxygen concentration of the water in the jar was monitored before and after each waterlogging treatments. After this waterlogging treatment, the paper roll containing the seedlings was drained and returned to the plastic bag and incubated at 25°C for a period of recovery. The recovery period was adjusted so that the total experimental period was 72, 96 or 120 hours for each waterlogging treatment. At the end of these times the roots were harvested and measurements of root length, fresh weight and dry weight were made. For the dry weight, the roots were harvested and dried in an oven for 48 hours at 70°C. In separate experiments the tips of the roots were harvested for measurement of their respiration rates.

Seedlings were also given waterlogging treatments in aerated water. The rolled filter paper along with its contents was taken from its plastic bag and placed in to a tank (44 cm height x 25 cm length x 22 cm wide) filled with water up to 40 cm. This was enough to cover the roll and its contents. The tank was maintained at 25°C. It also contained a stirrer which circulated the water to help maintain oxygen levels in the water. The water was aerated by bubbling fresh air through it from the laboratory compressed air line. The oxygen concentration was monitored at 3, 6, 12 and 24 hours and maintained at 100% ± 5%. At these times, the paper roll containing the seedlings was drained, returned to its plastic bag and incubated at 25°C for a period of recovery. The recovery period was adjusted so that the total experimental period was 72, 96 or 120 hours for each waterlogging treatment.

The oxygen concentration of the water in the waterlogging treatments was measured using an oxygen electrode (Model PHOX62, (PHOX System Ltd., Bedfordshire, U.K.). The instrument was calibrated

with distilled water saturated with 100% oxygen at 25°C. The sensor of the oxygen meter was immersed in the water and rotated to ensure adequate water flow past the sensor membrane. The oxygen concentration was measured in units of mgl^{-1}.

2.9 Measurement of oxygen uptake by root tips

The respiration rate of the root tip segments was measured with a Clark-type oxygen electrode connected to a chart recorder (Kipp & Zonen, Model DB-111, Talbot Scientific Ltd., Cheshire, U.K.). The water jacket of the electrode was connected to a temperature controlled water bath to maintain it at 25°C. Five approximately 5mm long tips were used for each measurement. Two cm^3 buffer (20mM MES-KOH buffer, pH 6.5 + 2mM $CaSO_4$) were placed in the sample compartment of the electrode and the tissue was added. The sample compartment was then closed and oxygen uptake was measured over a 20 minute period. The results are presented in units of nmoles min^{-1} $5tips^{-1}$.

2.10 Radiolabelling of roots

The uptake and incorporation of radio-labelled (Me-^{14}C) choline by cotton roots in the absence of saturating concentrations of unlabelled carrier choline was measured according to the method of Varty (1975). Roots from germinated seedlings were washed, damp dried and placed in a sterile 100 cm^3 conical flask containing 10 cm^3 of sterile labelling medium. The flask was then plugged with sterile cotton wool. The labelling medium, consisted of 50mM Tris-maleate buffer, 50µg cm^{-3} chloramphenicol and 1µCi (Me-^{14}C) choline (specific activity 55mCi $mmol^{-1}$). The pH of the medium and period of incubation were varied according to the experimental design. The flasks and their contents were incubated at 25, 30, 35, 40, 45 and 50°C in a

reciprocating water bath operating at 175 oscillation per minute. At the end of the radiolabelling period, the medium was removed from the roots by vacuum filtration through a sintered-glass funnel. The roots were then washed three times with distilled water and re-incubated for 5 minutes at room temperature in 100cm^3, 10mM unlabelled choline chloride to remove radiolabelled material from the tissue cell walls. At the end of this incubation, the roots were washed several times with distilled water. The roots were then extracted with boiling water-saturated butanol (WSB).

2.11 Extraction of lipids

In this investigation it was important to follow an efficient and reproducible procedure for the extraction of lipids from the tissues. A suitable lipid extraction procedure must ensure that lipoprotein bonds are broken and it must minimize degradative enzyme activity during extraction. This latter factor is particularly important in plants, which may contain phospholipases and other phosphatases that are activated by organic solvents (Kates, 1970; Colborne, 1974).

Hot water-saturated butanol (WSB) has been reported to be the most thorough lipid extraction solvent for wheat products (MacMurray and Morrison 1970) and its use has been advocated for vegetative plant tissues by Colborne and Laidman (1975). In the light of this work, the hot WSB extraction procedure was adopted for the present experiments. The tissue was thus plunged into 150 cm^3 boiling WSB in a 300 cm^3 tall-form beaker and boiled for 3-5 minutes to inactivate enzymes (Colborne, 1974). The tissue was then cut into small segments (<1cm) and homogenised for 5 minutes using a Polytron top drive

homogeniser at speed mark 3. After the particulate material had settled, the supernatant was filtered through 2 layers of Whatman GF/A glass microfibre filters placed in a sintered-glass filter funnel. The residue was further extracted twice with WSB and once with a mixture of chloroform and methanol (1:1, v/v). The final extraction prevents bumping when the combined filtrate is reduced to dryness in a rotary evaporator at <50°C. The residue from the rotary evaporation was dissolved in 30 cm^3 organic phase (Section 2.12) followed by 10 drops of distilled water (This breaks lipo-protein bonds present in the mixture). The mixture was then evaporated to dryness again, 25 cm^3 ethanol (to remove traces of water) were added and the whole was evaporated once more. The flask and its contents were then removed from the rotary evaporator and a stream of nitrogen was blown over the contents to make sure that they were completely dry. Finally, the sample was taken up into 5 cm^3 organic phase for purification by partition chromatography on a column of Sephadex G-25. If not used immediately, it was stored under nitrogen at -20°C for as short a time as possible.

2.12 Purification of crude lipid extract by partition chromatography on Sephadex G-25

Two solvent systems were used for partition chromatography on Sephadex G-25, the organic phase and the aqueous phase (Mirbahar, 1981). To prepare the two phases, chloroform, methanol and 0.58% aqueous sodium chloride were mixed in the ratio of 8:4:3 (v/v) in a separating funnel, shaken thoroughly and left for 4-5 hours. The mixture separated into two phases, in which the upper phase was the aqueous phase and lower one was the organic phase. Each phase was

transferred to separate stoppered bottles and stored until needed.

The crude lipid extract contains both lipid and non-lipid material (amino acids, sugars, nucleotides, inorganic salts *etc.*). It is necessary to remove these non-lipid components prior to lipid analysis. The lipid extract was therefore purified by partition chromatography on a column of Sephadex G-25, using a modification of the method of Rouser *et al.* (1967).

The column was prepared as follows. About 3g Sephadex G-25 (coarse grain) were briefly degassed in 30 cm^3 aqueous phase and the grains were allowed to swell in the liquid for 3 hours. The resulting slurry of beads was poured into a Whatman chromatography column (Whatman Ltd. Springfield Mill, Maidstone, Kent U.K.) of 1 cm internal diameter and allowed to settle to a height of 11 to 12 cm. The column was then pre-washed using the following sequence:-

Aqueous phase: 50 cm^3 at a flow rate of 1 $cm^3 min^{-1}$.

Organic phase: 20 cm^3 at a flow rate of 0.5 cm^3 min^{-1}.

Aqueous phase: 50 cm^3 at a flow rate of 1 cm^3 min^{-1}.

Immediately before application of the sample, the column was eluted with 20 cm^3 of the organic phase at a flow rate of 0.5 cm^3 min^{-1}. The crude lipid extract was then applied from its sample vial to the column in a small volume of organic phase using a Pasteur pipette. This was allowed to run slowly into the column bed. The vial was rinsed three times with small volumes of organic phase and these in turn were applied and allowed to run onto the column bed. The lipids were then eluted with 20 cm^3 organic phase at a flow rate of 0.5 cm3 min^{-1}. and collected in a 100 cm^3 round-bottomed flask containing two drops of ethanol. This fraction is termed the purified lipid extract. It was taken to dryness in a rotary evaporator at a temperature of

<50°C. It was then transferred in 2 cm^3 chloroform to a glass sample vial, evaporated to dryness under nitrogen and stored at -20°C for later analysis.

After use, the column could be regenerated by removing water-soluble material with 50 cm^3 of aqueous phase at a flow rate of 1 cm^3 min^{-1}. It could be repeatedly used if not allowed to run dry. The column could be left standing at room temperature with aqueous phase for a week or more between uses. To prevent bacterial growth on the Sephadex, 0.02% (w/v) sodium azide was added to the aqueous phase when the column was not in use.

2.13 Separation of free fatty acids and esterified fatty acids

The separation of free fatty acids from the esterified fatty acids was performed according to the method of Draper (1969). The purified lipid extract (Section 2.12) was dissolved in 25 cm^3 of diethyl ether-hexane (1:1, v/v) and placed in a separating funnel. The free fatty acids were then extracted with 4 separate 10 cm^3 aliquots of 1% aqueous sodium carbonate. The combined aqueous extracts were acidified with concentrated phosphoric acid to pH 1 and the free fatty acids were re-extracted with 50 cm^3 of diethyl ether-hexane mixture (4:1, v/v). This fraction was dried by rotary evaporation and redissolved in 2 cm^3 chloroform prior to further analysis. The organic phase remaining after the extraction with sodium carbonate was dried by rotary evaporation and redissolved in 2 cm^3 chloroform for further analysis.

2.14 Chromatography on silicic acid columns

Disposable Sep-Pak cartridges (Section 2.3) containing a column

of silicic acid (2.5 cm height x 1.0 cm diameter) were used to separate the esterified lipids obtained by partition between hexane:ether and sodium carbonate solution (Section 2.13) into neutral lipid, glycolipid and phospholipid fractions. The column was attached to a solvent reservoir consisting of the barrel of a 50 cm^3 syringe without its plunger. The column bed was washed with 25 cm^3 of chloroform at its natural flow rate. The esterified lipid sample was then carefully transferred into the syringe barrel and allowed to run into the column bed. This procedure was repeated 3 times with 2 cm^3 aliquots of chloroform to ensure that all lipid was transferred. Lipid fractions were then eluted according to following sequence.

Neutral lipid: 30 cm^3 chloroform at a natural flow rate.

Glycolipid: 25 cm^3 acetone at a natural flow rate.

Phospholipid: 30 cm^3 methanol at a 1 cm^3 per minute.

The natural flow rate for methanol was found to be very slow. This was improved by using the piston of the syringe to force the solvent through the column. The fractions were collected separately in small round-bottomed flasks, reduced to dryness on a rotary evaporator and transferred using chloroform to glass sample vials. The samples in their vials were stored under nitrogen at -20°C.

The efficiency of the method was checked using (Me-^{14}C) phospatidyl choline. Over 95 percent recovery of the phospholipid was obtained.

2.15 Determination of lipid phosphorus

An adaptation of the method of Rouser *et al.* (1966) was used to determine the amounts of lipid phosphorus in the purified lipid extracts. An aliquot of the lipid fraction from Sephadex G-25,

containing 2-8 µg of lipid phosphorus, was transferred to a 20 cm^3 reductase tube and reduced to dryness under nitrogen. The samples was then digested with 0.7 cm^3 perchloric acid at 200°C using a dry heating block (Model DB-4, Tekhne Cambridge Ltd., Duxford, Cambridge, U.K.). When the digestion became clear (about 90 minutes), it was cooled and a further 0.7 cm^3 perchloric acid was added followed by 6.6 cm^3 distilled water and 1 cm^3 2.5% ammonium molybdate. After mixing thoroughly, 1 cm^3 10% ascorbic acid was added, and the whole was mixed and heated for 5 minutes in a boiling water bath. After cooling, the optical absorbance was measured at 800nm. A calibration curve was prepared using standard solutions of potassium orthophosphate containing 0-10 µg phosphorus (Figure 2.1).

2.16 Determination of radioactivity

Uptake of radioactivity into the tissue was determined as the radioactivity contained in an aliquot taken from the WSB extract (crude lipid extract). Incorporation of radioactivity into phosphatidyl choline was determined as the radioactivity contained in the purified total lipid fraction obtained from Sephadex G-25 chromatography.

For these determinations, the lipid samples were transferred to scintillation vials, dried under nitrogen and dissolved in 5 cm^3 of scintillation fluid (6 cm^3 plastic vials inserted in 22 cm^3 glass scintillation vials were used). The scintillant used was Scintillant Cocktail T (BDH Ltd. Poole, Dorset, U.K.). Radioactivity was counted in a Beckman Model LS 7500 spectrometer (Beckman-IRIC Ltd. High Wycombe, Bucks, U.K.). Raw counting data were converted from counts per minute (cpm) to disintegration per minute (dpm) by the

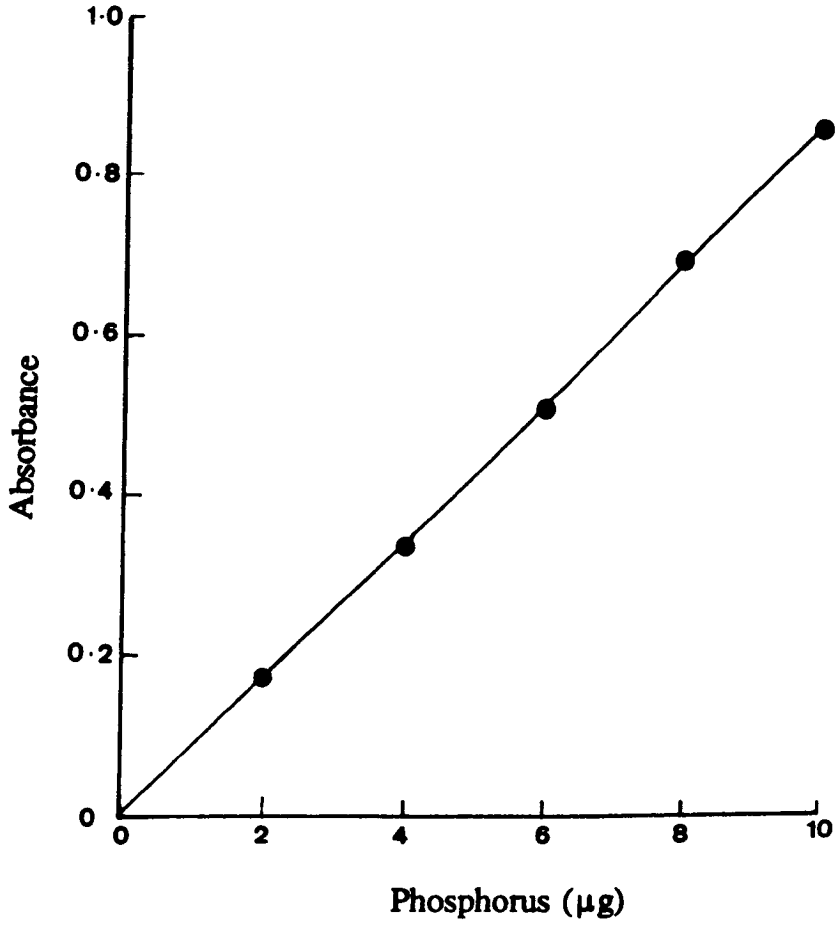


Figure 2.1 Calibration curve for the determination of lipid phosphorus.

spectrometer using the Crompton end effect.

2.17 Gas-liquid chromatography of fatty acid methyl esters

The free fatty acid fraction (Section 2.13) and the lipid fractions from silicic acid chromatography (Section 2.14) were converted to fatty acid methyl esters according to the method of Morrison and Smith (1964). The lipid samples in 4 cm^3 screw-capped sample vials (Phase Separations Ltd. Deeside, Clywd, U.K.) were reduced to dryness under nitrogen and then placed in a desiccator under vacuum for an hour to ensure complete dryness. One cm^3 of 14% boron trifloride in anhydrous methanol was then added. The samples were heated in a dry block heater (Model DB-4, Techne Cambridge Ltd. Cambridge, U.K.) at 60°C for 30 minutes and then cooled to room temperature. Two cm^3 hexane and 1 cm^3 distilled water were then added, the mixtures were briefly shaken and they were then centrifuged for 5 minutes at 1500 rpm in a bench centrifuge. The upper hexane layer was transferred by Pasteur pipette to a clean sample vial. The remaining aqueous phase was reextracted with 2 x 2 cm^3 hexane. The combined hexane extract was dried completely under nitrogen and the fatty acid methyl esters were redissolved in 1 cm^3 of dichloromethane prior to gas chromatographic analysis. At this stage, the methyl esters could be stored at -20°C and they were found to be stable for several weeks.

The fatty acids methyl ester mixture was analyzed using a Pye Model 104 gas chromatograph fitted with a flame ionization detector. Five or 10 µl samples in dichloromethane were analyzed using a silanized glass column (5.0 ft x 0.4 mm i.d.) packed with 10% diethylene glycol succinate (DEGS) on 100-120 mesh Chromosorb W-AW-DMCS as support phase. The chromatography was carried out at 190°C

using oxygen free nitrogen at 40 cm^3 per minute as the carrier gas. The hydrogen and air flow rates were 40 cm^3 per minute and 150 cm^3 per minute respectively. Before and after each 3 hours of work, the chromatograph was re-calibrated using a mixture of authentic, pure fatty acid methyl esters. This allows the peaks corresponding to palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) to be identified in the chromatograms from the cotton lipid fractions (Figure 2.2). In this method, the area under each peak was calculated by triangulation (i.e. peak height x width at half peak height). Another standard of heptadecanoic acid (C17:0) (1 mg cm^{-3}) was also applied after each 3 hours of work, this was the quantitative standard from which the amount of each fatty acid methyl ester in the unknown samples could be calculated. From these areas and that of the C17:0 standard the percentage of each fatty acid was calculated.

2.18 Statistical analysis

All means, standard deviation and standard error values were calculated using a pocket scientific calculator (Casio Model fx-82c). 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.

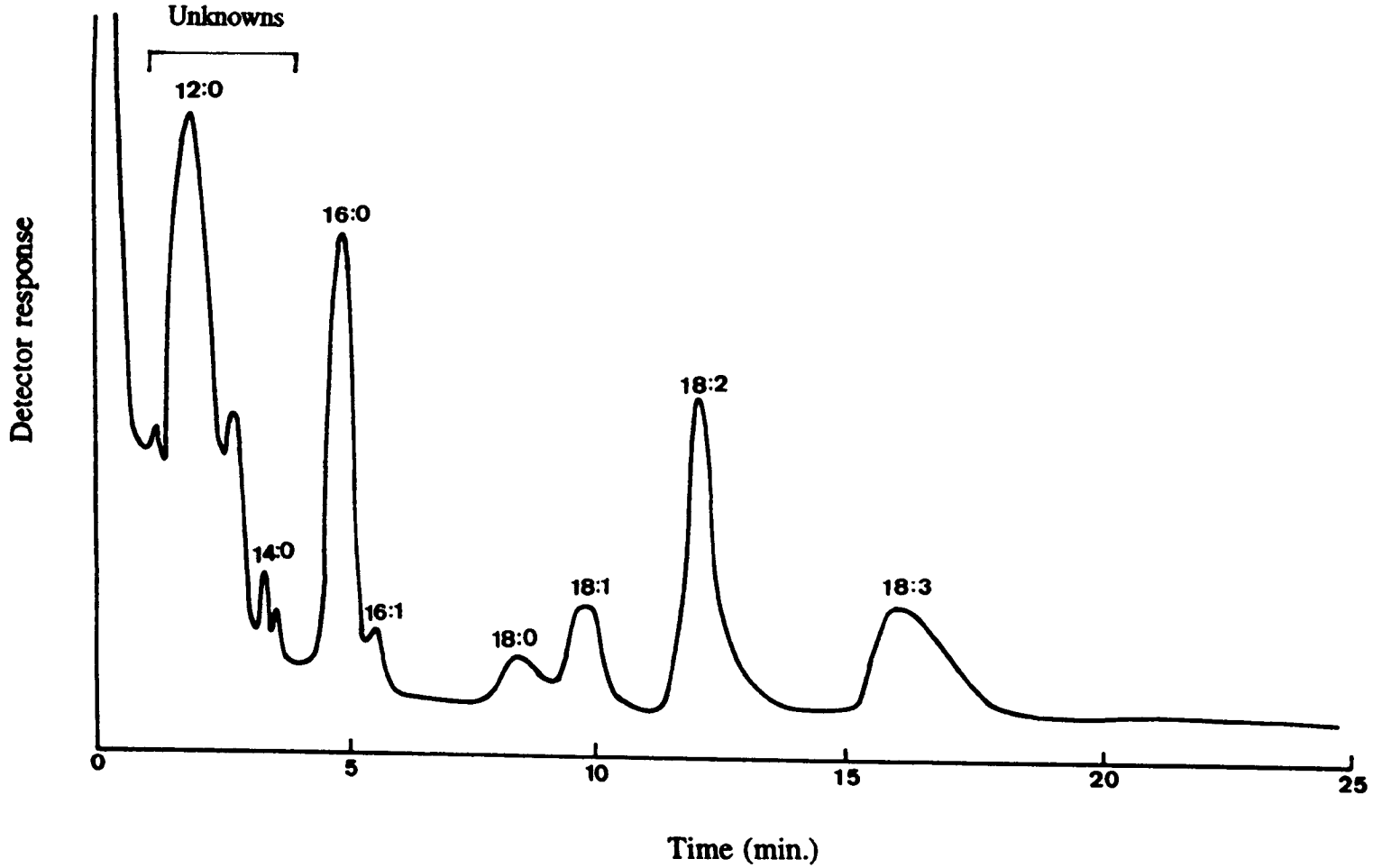


Figure 2.2 Gas-liquid chromatographic separation of fatty acid methyl esters.

CHAPTER THREE

Chapter Three

Effects of Heat Shock on Root Development

3.1 Introduction

Numerous investigators have reported that temperatures above or below the optimum level inhibit root and shoot growth (Kailasanthan *et al.*, 1976; Abdel Magid and Osman, 1977; Garcia-Huidobro *et al.*, 1985; Murtadha *et al.*, 1989). Even a 2 minute heat-shock at 47.5°C applied to maize roots inhibited shoot and root growth (Caers *et al.*, 1985). The period between planting and emergence is a critical time in the development of the cotton plant. In countries such as Pakistan and India the temperature often reaches 40°C or more during this period. Such temperatures may cause severe damage to both the root and the shoot of the seedlings. Haugen and Smith (1992) reported that a temperature of 38°C brings marked differences in the morphology and growth of the root systems of mungbean.

Pollock (1990) pointed out that temperature change alters the rates of chemical reactions, and living organisms are the products of many such reactions occurring within their cells. Exposure of young seedlings to high temperature thus causes a variety of changes in physiological and metabolic processes. Heating pea roots caused modification in the structure of membrane systems (Buttrose and Swift, 1975). Exposure of maize kernels to 46°C produces the appearance of corpuscles in the cytoplasm and in the mitochondrial matrix (Fransolet *et al.*, 1979). This is due to changes in membrane lipids (Rivera and Penner, 1978). These workers further reported that an increase in temperature from 15 to 30°C caused increases in palmitic and stearic acids and decreases in oleic, linoleic and linolenic acid contents in both the plasmalemma and mitochondrial fractions.

After sowing and onwards, the soil temperature plays an important role in the rate and duration of plant growth. Soil temperature clearly affects root development in cotton and soybean as found by Bland (1993). Robinson (1949) reported that the soil temperature is closely related to the air temperature. The cotton seed is sown 2-4 cm below the soil surface. Brady (1984) stated that average monthly soil temperature at a 7.5-cm depth is higher than the air temperature throughout the year. He further added that, on average, the surface 15-cm layer of soil is warmer than the air at every season of the year.

Once cotton seed has germinated, the temperature optimum for growth probably changes with different stages of seedling growth (Arndt, 1945). Radicle elongation begins before hypocotyl elongation and proceeds at a faster rate. Before appearance of lateral roots, the radicle elongates at a constant rate. It has been reported that the rate of elongation of both radicle and hypocotyl in cotton increase between 16 and 32°C and begins to decrease at 38°C (Wanjura and Buxton, 1972). This was in accord with the results of Arndt (1945) who showed that increasing elongation occurred between 18 and 33°C with a decreased toward 39°C. Wanjura and Buxton (1972) further stated that growth and elongation of the radicle is limited to the tips where cell division and elongation are concentrated.

Meristematic cells are more sensitive to heat stress. In wheat leaf meristems, for example, temperature stress at around 45°C causes inhibition of both mitosis and cell division (Kharlamov and Mousienko, 1992) followed by a cessation of root elongation (Sattelmacher *et al.*, 1990). Sattelmacher *et al.* (1990) further reported that bending of the apical root zone occurred together with formation of root hairs on the concave side and lateral roots on the convex side of the root. Root

extension is also significantly affected by temperatures (Bowen, 1991).

Safaralieva *et al.* (1980) reported that high temperature increased the abscisic acid activity in roots, decreasing the root's rate of cell formation and cell elongation. Increase in temperature also increase uptake of mineral nutrients (P, N and K) (Natr and Purs, 1971).

The main objective of the present experiment was to evaluate the effect of high temperature on root growth in the cotton species *Gossypium hirsutum*. The chapter describes preliminary experiment carried out to find suitable procedures for heat shock treatment and to study the effects of temperature on seedlings development before proceeding to further biochemical studies.

3.2 Methods

The preliminary experiments were carried out on the cultivars MNH-93 and Qalandari from the 1989-90 harvest (Section 2.1). An adaptation of the method of Sethar (1993) was used to germinate the cotton seeds in rolled filter paper. The details of the procedure and the selection of seedling quartiles are described in Section 2.6.

Seedlings that had been germinated for 48 hours at 25°C were heat shocked at 25°C (control), 30, 35, 37.5, 40, 42.5 and 45°C. Two procedures were used for this. In the case of the root length data, the seedlings were heat shocked for 3 hours in an air incubator, whereas for the fresh and dry weight determinations the seedlings were heat shocked at 35, 40, 42.5 and 45°C for 2 hours in a water bath (Section 2.7). After the heat-shock treatment, they were returned to the incubator at 25°C to recover for up to 72 hours. A further 5 cm^3 aliquot of sterile distilled water was added to each batch of

seedlings at 48 hours. Two further controls consisted of seeds germinated for 48 hours at 25°C (control 1) and for 72 hours at 25°C (control 2), that is without removal from the 25°C incubator.

At the end of their incubations, the seedlings were harvested and their roots were detached from the cotyledons using a scalpel. The isolated roots were sorted into quartiles according to their root length (Section 2.6) and the length of each root was measured using a ruler. For fresh weight determination, the seedlings in each quartile were bulked and weighed on a five-place analytical balance. The quartiles were then dried for 48 hours in an oven at 70°C and re-weighed to determine their dry weight. The percent moisture contents of the quartiles were calculated from the dry weight and fresh weight data.

The resulting data were analysed statistically as described in Section 2.18. Best fit curves were drawn to data points using a personal computer with the Systat Sygraph *DWLS* (Distance Weighted Least Square) software programme.

3.3 Results

3.3.1 Effect of heat shock on root growth

The results of this experiment are presented in Tables 3.1 to 3.3 and in Figures 3.1 and 3.2. Large variations in root growth rate were noted. Due to this variation in growth rate, which occurred even within a single seed lot, relatively large samples (100 seeds) were used for each analysis. Even with samples of 100 seeds large standard deviation values were still obtained. Therefore, it was decided to divide the roots into four quartiles according to their length. This reduced the standard deviation values considerably (see Tables 3.2 and 3.3 and Figures 3.1 and 3.2). Only the mean values ±sd and ±se for the

first, second and third quartiles are presented in this thesis. Due to poor germination percentages, values for the fourth quartile were not considered reliable (variable numbers of seeds in the quartile had not germinated at all). This quartile was therefore discarded.

The data for the whole root samples (i.e. before division into quartiles) will be described first (Table 3.1, Figures 3.1A and 3.2A). It is quite clear from these data that, in the first phase of growth from zero hour to 48 hours at 25°C, the roots of the MNH-93 grew faster than those of the cultivar Qalandari (cf. control 1 of MNH-93 and control 1 of Qalandari in Table 3.1). In the second phase, from 48 to 72 hours, the pattern was completely different however. The roots of Qalandari grew faster than those of MNH-93 at 25°C. (cf. the difference between control 1 and control 2 for each cultivar in Table 3.1).

When ratios (control 2: control 1) of the growth of both cultivars were calculated from the data, it was quite obvious that the ratio for two cultivars, MNH-93 and Qalandari were 1.7 and 2.5-fold respectively.

In the case of MNH-93 (Figure 3.1A), the lengths of the roots subjected to heat shock at 25, 30, 35 and 37.5°C remained variable (large sd values) but the means were not obviously affected by these treatments. Above 37.5°C, however, there was a noticeable effect on root growth. As temperature increased, the root length decreased. At 45°C, root growth was nearly stopped. Cultivar Qalandari behaved only slightly different from MNH-93 in this respect (Figure 3.2A). In this cultivar, heat shock at 25 and 30°C had no significant effect on growth. Heat shock at higher temperatures, however, produced increasingly large reductions in mean root length. Root growth was reduced greatly after heat shock at 40°C and growth nearly ceased

Table 3.1 Effect of heat shock on root growth of two cotton cultivars.

Heat-shock temp. (°C)	MNH-93			Qalandari		
	mean	±sd	±se	mean	±sd	±se
25.0	66.4	23.7	1.4	59.4	29.0	2.0
30.0	64.7	23.8	1.4	64.6	30.3	1.9
35.0	66.0	25.6	1.5	56.5	24.8	1.7
37.5	63.0	24.8	1.6	53.9	24.0	1.6
40.0	46.7	19.4	1.2	48.0	22.3	1.4
42.5	44.0	17.5	1.1	43.7	21.6	1.4
45.0	41.2	13.9	0.8	32.4	14.2	0.9
Control 1	36.9	11.9	0.7	27.4	12.1	0.8
Control 2	64.1	24.5	1.5	61.8	25.6	1.6

Each value is the mean length (mm) ±sd and ±se from 300 seeds.
Control 1: Seeds germinated for 48h at 25°C without heat shock.
Control 2: Seeds germinated for 72h at 25°C without heat shock.

Table 3.2 Effect of heat shock on root growth of the cultivar MNH-93.

Heat-shock temp. (°C)	First quartile			Second quartile			Third quartile		
	mean	±sd	±se	mean	±sd	±se	mean	±sd	±se
25.0	93.6	8.2	0.9	75.5	5.9	0.7	57.6	8.3	1.0
30.0	94.6	8.9	1.0	71.6	6.8	0.8	52.8	7.1	0.8
35.0	93.9	9.1	1.0	75.4	10.9	1.3	56.2	10.5	1.2
37.5	90.0	13.9	1.6	70.5	12.9	1.5	54.7	12.5	1.4
40.0	68.5	11.6	1.3	53.1	8.6	1.0	39.3	6.0	0.7
42.5	65.1	10.7	1.2	47.6	3.4	0.4	36.3	4.4	0.5
45.0	55.6	5.7	0.7	46.0	5.4	0.6	36.6	7.3	0.8
Control 1	50.9	4.3	0.5	41.4	2.3	0.3	33.2	3.3	0.4
Control 2	93.2	12.3	1.4	72.1	9.2	1.1	51.7	6.3	0.7

Each value is the mean length (mm) ±sd and ±se from 300 seeds.
Control 1: Seeds germinated for 48h at 25°C without heat shock.
Control 2: Seeds germinated for 72h at 25°C without heat shock.

Table 3.3 Effect of heat shock on root growth of the cultivar Qalandari.

Heat-shock temp. (°C)	First quartile			Second quartile			Third quartile		
	mean	±sd	±se	mean	±sd	±se	mean	±sd	±se
25.0	88.3	13.2	1.5	59.8	11.6	1.3	23.1	13.3	1.7
30.0	95.8	13.1	0.7	72.3	11.6	1.3	44.3	16.3	1.9
35.0	80.4	11.3	1.3	57.9	9.6	1.1	26.7	14.8	1.9
37.5	76.9	11.4	1.3	54.7	11.5	1.3	27.2	15.0	2.0
40.0	71.5	8.7	1.1	54.8	8.7	1.0	33.5	11.2	1.3
42.5	61.2	17.5	2.0	46.1	12.3	1.4	25.9	13.2	1.6
45.0	46.5	8.6	1.0	34.9	5.2	0.6	20.9	8.0	0.9
Control 1	39.6	5.1	0.6	27.1	4.5	0.5	11.9	5.8	0.8
Control 2	88.7	9.1	1.1	67.2	8.5	1.0	44.6	12.9	1.5

Each value is the mean length (mm) ±sd and ±se from 300 seeds.
Control 1: Seeds germinated for 48h at 25°C without heat shock.
Control 2: Seeds germinated for 72h at 25°C without heat shock.

Table 3.4 Temperature at which root growth is inhibited by 50% (TIG-50) in the two cotton cultivars.

	MNH-93 TIG-50	Qalandari TIG-50
Total roots	41.0	41.5
First quartile	41.4	41.5
Second quartile	41.0	41.8
Third quartile	41.3	40.6

The TIG-50 values (°C) are calculated from Figures 3.1 and 3.2.

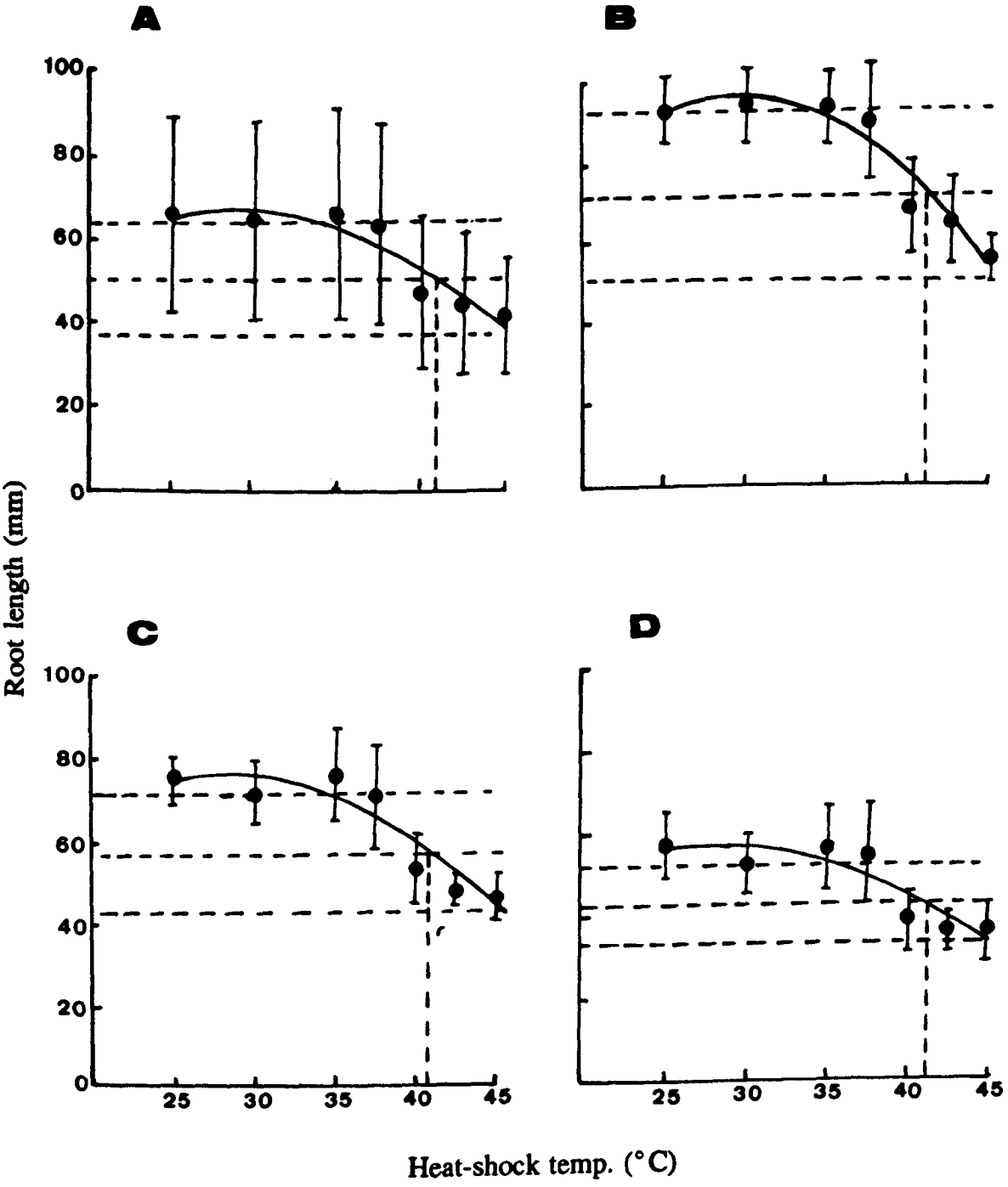


Figure 3.1 Effect of heat shock on root growth in cultivar MNH-93. (A) all seeds, (B) first quartile, (C) second quartile, (D) third quartile. Vertical bars indicate standard deviation values.

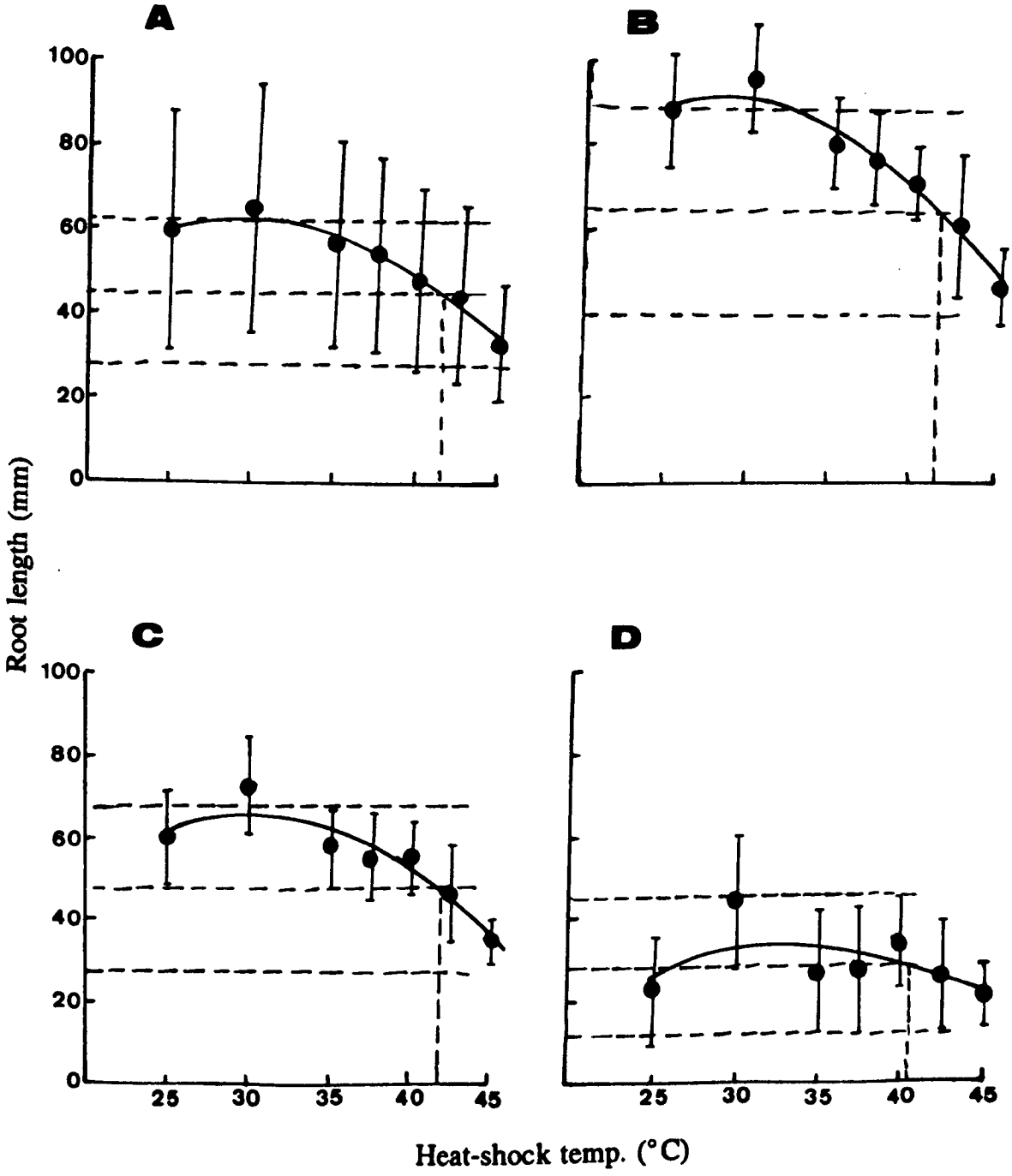


Figure 3.2 Effect of heat shock on root growth in cultivar Qalandari. (A) all seeds, (B) first quartile, (C) second quartile, (D) third quartile.
Vertical bars indicate standard deviation values.

following treatment at 45°C.

Because of the large standard deviation values, it was difficult to prove significant differences between two cultivars (cf Figures 3.1A and 3.2A). In an attempt to overcome this problem, TIG-50 values were calculated for each cultivar. The TIG-50 value is the temperature at which root growth is inhibited by 50%. It was calculated as follows: On each graph, two horizontal lines were drawn representing the control 1 values (48 hour germination at 25°C) and the control 2 values (72 hour germination at 25°C). A third horizontal line was then drawn half way between the two control lines. Where this line cut the curve, a vertical line was dropped to the x axis. The point where this vertical line intercepts the x axis is the temperature at which growth is inhibited by 50% (the TIG-50 value).

From the results shown in Table 3.4 and in Figures 3.1A and 3.2A, the TIG-50 for MNH-93 was 41°C and the value for Qalandari was 41.5°C. It can be seen that there is only about 0.5°C difference between the values for the two cultivars. The cultivar Qalandari was possibly slightly more heat tolerant than MNH-93, but the difference is not statistically significant.

The data for the first, second and third quartiles are presented in Tables 3.2 and 3.3 and in Figures 3.1B to D and 3.2B to D. When these results are compared with the data for whole seedling samples, the general pattern was found to be very similar in all cases. In the case of MNH-93, heat shock at temperature up to 37.5°C had no significant effect on any of the quartiles (statistical probability $P>0.06$). Heat shock at temperature above 37.5°C had progressively greater effects on root growth and the effects were highly significant ($P<0.001$). In the case of Qalandari, root growth was significantly reduced at 35°C and above ($P<0.001$) suggesting that this cultivar is

more heat sensitive than MNH-93. However, there were no significant differences between the TIG-50 values for the first, second and third quartiles of the two cultivars (Table 3.4). All of the TIG-50 value for both cultivars lay in a narrow range within less than one degree of 41°C.

In the data for the different quartiles and the data for whole samples, two more important observations can be made. The values for the standard deviations obtained from the quartiles data were much smaller than from the whole sample data. Standard error values were relatively low in all cases, however, due to the large sample size employed.

3.3.2 Effect of heat shock on the fresh and dry weights

The determination of fresh weight and dry weight was carried out only for the cultivar MNH-93. The results are presented in Tables 3.5 and 3.6 and Figures 3.3 and 3.4. The results for fresh weights of the roots show a similar pattern to the data for root length. Thus, the fresh weight in all the three quartiles almost doubled between 48 and 72 hour in seeds germinated at 25°C (cf control 1 and control 2 in Table 3.5). Seedlings heat shocked at 35°C or above showed obvious reductions in fresh weight in all the three quartiles. The fresh weights of all the three quartiles were highly affected following heat shock at 42.5 and 45°C. Moreover, it was observed that the seedlings heat shocked at these temperatures showed actual reductions in root fresh weight compared with the 48 hour control. Clearly, this treatment even caused a significant loss of fresh weight presumably due to dehydration of the roots following severe heat-shock damage.

When TIG-50 values were calculated from the graphs (Figure 3.3 and Table 3.7), somewhat different results were obtained compared with

Table 3.5 Effect of heat shock on root fresh weight gain of the cultivar MNH-93.

Heat-shock temp. (°C)	First quartile			Second quartile			Third quartile		
	mean	±sd	±se	mean	±sd	±se	mean	±sd	±se
25.0	3.4	0.2	0.10	2.7	0.1	0.06	2.0	0.2	0.10
35.0	2.9	0.1	0.06	2.3	0.1	0.06	1.6	0.2	0.10
40.0	2.5	0.3	0.20	1.8	0.4	0.20	1.2	0.2	0.10
42.5	1.3	0.2	0.10	0.8	0.3	0.20	0.5	0.2	0.10
45.0	1.3	0.1	0.06	0.9	0.1	0.03	0.5	0.1	0.04
Control 1	1.8	0.1	0.04	1.4	0.0	0.00	1.0	0.3	0.20
Control 2	3.4	0.2	0.10	2.7	0.1	0.06	2.0	0.2	0.10

Each value is the mean weight (g (25 roots)$^{-1}$) ±sd and ±se from 3 or 4 experiments.
Control 1: Seeds germinated for 48h at 25°C without heat shock.
Control 2: Seeds germinated for 72h at 25°C without heat shock.

Table 3.6 Effect of heat shock on root dry weight gain of the cultivar MNH-93.

Heat-shock temp. (°C)	First quartile		Second quartile		Third quartile	
	mean	±sd	mean	±sd	mean	±sd
25.0	0.23	0.00	0.18	0.00	0.15	0.02
35.0	0.20	0.01	0.17	0.01	0.12	0.01
40.0	0.17	0.02	0.12	0.03	0.09	0.02
42.5	0.11	0.01	0.07	0.01	0.05	0.01
45.0	0.07	0.01	0.07	0.01	0.05	0.01
Control 1	0.13	0.00	0.11	0.01	0.08	0.02
Control 2	0.23	0.00	0.18	0.00	0.15	0.02

Each value is the mean weight (g (25 roots)$^{-1}$) ±sd from 3 or 4 experiments.
Control 1: Seeds germinated for 48h at 25°C without heat shock.
Control 2: Seeds germinated for 72h at 25°C without heat shock.

Table 3.7 Temperature at which fresh and dry weights gains are inhibited by 50% (TIG-50) in the cultivar MNH-93.

	Fresh weight TIG-50	Dry weight TIG-50
First quartile	38.0	38.0
Second quartile	37.3	37.3
Third quartile	36.3	35.6

The TIG-50 values (°C) are calculated from Figures 3.3 and 3.4.

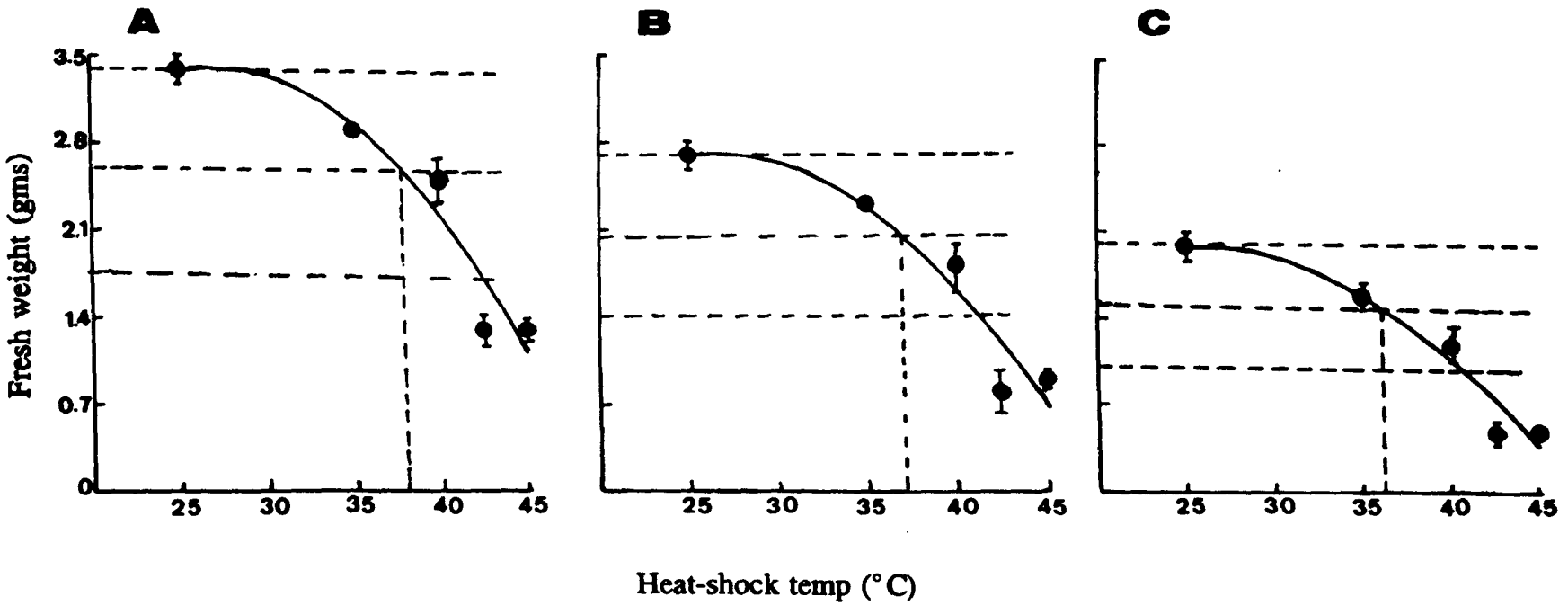


Figure 3.3 Effect of heat shock on root fresh weight gain in cultivar MNH-93. (A) first quartile, (B) second quartile, (C) third quartile. Vertical bars indicate standard error values.

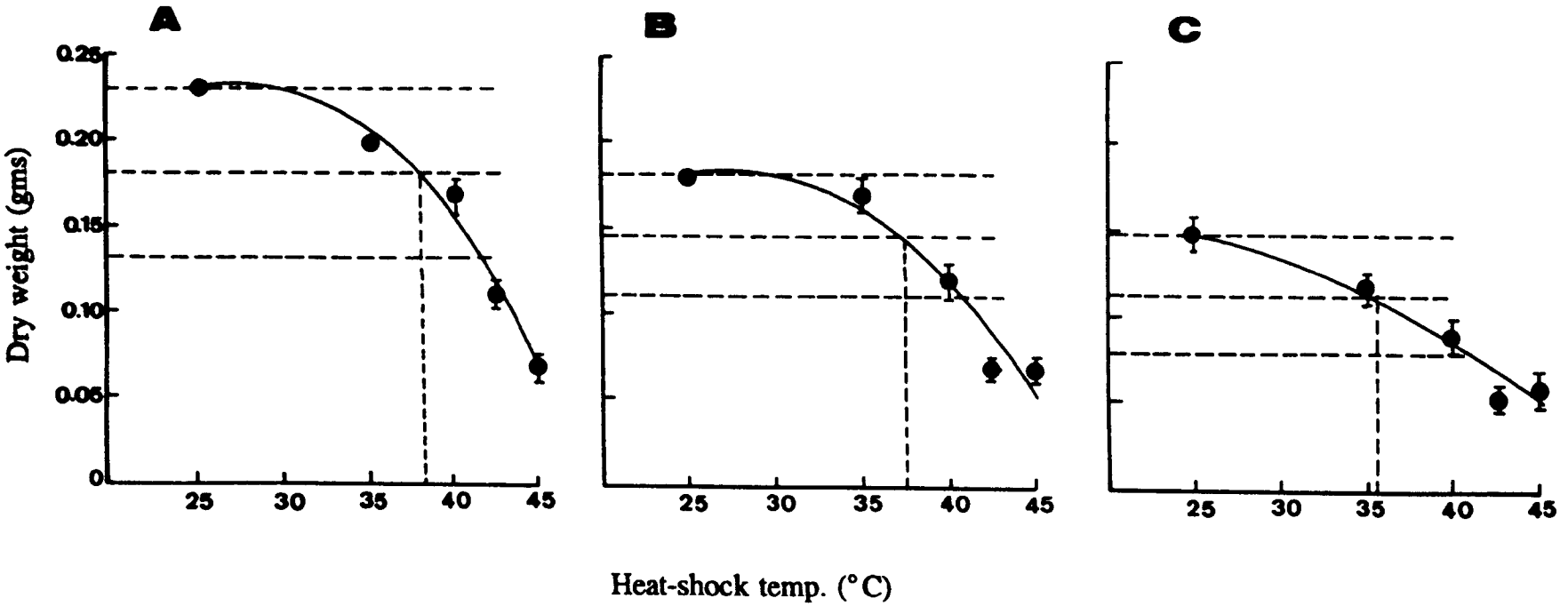


Figure 3.4 Effect of heat shock on root dry weight gain in cultivar MNH-93. (A) first quartile, (B) second quartile, (C) third quartile. Vertical bars indicate standard error values.

the TIG-50 values obtained from the root length data. The fresh weight TIG-50 values for the first, second and third quartiles were 38.0, 37.3 and 36.3°C, respectively. These values cover a range of only about 1°C on either side of 37°C and the differences are almost certainly not significant.

The dry weight values are presented in Table 3.6 and in Figure 3.4. The dry weight patterns for the roots of heat shocked seedlings were qualitatively similar to the fresh weight and the root length pictures. Once again, there were significant reductions in the dry weight values for all three quartiles following heat shock at temperature of 40°C or above. The dry weight of all three quartiles were highly affected following heat shock at 42.5 and 45°C.

Statistical analysis by T-test of the data in Tables 3.5 and 3.6 showed that heat shock at temperatures of 40°C and above significantly affected both fresh weight and dry weight values ($P<0.005$).

When TIG-50 values were calculated from the dry weight data, they were very similar to those obtained from fresh weight data, i.e. the TIG-50 all lay within about 1°C of 37°C (Table 3.7).

From the Figures 3.1 to 3.4, it can be seen that there is about 3 to 4°C difference between TIG-50 values derived from root lengths and those derived from fresh and dry weight data. The TIG-50 values of root length are in the range of 40.6 to 41.8°C, whereas those for the fresh and dry weight data are in the range between 35.6 to 38.0°C.

Discussion

All germinating seeds have similar patterns of root growth. Under optimum conditions, the rate of growth increases with increasing germination time. In the case of cotton germination at 25°C, the root length shows a progressive increase during the first 72

hours (Figure 6.2). Interestingly, cultivar MNH-93 showed faster root growth in the first phase from zero to 48hour compared with Qalandari. In the second phase, from 48 to 72 hour, the root growth of the Qalandari was much faster than that of MNH-93. There are two possible explanations for this, firstly seed coat permeability and secondly seed quality. In favour of the first explanation, Christiansen and Moore (1959) found that most species of wild cotton and even a few domestic varieties produce seeds with seed coats which upon drying become hard and impermeable to water. This in turn delays germination. The seeds of the cultivar Qalandari may have a slightly harder seed coat so that swelling and opening the chalazal aperture takes a little longer. In support of the second explanation, Aref *et al.* (1973) reported that seed quality is one of the important factors that determine the development of the seedlings. Emergence of the radicle, for example, is mainly dependent on seed vigour. Seed of poor quality and vigour may perform poorly even under ideal planting and growing conditions, whereas seeds of high quality and vigour generally perform well under relatively adverse environmental conditions (Aref *et al.*, 1973). The environment in which seeds are produced can affect the seed vigour and hence seedling performance (Peacock and Hawkins, 1970). The seed of cultivar MNH-93 may be of better quality because it showed the highest germination percentage and the root emerged faster than Qalandari under normal growing conditions at 25°C.

The results presented in this chapter reveal that root growth was affected by high temperature. The cultivar MNH-93 showed no significant effect up to temperature 37.5°C, while Qalandari was significantly affected at 35°C and above. Visible damage was observed at a temperature of 40°C or above. After heat shock at 40°C, the root tips were generally narrow and pointed and they became a yellowish. At

42.5°C the upper half of the roots became damaged. At 45°C, most of the roots were completely damaged. They were generally blackened and they were obviously dead. It is clear that exposure to 45°C is lethal for cotton roots. These results are in agreement with other workers who reported that temperatures above 42°C are lethal for cotton (Berckley and Berckley, 1933; Sethar, 1993). Glady (1938) has reported that even 35°C is beyond the range of optimal temperature for the growth of cotton root tips.

In the case of the fresh and dry weights, the results showed a different pattern to that shown by the root length data. Heat shock at 40°C caused significant reductions in fresh and dry weights of the cotton roots. Above 40°C, the change in fresh and dry weight was actually negative; that is, there were losses in weight compared with the controls. This was probably due to severe damage and even death of the cells leading to loss of turgor and shrivelling of the tissues. There are some other reports which support these findings that high temperature stress reduces fresh and dry weight accumulation (Adelusi and Lawanson, 1978; Salim and Saxena, 1991).

Due to the variability in the rate of growth of individual cotton seedlings, it was difficult to find significant differences between the two cultivars studied. To overcome this problem, root length data were dealt with by separating them in quartiles and TIG-50 values were introduced. From the TIG-50 values obtained from the whole samples and first, second and third quartiles, it is clear that there were only slight difference between the two cultivars. Similarly Sethar (1993) working on cotton, reported little difference between various cultivars of the cotton obtained from Pakistan.

When the TIG-50 values from root length data and those obtained from fresh and dry weight data were compared, there was about 3 to 4°C

difference between them. This could be due to the nature of the different heat shock treatments applied to the seedlings. (The root length data were obtained from seedlings heat shocked in an air incubator, while the data for fresh weight and dry weight were from seedlings heat shocked in a water bath). The seedlings heat shocked in the water bath were more badly damaged than the seedlings heat shocked in the air incubator.

From the TIG-50 values of fresh and dry weights, we can speculate that the seedlings of the first quartile might be more heat tolerant than the second quartile and the seedlings of the second quartile might be more heat tolerant than the third quartile. These differences were small however. To confirm this pattern, it was decided to carry out more detailed determinations of fresh and dry weights at different times following heat shock to see the effect on cotton seedling roots. These results will be discussed in Chapter 4.

It was also noted that the seedling treated in the air incubator took one hour to reach the desired heat shock temperature, whereas the seedlings in the water bath treatment took only 3 to 5 minutes to reach the heat shock temperature. It was therefore decided to use the water bath heat shock treatment in all further experiments.

CHAPTER FOUR

Chapter Four

Effects of Heat Shock on Phospholipids

4.1 Introduction

Numerous investigations have shown that cellular membranes are primarily involved in injury caused to plant cells by high temperature (Ordin *et al.*, 1974; Raison *et al.*, 1980; Thebud and Santarius, 1982). It is a well established fact that the physical phase of lipids, mainly phospholipids, play an important role in the structure and physical functions of biomembranes (Simon, 1974; Mazliak, 1977). Increasing the growing temperature caused increases in the saturation of fatty acids (Rivera and Penner, 1978). The greater fatty acid saturation leads to decreased membrane fluidity (Thompson, 1979) and this decreased fluidity of the membrane can affect the activity of the fatty acyl desaturase enzyme (Brenner, 1984). Recent investigations have suggested that changes in the lipid phase of membranes, such as decomposition of lipid molecules and release of free fatty acids, are also involved in altering the physical state of the membranes during heat treatment (Berry *et al.*, 1975; Murata *et al.*, 1975; Fork and Murata, 1977).

Plasma membranes contain a high ratio of phospholipids (Parsons, 1975). It has been reported that these membranes are highly susceptible to high temperature stress (Thebud and Santarius, 1982). Death of the plant due to heat may arise due to liquefaction of protoplasmic lipids (Belehdradck, 1935). Actual changes in the levels of lipids during heat treatment are less well documented however. Quinn (1988) reported that changes in the growth temperature are associated with changes in the membrane lipid composition. Exposure of tobacco leaves to high temperature for 2 minutes at 47.5°C inhibited

the synthesis of phospholipids (Benzioni and Itai, 1973).

Membranes play an important role in maintaining the transport processes in the cell. In normal growing conditions, the lipids (mainly phospholipids) of the membranes are arranged in a fluid bilayer configuration which acts as a matrix for the intrinsic and extrinsic proteins (Quinn, 1988). In this configuration the membranes have a low permeability to small polar molecules. When the temperature is changed to either above or below the limits of normal growth, the membranes loose their normal configuration and become permeable (Berry and Bjorkman, 1980; Raison *et al.*, 1980). In tobacco roots, for example, the efflux of chloride ions and ^{14}C-labelled metabolites increased when the roots were treated for only 2 minutes at 47.5°C (Benzioni and Itai, 1973). In addition to this, they further reported that damage to the membranes will affect the activity of bound membrane enzymes as well as compartmentation in the tissue, which may in turn cause a rise in catabolic activity.

Several scientists have studied the effects of high temperature on chloroplast membranes. Adelusi and Lawanson (1978) found that chloroplast formation in maize seedlings and cowpea is retarded when seedlings were heat stressed for 8 hours at 45°C. Gounaris *et al.* (1983a and b) reported that incubation of bean chloroplasts at 35-45°C caused complete destacking of the grana, but no alteration in the distribution of the membrane-associated particles between the exoplasmic and protoplasmic fracture faces in freeze fracture studies. Heating above 45°C leads to an extensive separation of non-phospholipid lipids into aggregates of cylindrical inverted micelles. They further added that a 5 minute incubation at 50°C leads to 75% of the chloroplasts containing non-bilayer structures.

Skogqvist (1974) working on wheat seedlings showed that high

temperature is also damaging to the endo-membrane structure. She reported that a 2 minute heat shock at 45°C caused an expansion of the endoplasmic reticulum cisternae giving rise to small irregular vacuoles. In addition to these she further added that mitochondrial structure becomes irregular with fewer tubules and adhering membrane curls. In pea roots, 2 hours of heat-shock treatment at 41°C induced a 50% decrease of respiration with no change in ATP content (Nikulina, 1985). Heating for more than 5 hours killed pea leaves and abruptly increased oxygen uptake by increasing substrate contact with released enzymes (Semikhotova and Nikulina, 1987).

It has been reported that phospholipid deterioration and consequent loss of membrane integrity represents an important physiological event associated with the loss of seed viability. Very little work has been done in this context on the effects of high temperature on membrane lipid metabolism. The general lack of information is especially apparent in the case of cotton. The experiments in this chapter were therefore performed to determine the effects of high temperature on phospholipid levels during germination, and on the synthesis of phosphatidyl choline in cotton seedlings.

4.2 Methods

4.2.1 Plant material

In this section the seeds of four following cultivars were used: MNH-93 and Qalandari from the 1989-90 harvests and Rehmani and S-12 from the 1990-91 harvests. For the phospholipid determinations, seed of one cultivar MNH-93, was used. The preliminary experiments to measure (Me-^{14}C) choline uptake and incorporation were also carried out on the cultivar MNH-93. Due to shortage of seed from the same seed lot, another lot of seed from the same cultivar (MNH-93) was used to

measure the uptake and incorporation of (Me-^{14}C) choline in the main experiment.

4.2.2 Application of heat stress

The detailed procedures for these experiments are described in the Material and Methods chapter. Briefly, seedlings that had been germinated for 48 hours at 25°C were heat-shocked for 2 hours at 35, 40, 42.5 and 45°C in a water bath (Section 2.7). After the heat-shock treatment, they were returned to the incubator at 25°C for recovery. The recovery period was adjusted so that the total experimental period was 54, 60, 66 and 72 hours for each heat shock treatment. Controls consisted of seeds germinated up to 72 hours at 25°C in the same incubator. At the end of the experimental treatments, seedlings were harvested and their roots were detached from the seeds using a scalpel. The isolated roots were sorted into quartiles according to their lengths (Section 2.6) and their fresh weight measured. Their lipids were then extracted by hot, water-saturated butanol (Section 2.11). After purification by chromatography on columns of Sephadex G-25, the total lipid fraction was assayed for its lipid phosphorus content (Section 2.12 and 2.15). The dry weight values were determined from separate experiments as in section 3.2.

4.2.3 Measurement of uptake and incorporation of (Me-^{14}C) choline

The detailed procedures for this experiment have been described in Chapter 2 (Sections 2.5 and 2.6 and 2.10 to 2.12). Briefly, the procedures were as follows. For the measurement of (Me-^{14}C) choline uptake and incorporation into phosphatidyl choline in the absence of saturating concentration of unlabelled carrier choline, 25 roots were placed in 100 cm^3 50mM tris-maleate buffer containing 50 µg cm^{-3}

chloramphenicol and 1 μCi (Me-^{14}C) choline (specific activity 55 mCi mmol^{-1}). In preliminary experiments, the pH was varied between 6.2 and 7.7. In the main experiments the pH was fixed at 7.2 (see results below). The tissues were incubated for 15, 30 or 60 minutes in a reciprocating water bath operating at 175 oscillation per minute. After incubation, the roots were washed three times with distilled water and then incubated for 5 minutes in 100 cm^3, 100mM unlabelled choline chloride to remove radioactive material from the free spaces of the cell walls. The roots were finally washed 5 times with distilled water and their lipids were extracted with hot water-saturated butanol (Section 2.11). After extraction, the crude lipid extract was purified by chromatography on columns of Sephadex G-25 (Section 2.12).

Uptake of radioactivity into the tissue was determined as the radioactivity in an aliquot taken from the crude lipid extract. Incorporation of radioactivity into phosphatidyl choline was determined as the radioactivity contained in the purified total lipids from Sephadex G-25 chromatography.

All the data were analysed statistically as described in Section 2.18. Best fit curves were drawn to data points using the personal computer with the Systat/Sygraph DWLS (Distance Weighted Least Square) software programme.

4.3 Results

The results from the experiments in which fresh weight, dry weight and total phospholipid levels were determined are presented in Tables 4.1 to 4.3. and Figures 4.1 to 4.3. In seeds germinated at 25°C (controls), fresh weight, dry weight and total phospholipid contents of the roots all progressively increased with increasing germination

time. In each case the curves followed a similar pattern. The effect of heat shock on each of these experimental parameters will now be discussed in turn.

4.3.1 Effect of heat shock on fresh and dry weights

The results for fresh weight are presented in Table 4.1 and in Figure 4.1. The fresh weight of roots in the first quartile increased from 0.1g at 24 hour up to 3.5g at 72 hour in the control seedlings germinated at 25°C. In seedlings that had been heat shocked at 35°C, there was no clear differences between the 72 hour fresh weight value and the 72 hour control value. It is more difficult, however, to decide whether or not the shock treatment had an effect at intermediate times between 48 and 72 hours. Roots heat shocked at 40°C or above showed more obvious reductions in fresh weight. After treatment at 40, 42.5 and 45°C, fresh weight was reduced to 2.5, 1.4 and 1.0g respectively at 72 hours. Also, it was observed that these roots clearly showed deleterious effects (browning of the tissue) at 54, 60 and 66 hours (Figure 4.1A).

In the case of the second quartile, the patterns of the growth curves were very similar to those for the first quartile, but all the values were lower. In this quartile, the general appearence of the seedlings heat stressed at 35°C were also significantly affected as compared with controls treated at 25°C. Heat stress at 40°C and above caused further reductions in the fresh weight gains of the roots compared with the control (Figure 4.1B).

The effect of heat stress on the third quartile was also qualitatively the same as that on the first quartile (Figure 4.1C). The general difference was that the fresh weight values were generally lower than those of the second and first quartiles.

Table 4.1 Effect of heat shock on root fresh weight.

Heat-shock temp.(°C)	Total germ. time(h)	First quartile	Second quartile	Third quartile
25	24	0.10 ±0.02	— —	— —
	36	0.65 ±0.08	— —	— —
	42	1.30 ±0.05	0.80 ±0.05	— —
	48	2.10 ±0.20	1.60 ±0.20	1.10 ±0.10
	60	2.50 ±0.20	1.90 ±0.30	1.30 ±0.10
	72	3.50 ±0.40	3.10 ±0.40	2.50 ±0.20
35	54	2.40 ±0.06	1.80 ±0.10	1.20 ±0.20
	60	2.30 ±0.05	1.80 ±0.10	1.40 ±0.20
	66	2.90 ±0.60	2.30 ±0.40	1.60 ±0.30
	72	3.50 ±0.10	2.70 ±0.20	1.90 ±0.40
40	54	1.90 ±0.30	1.50 ±0.20	1.00 ±0.30
	60	2.20 ±0.30	1.50 ±0.30	1.00 ±0.20
	66	2.40 ±0.20	1.80 ±0.20	1.10 ±0.20
	72	2.50 ±0.20	1.70 ±0.20	1.10 ±0.30
42.5	54	1.60 ±0.20	1.30 ±0.10	0.90 ±0.01
	60	1.40 ±0.00	1.10 ±0.10	0.70 ±0.06
	66	1.50 ±0.40	1.10 ±0.20	0.70 ±0.20
	72	1.40 ±0.30	1.10 ±0.20	0.70 ±0.20
45	54	1.70 ±0.05	1.30 ±0.20	0.90 ±0.20
	60	1.70 ±0.20	1.10 ±0.20	0.60 ±0.20
	66	1.80 ±0.10	1.20 ±0.40	0.70 ±0.10
	72	1.00 ±0.30	0.70 ±0.10	0.40 ±0.10

Each value is the mean weight (g (25 roots)$^{-1}$) ±sd from 3 or 4 separate experiments.
The experiment was carried out on Cv. MNH-93.

Table 4.2 Effect of heat shock on root dry weight.

Heat-shock temp.(°C)	Total germ. time	First quartile	Second quartile	Third quartile
25	24	0.03 ±0.000	-- --	-- --
	36	0.07 ±0.004	-- --	-- --
	42	0.09 ±0.006	0.06 ±0.005	-- --
	48	0.15 ±0.001	0.11 ±0.007	0.08 ±0.020
	60	0.18 ±0.020	0.13 ±0.010	0.10 ±0.010
	72	0.25 ±0.030	0.18 ±0.020	0.18 ±0.020
35	54	0.15 ±0.008	0.12 ±0.007	0.09 ±0.010
	60	0.16 ±0.004	0.13 ±0.005	0.11 ±0.020
	66	0.19 ±0.030	0.15 ±0.020	0.12 ±0.010
	72	0.24 ±0.010	0.19 ±0.010	0.15 ±0.030
40	54	0.12 ±0.010	0.10 ±0.007	0.08 ±0.010
	60	0.15 ±0.020	0.11 ±0.020	0.08 ±0.020
	66	0.16 ±0.010	0.12 ±0.010	0.08 ±0.008
	72	0.18 ±0.020	0.11 ±0.010	0.08 ±0.020
42.5	54	0.10 ±0.001	0.09 ±0.003	0.07 ±0.004
	60	0.10 ±0.000	0.07 ±0.007	0.05 ±0.005
	66	0.10 ±0.020	0.07 ±0.010	0.06 ±0.020
	72	0.11 ±0.020	0.10 ±0.020	0.07 ±0.020
45	54	0.10 ±0.003	0.08 ±0.005	0.06 ±0.008
	60	0.10 ±0.010	0.06 ±0.010	0.05 ±0.002
	66	0.09 ±0.010	0.07 ±0.006	0.05 ±0.003
	72	0.06 ±0.010	0.04 ±0.007	0.03 ±0.008

Each value is the mean weight (g (25 roots)$^{-1}$) ±sd from 3 or 4 separate experiments.
The experiment was carried out on Cv. MNH-93.

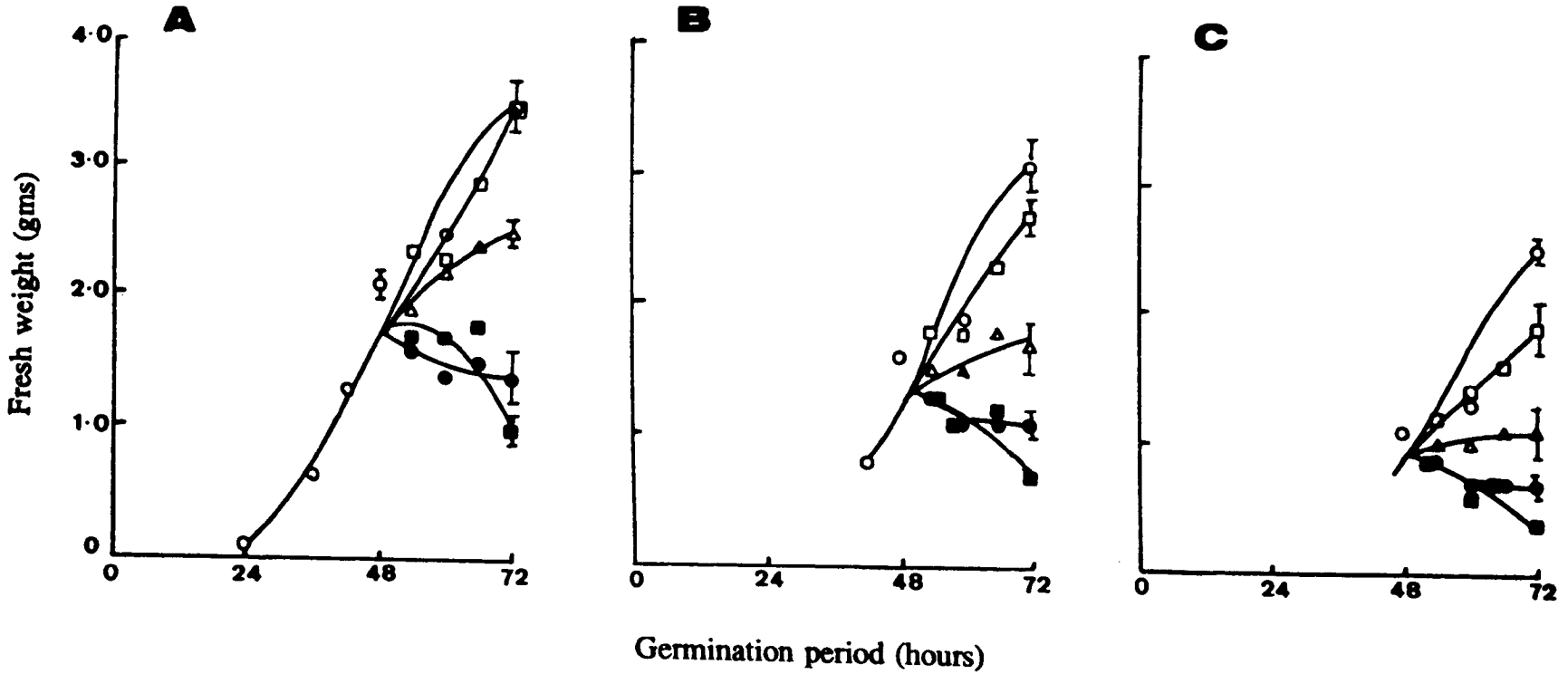


Figure 4.1 Effect of heat shock on root fresh weight in cultivar MNH-93. (A) first quartile, (B) second quartile, (C) third quartile. ○, 25°C; □, 35°C; △, 40°C; ●, 42.5°C; ■, 45°C.
Vertical bars indicate standard error values.

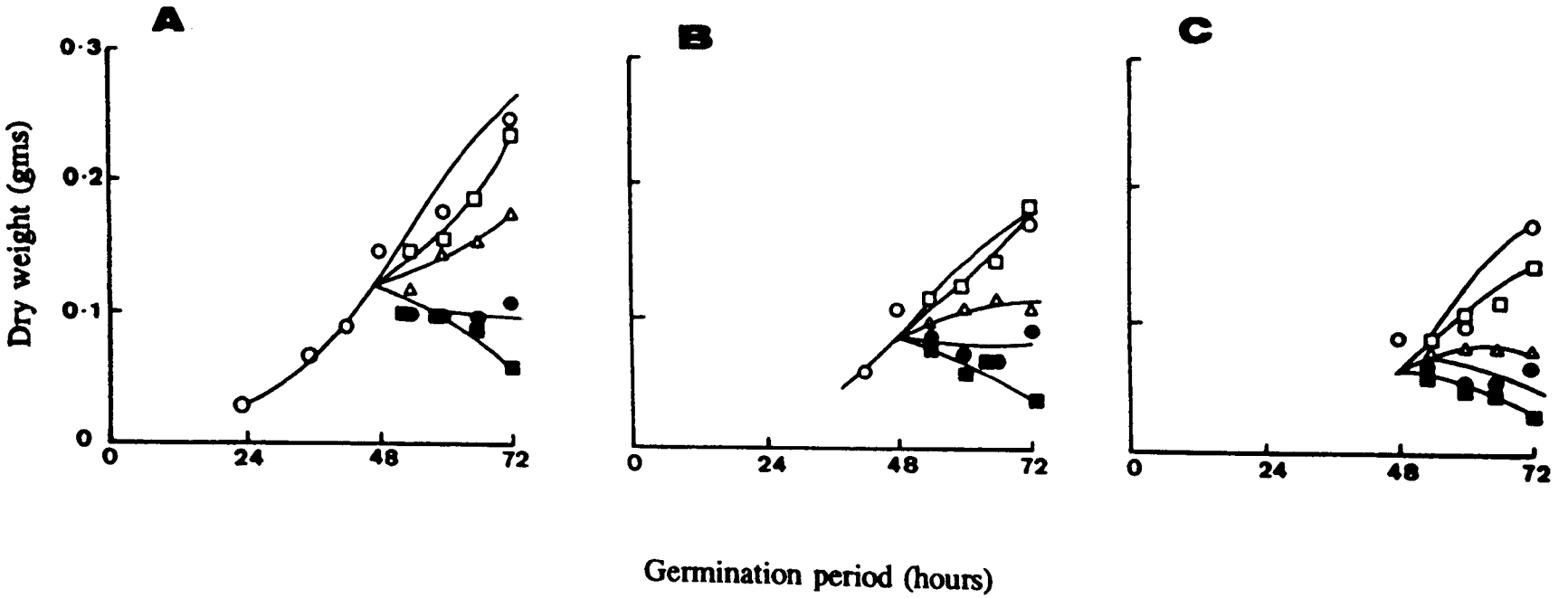


Figure 4.2 Effect of heat shock on seedling dry weight in cultivar MNH-93. (A) first quartile, (B) second quartile, (C) third quartile. ○, 25°C; □, 35°C; △, 40°C; ●, 42.5°C; ■, 45°C.

A statistical analysis of the results for the first, second and third quartiles shows that heat stress at 35°C had no significant effect in any quartile ($P>0.08$). The effects of heat stress at 40, 42.5 and 45°C were always highly significant ($P<0.01$).

The dry weight results are shown in Table 4.2 and in Figure 4.2. The dry weight patterns for the roots of heat stressed seedlings in all the three quartiles were qualitatively very similar to the fresh weight patterns. In all the quartiles, the seedlings heat stressed at 35°C did not show significant differences at 72 hours compared with the 25°C control ($P>0.32$). At shock temperatures of 40°C and above, the dry weights of all three quartiles were affected in a very similar way to that seen for fresh weight. The latter effects were highly significant ($P<0.01$).

4.3.2 Effect of heat shock on total phospholipid levels

The results for the three quartiles are presented in Table 4.3 Figure 4.3. The general pattern for total phospholipid levels was very similar in all the three quartiles. In the first quartile, the phospholipid level rose from 1.3 µmoles $(25 \text{ roots})^{-1}$ at 24 hours to 6.5 µmoles $(25 \text{ roots})^{-1}$ at 72 hours in the control seedlings germinated at 25°C. Seedlings heat stressed at 35°C, however, did not show a significant difference in this pattern ($P=0.9$). The final phospholipid level at 72 hours was possibly significantly reduced by heat-shock at 40°C, however, ($P=0.06$) and above 40°C, the effect was very large and significant ($P<0.001$). Following shock at 45°C, there was actually a loss of phospholipid and the total phospholipid at 72 hours was reduced by 50%.

In the second quartile, the total phospholipid level rose from 2.9 µmoles $(25 \text{ roots})^{-1}$ at 42 hour to 6.0 µmoles $(25 \text{ roots})^{-1}$ at 72

Table 4.3 Effect of heat shock on total phospholipid levels.

Heat-shock temp.(°C)	Total Germ. time(h)	First quartile	Second quartile	Third quartile
25	24	1.30 ±0.15	— —	— —
	36	2.70 ±0.20	— —	— —
	42	4.20 ±0.40	2.90 ±0.30	— —
	48	4.90 ±0.30	4.00 ±0.30	3.20 ±0.20
	60	6.10 ±0.40	4.60 ±0.30	3.50 ±0.30
	72	6.50 ±1.30	6.00 ±1.20	4.60 ±1.10
35	60	6.30 ±0.30	5.00 ±0.30	4.20 ±0.40
	72	6.60 ±0.40	4.70 ±0.90	4.10 ±0.60
40	60	5.60 ±0.50	4.60 ±0.60	3.20 ±0.30
	72	5.20 ±1.00	4.30 ±0.20	3.00 ±0.40
42.5	60	4.40 ±0.20	3.60 ±0.10	2.60 ±0.20
	72	4.60 ±0.30	3.20 ±0.60	3.20 ±0.40
45	60	3.80 ±0.30	2.90 ±0.30	2.20 ±0.30
	72	3.30 ±0.50	2.80 ±0.30	2.00 ±0.40

Each value is the mean lipid phosphorus level(μmoles (25 roots)$^{-1}$) ±sd from 3 to 5 separate experiments.
The experiment was carried out on Cv. MNH-93.

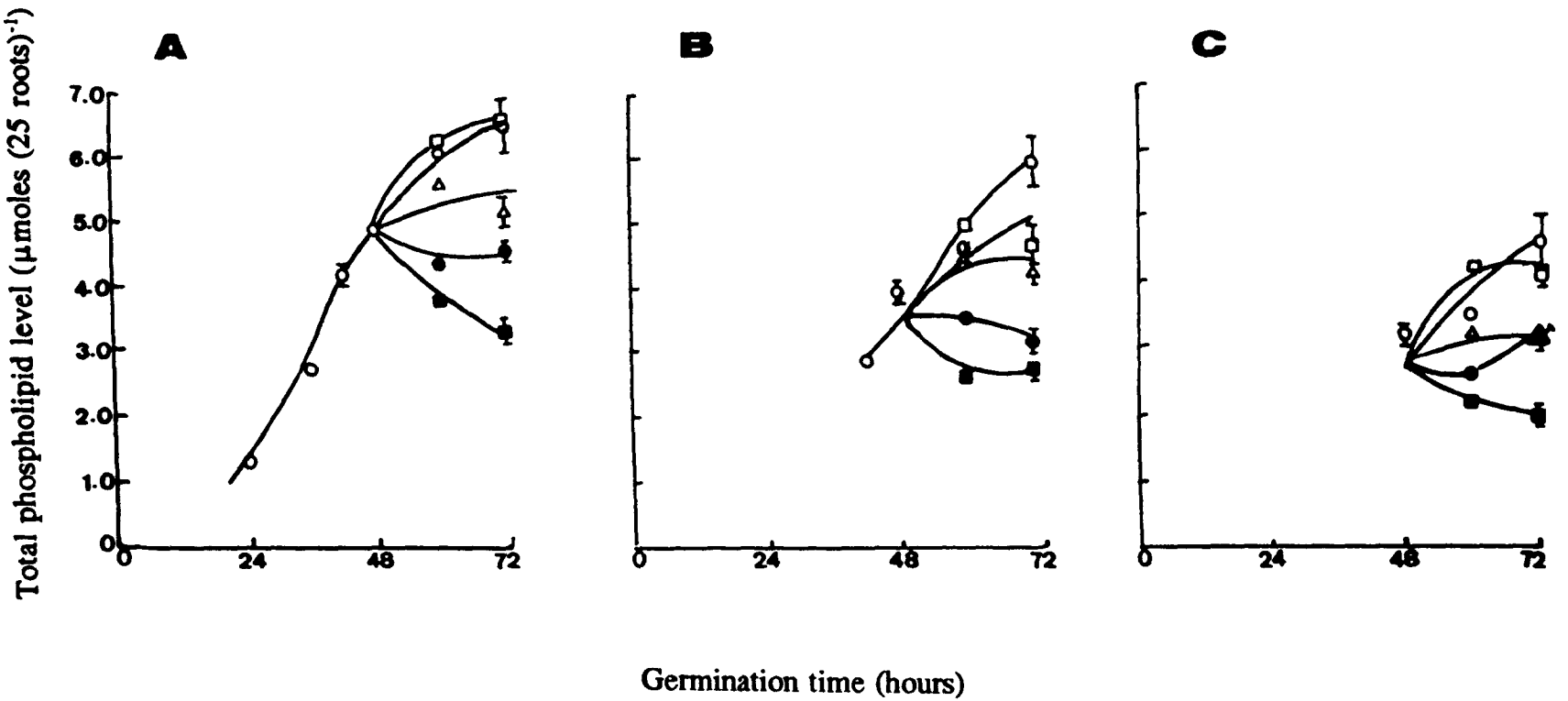


Figure 4.3 Effect of heat shock on total phospholipid levels in cultivar MNH-93. (A) first quartile, (B) second quartile, (C) third quartile.
○, 25°C; □, 35°C; △, 40°C; ●, 42.5°C; ■, 45°C.
Vertical bars indicate standard error values.

hours in the seedlings germinated at 25°C. The effect of heat stress on this quartile was similar to that in the first quartile but with one difference. In this quartile, the phospholipid level at 60 hour was apparently higher in the seedlings heat stressed at 35°C as compared to the corresponding control (P=0.48). At 72 hours, however, the accumulation of total phospholipid was negatively and significantly affected by the heat stress treatment (P<0.03). Following treatment at above 35°C the reduction was very marked at 72 hours. Following shock at 45°C, the total phospholipid was reduced by 53% as compared to its control (P<0.001).

In the case of third quartile, the curves were very similar to the other two quartiles. In the control, seedlings germinated for 48 hours at 25°C showed 3.2 μmoles (25 roots)$^{-1}$ total phospholipid level, which increased to 4.6 μmoles (25 roots)$^{-1}$ at 72 hours. Heat stressed seedlings at 35°C showed a significantly higher phospholipid level at 60 hours as compared with the control (P=0.008). At 72 hours, however, the difference had disappeared. Seedlings heat stressed at 40°C showed clear reductions in phospholipid levels at all times (P<0.006). After treatment at 45°C, the phospholipid was reduced by 57% as compared to the control (P<0.001).

When the results for total phospholipid levels are compared with those for fresh weight and dry weight (see Figures 4.1 and 4.2 and Chapter 3), it can be seen that they are all very similar in all quartiles. Thus, as the shock temperature was increased, the fresh weight, dry weight and total phospholipid levels decreased. The effects became progressively more pronounced as the shock temperature was increased above 35°C.

4.3.3 Preliminary experiments on the uptake and incorporation of (Me-^{14}C) choline

A series of preliminary experiments were carried out to determine the best experimental conditions in which to measure the uptake and incorporation of (Me-^{14}C) choline into the cotton seedling roots. In the first of these experiments, roots were incubated for 15, 30 and 60 minutes in the presence of the radiolabelled precursor. This experiment was carried out at pH 6.7 (see Vakharia, 1986). The results obtained for choline uptake and incorporation are presented in Table 4.4 and in Figure 4.4. Both the uptake and incorporation increased with the increasing incubation time. Thus, at 15, 30 and 60 minutes the uptake was 37250, 50725 and 114913 dpm (25 roots)$^{-1}$ respectively producing a straight line on the graph. The graph for incorporation into phosphatidyl choline showed a hyperbolic curve, however, and incorporation was much slower after 60 minutes of incubation. The incorporation values were 4940, 8870 and 11420 dpm 25 (roots)$^{-1}$ at 15, 30 and 60 minutes respectively. The incorporation/uptake ratio was also reduced at 60 minute compared with 30 minute. A 30 minute incubation period was therefore chosen for the next experiment.

Because the uptake and incorporation values were very low compared with those in wheat (Vakharia, 1986), a further experiment was carried out to examine the effect of pH on the two activities. In this experiment, both uptake and incorporation were measured over a 30 minute period at pH 6.2, 6.7, 7.2 and 7.7. The results are presented in Table 4.5 and in Figure 4.5. Uptake did not vary between pH 6.2 and 6.7 showing values of 50511 and 50725 dpm (25 roots)$^{-1}$ respectively. Uptake increased slightly at pH 7.2 (i.e. 53794 dpm (25 roots)$^{-1}$), but it was sharply reduced at pH 7.7, where it was 15911 dpm (25 roots)$^{-1}$. Choline incorporation into phosphatidyl choline at pH 6.2 and 6.7 also

Table 4.4 Uptake and incorporation of (Me-^{14}C) choline at pH 6.7

	15 min.	30 min.	60 min.
Uptake	37.3±18.0	50.7±6.7	115.0±24.3
Incorporation	4.9±1.3	8.9±1.7	11.4±2.2
I/U ratio	0.15±0.04	0.18±0.04	0.10±0.00

Each value is the mean (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 5 experiments.
The experiment was carried out on Cv. MNH-93.

Table 4.5 Effect of pH on the uptake and incorporation of (Me-^{14}C) choline

	6.2	6.7	7.2	7.7
Uptake	50.5±12.6	50.7±6.7	53.8±8.3	15.9±11.7
Incorporation	9.1±1.9	8.9±1.7	13.7±3.2	4.2±3.4
I/U ratio	0.19±0.04	0.18±0.04	0.26±0.08	0.26±0.02

Each value is the mean uptake or incorporation (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 5 experiments.
The experiment was carried out on Cv. MNH-93.

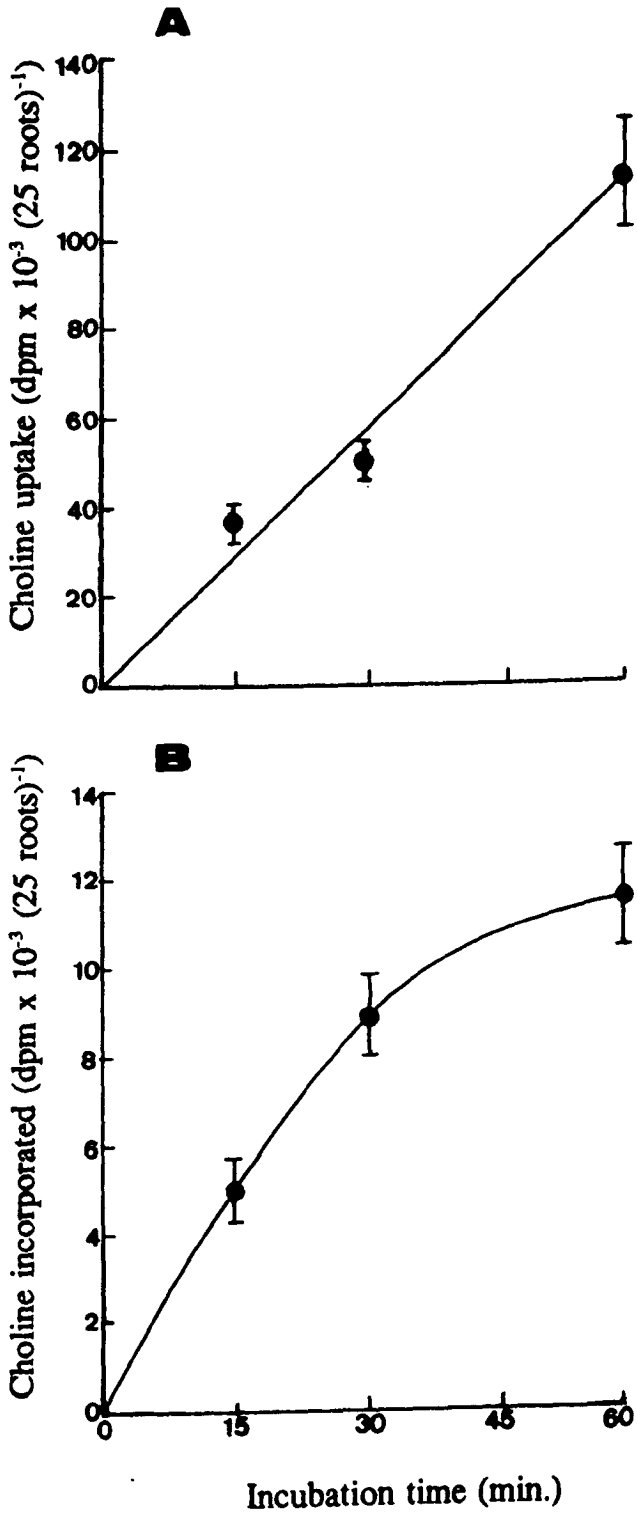


Figure 4.4 Uptake and incorporation of (Me-^{14}C) choline at pH 6.7.(A) uptake, (B) incorporation in cultivar MNH-93.
Vertical bars indicate standard error values.

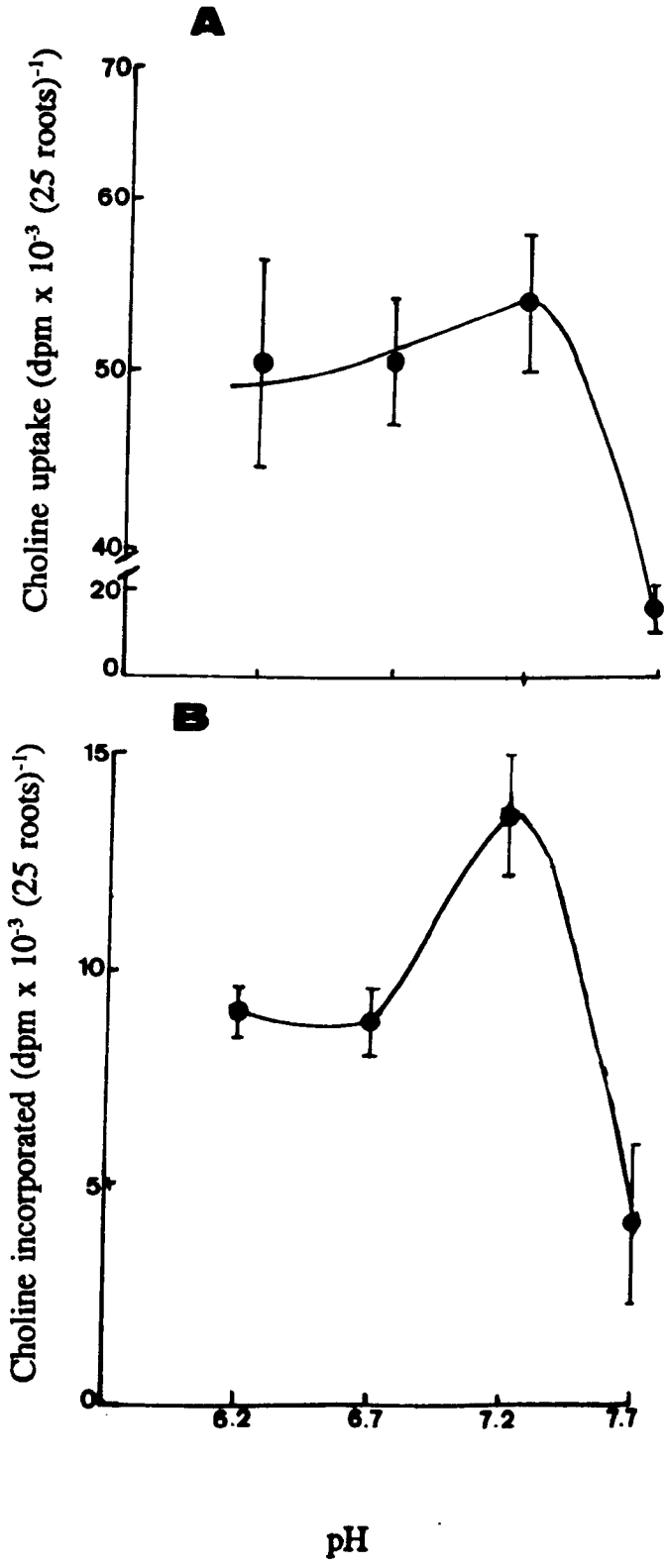


Figure 4.5 Effect of pH on uptake and incorporation of (Me-^{14}C) choline.
(A) uptake, (B) incorporation in cultivar MNH-93.
Vertical bars indicate standard error values.

did not differ, whereas at pH 7.2 it increased reaching a peak of 13707 dpm (25 roots)$^{-1}$. The incorporation was reduced again to 4249 dpm (25 roots)$^{-1}$ at pH 7.7. It is clear from this experiment that the optimum pH for cotton roots is different from the value reported by Vakharia (1986) for wheat aleurone tissue. Therefore, it was decided to carry out all further experiments on cotton at pH 7.2.

The next preliminary experiment was carried out to measure the time course for the uptake and incorporation of (Me-^{14}C) choline at pH 7.2 (the optimum pH for cotton). This experiment was carried out on cotton roots incubated for 15, 30 and 60 minutes at 25°C. Only roots from the first quartile were studied and the results are presented in Table 4.6 and in Figure 4.6. Both the uptake and the incorporation of radioactivity increased with increasing incubation time. At 15, 30 and 60 minutes, the uptake was 22919, 53794 and 95788 dpm (25 roots)$^{-1}$ producing a straight line on the graph. The incorporation values were 5597, 13707 and 24618 dpm (25 roots)$^{-1}$ respectively at 15, 30 and 60 minutes, which also produced a straight line graph. Thus, both uptake and incorporation increased linearly with the incubation time at pH 7.2.

An experiment was also carried out in which a source of calcium or magnesium was presented in an attempt to enhance the uptake and incorporation of the (Me-^{14}C) choline into the roots. Various concentrations of calcium and 0.5 mM magnesium chloride were used in separate experiments. The results of this experiment are shown in Table 4.7. They show that magnesium chloride at the concentration employed inhibited both uptake and incorporation. Calcium chloride at 5mM and 10mM did not have any effect, but calcium sulphate at 0.5mM increased uptake from 53794 to 69356 dpm (25 roots)$^{-1}$. It did not produce any difference in the incorporation of (Me-^{14}C) choline into

Table 4.6 Uptake and incorporation of (Me-^{14}C) choline at pH 7.2

	15 min.	30 min.	60 min.
Uptake	22.9±0.8	53.8±8.3	95.8±2.8
Incorporation	5.6±0.8	13.7±3.2	24.6±0.4
I/U ratio	0.24±0.03	0.26±0.08	0.26±0.01

Each value is the mean uptake or incorporation (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 5 experiments.
The experiment was carried out on Cv. MNH-93.

Table 4.7 Effect of Ca^{++} and Mg^{++} ions on the uptake and incorporation of (Me-^{14}C) choline

	Control	$CaSO_4$ 0.5mM	$CaCl_2$ 5mM	$CaCl_2$ 10mM	$MgCl_2$ 0.5mM
Uptake	53.8±8.3	69.4±5.6	55.3±4.7	46.4±7.0	40.0±2.5
Incorporation	13.7±3.2	12.8±0.5	11.9±1.1	10.7±1.7	9.3±0.8
I/U ratio	0.26±0.08	0.19±0.02	0.22±0.01	0.23±0.02	0.23±0.02

Each value is the mean uptake or incorporation (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 4 experiments.
The experiment was carried out on Cv. MNH-93.

Table 4.8 Effect of pre-incubation on the uptake and incorporation of (Me-^{14}C) choline.

	Control	With pre-incubation
Uptake	53.8±8.3	34.1 29.4
Incorporation	13.7±3.2	7.5 6.2
I/U ratio	0.26±0.08	0.21 0.22

Each control value is the mean uptake or incorporation (dpm x 10^{-3} (25 roots)$^{-1}$) ±sd from 4 experiments.
With pre-incubation, the results of two seperate experiments are presented.
The experiments were carried out on Cv. MNH-93.

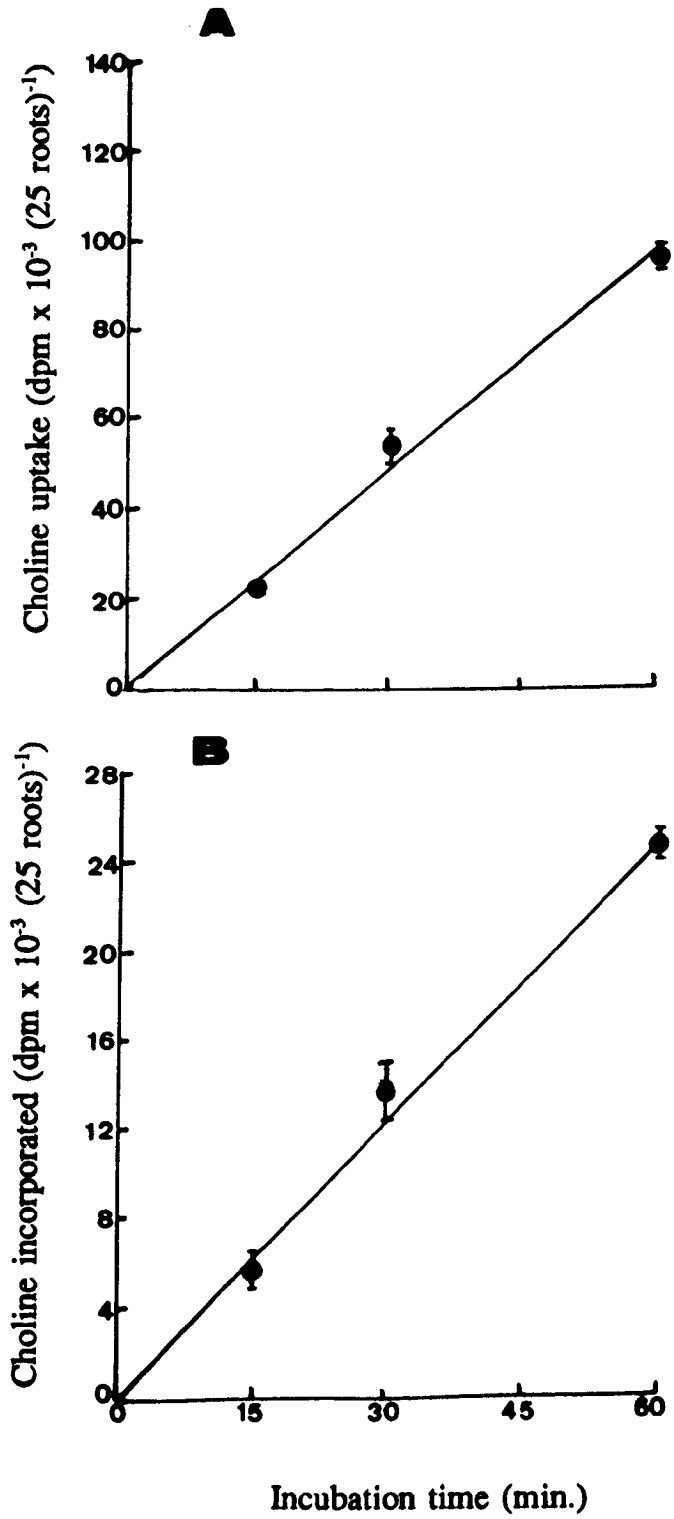


Figure 4.6 Uptake and incorporation of (Me-^{14}C) choline at pH 7.2. (A) uptake, (B) incorporation in cultivar MNH-93.
Vertical bars indicate standard error values.

phosphatidyl choline, however. (The use of the sulphate salt was coincidental and it is assumed that 0.5mM calcium chloride would have produced the same effect). Although its effect on (Me-^{14}C) choline incorporation was small and insignificant (P=0.70), it showed a significant effect on uptake (P=0.04). It was therefore decided to use 0.5mM calcium sulphate in all future experiments.

A final brief experiment was carried out in which the roots were pre-incubated for 30 minutes prior to radiolabelling. For this, the 48 hour seedling roots were placed in a 100 cm^3 sterile conical flask containing 10 cm^3, 50mM tris-maleate buffer, pH 7.2, 0.5mM calcium sulphate and 50 µg cm^3 chloramphenicol. The flask was incubated for 30 minutes at 25°C. After incubation, the roots were removed from the pre-incubated medium and radiolabelled according to the method described in Section 4.2.1. The results are presented in Table 4.8. It is quite obvious from this experiment that pre-incubation reduced the uptake and incorporation of (Me-^{14}C) choline by up to 50%. This step was therefore, not adopted as a routine part of the final procedure.

4.3.4 Effects of heat stress on the uptake and incorporation of (Me-^{14}C) choline in the cultivar MNH-93

Due to similar patterns of developing phospholipid levels being found in the first, second and third quartiles and the effect of temperature on them, it was decided to work only on the first and the third quartiles from the germinated seedlings (see Section 2.6). In this experiment, the heat shock was applied actually during the measurement of (Me-^{14}C) uptake. Thus, roots from 48 hour seedlings were radiolabelled for 15, 30 and 60 minutes at 25°C (control), and for 30 and 60 minutes at 30, 35, 40, 45 and 50°C. After the incubation the roots were washed and extracted as described earlier (Sections 2.10 to

2.12). The results are presented in Table 4.9 and in Figures 4.7 and 4.8.

In both quartiles, uptake and incorporation in the 25°C control increased linearly with increasing incubation time. Increasing the incubation temperature increased the uptake rate but the relationship between uptake and temperature was rather random. In the first quartile, for example, uptake increased with increasing temperature up to 40°C but then declined again at 45 and 50°C (Figures 4.7A & B). In the third quartile, uptake increased progressively with the incubation temperature, but the relationship was still not regular.

In contrast to the picture obtained for uptake, incorporation of (Me-^{14}C) choline into phosphatidyl choline was strongly affected by temperature. In the first quartile (Figure 4.7C), roots incubated at 30 and 35°C were not significantly affected ($P>0.07$) compared with the (25°C) control. The incorporation was significantly decreased at 40°C, however, ($P<0.006$) and above 40°C, the effect became very large ($P<0.001$). At 50°C, incorporation was reduced by 88% at the 60 minute incubation period.

The effect of temperature on the third quartile (Figure 4.7D) was very similar to that on the first quartile but with one minor difference; incorporation at 45°C decreased markedly after 60 minutes incubation so that a non-linear uptake pattern was obtained. At 50°C, incorporation was reduced by 83% over a 60 minute incubation.

There is a possibility that the data for the uptake of (Me-^{14}C) choline in Table 4.9 and Figure 4.7 are the results of combined effects of temperature on both uptake and incorporation. In order to get more accurate assessment of the effect of temperature on the incorporation alone, it was decided to calculate uptake/incorporation ratios. These values are given in Table 4.9 and they are presented

Table 4.9 Effect of temperature on the uptake and incorporation of (Me-^{14}C) choline

Temp. (°C)		First quartile		Third quartile	
		30 min.	60 min.	30 min.	60 min.
25	Uptake	49.4±7.3	112.3±19.2	33.5±5.8	83.0±12.5
	Incorp.	9.3±1.1	23.0±1.6	7.3±0.7	13.6±0.8
	I/U ratio	0.19±0.00	0.21±0.03	0.22±0.03	0.17±0.03
30	Uptake	-- --	143.0±12.0	-- --	110.0±15.2
	Incorp.	-- --	19.4±1.3	-- --	12.2±0.7
	I/U ratio	-- --	0.14±0.02	-- --	0.12±0.02
35	Uptake	-- --	136.6±15.8	-- --	98.6±10.7
	Incorp.	-- --	18.9±0.9	-- --	12.2±2.0
	I/U ratio	-- --	0.14±0.01	-- --	0.12±0.01
40	Uptake	80.4±10.0	182.3±5.3	56.0±14.4	118.5±5.6
	Incorp.	6.1±0.1	14.8±1.2	4.4±1.0	9.8±0.7
	I/U ratio	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
45	Uptake	68.6±10.3	136.6±7.5	49.6±3.8	125.2±11.6
	Incorp.	5.0±0.7	8.6±0.3	4.5±0.7	6.0±0.5
	I/U ratio	0.07±0.02	0.06±0.02	0.09±0.00	0.05±0.01
50	Uptake	68.0±5.8	125.8±18.3	50.2±5.8	138.2±6.8
	Incorp.	1.7±0.2	2.8±0.7	1.3±0.2	2.4±0.1
	I/U ratio	0.025±0.0	0.022±0.0	0.026±0.01	0.017±0.0

Each value is the mean uptake or incorporation (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 5 experiments.
The experiment was carried out on Cv. MNH-93.

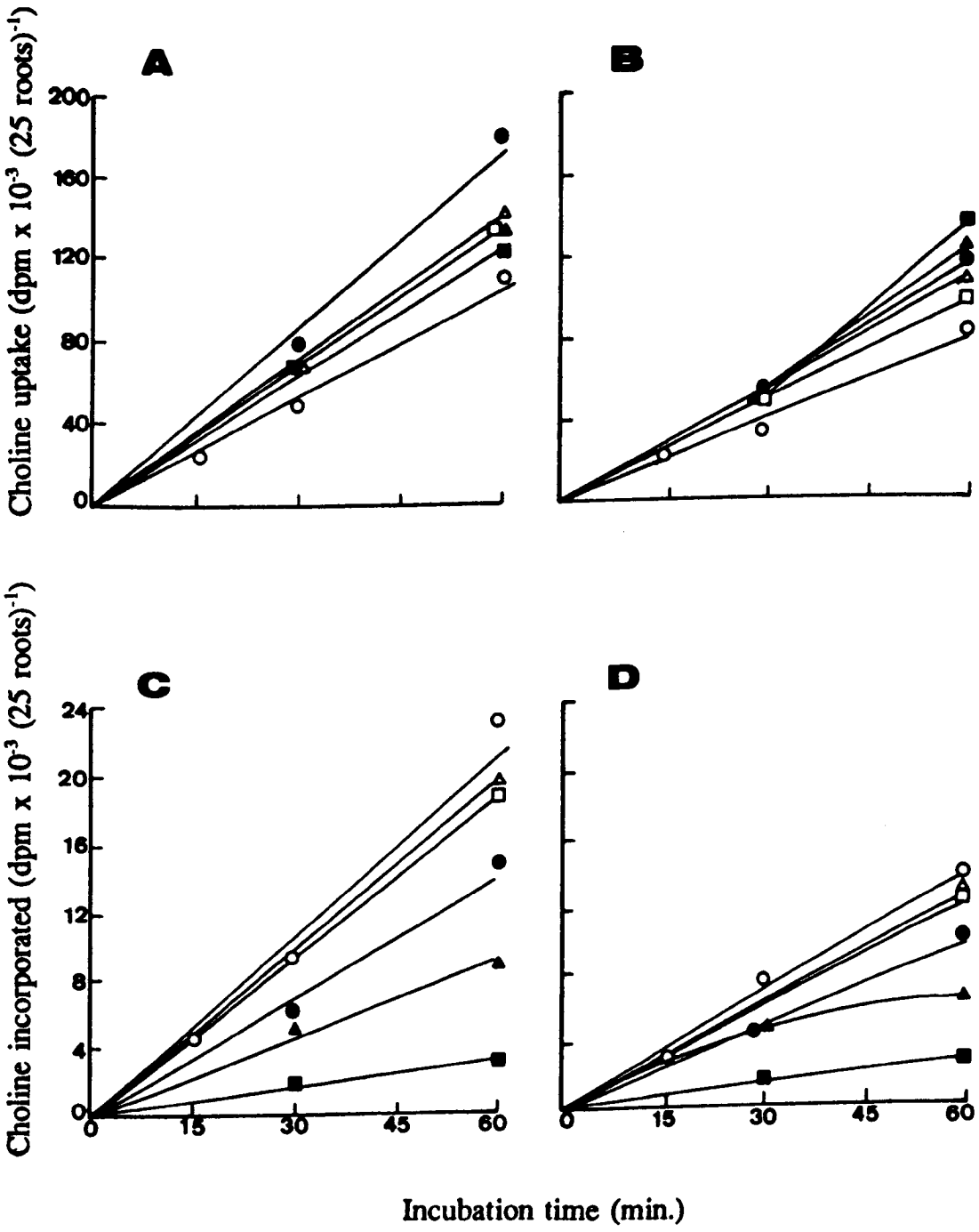


Figure 4.7 Effect of temperature on uptake and incorporation of (Me-^{14}C) choline in cultivar MNH-93. (A) uptake, first quartile, (B) uptake, third quartile, (C) incorporation, first quartile, (D) incorporation, third quartile. ○, 25°C; △, 30°C; □, 35°C; ●, 40°C; ▲, 45°C; ■, 50°C.

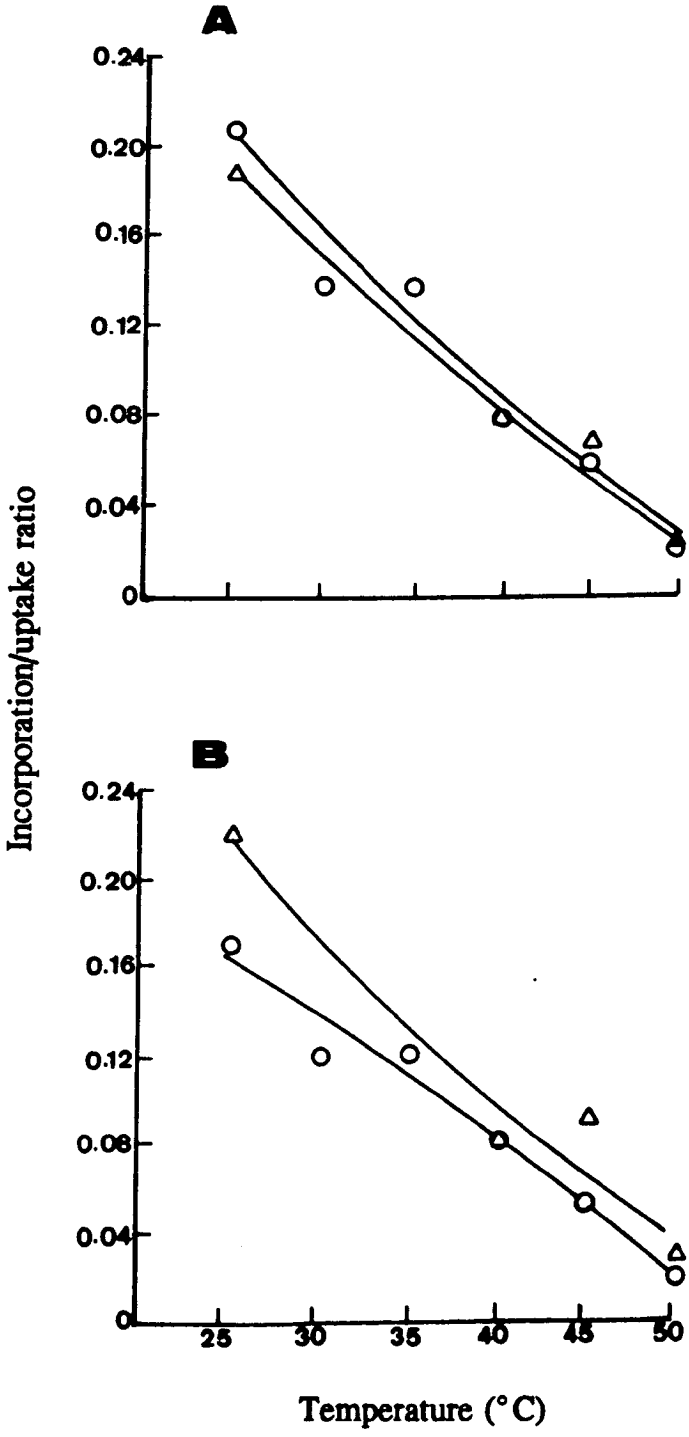


Figure 4.8 Effect of temperature on (Me-^{14}C) choline incorporation/uptake ratios in cultivar MNH-93. (A) first quartile, (B) third quartile. Δ, 30 min.; ○, 60 min.

graphically in Figure 4.8. It can be seen that the effects of heat stress on both quartiles were very similar, with the curves following the same pattern. Thus, as the temperature was increased above 30°C the incorporation/uptake ratio decreased progressively.

4.3.5 Effect of heat stress on the uptake and incorporation of (Me-^{14}C) choline in four different cotton cultivars

Because of the similar effect of heat stress on (Me-^{14}C) choline uptake and incorporation on the first and third quartiles of MNH-93 (Section 4.3.4 above), it was decided to estimate the rates of synthesis of phospholipids in only the first quartile of four cultivars of cotton. Since uptake and incorporation have been shown to be linear with time, it was also decided for this experiment to use only the 60 minutes incubation period. The results for the uptake and incorporation of (Me-^{14}C) choline in the different cultivars are presented in Table 4.10 and in Figures 4.9 and 4.10.

At the control temperature of 25°C, the different cultivars showed different uptake activities (Table 4.10 and Figure 4.9). Cultivars MNH-93 and Qalandari showed higher uptake, having values of 112303 and 105233 dpm (25 roots)$^{-1}$ respectively, while cultivars Rehmani and S-12 had lower uptake values of 73710 and 63303 dpm (25 roots)$^{-1}$ respectively. Uptake was affected at higher temperatures, but the effects were not clear cut and it was difficult to explain the results (see Figure 4.9). To overcome this problem, several curve-fitting methods from the Systat/Sygraph computer software package were tried. Best fit curves for the plots of uptake against temperature were eventually obtained using the DWLS (Distance Weighted Least Square) programme (Section 3.2). It can be seen from the graphs (Figure 4.9) that these curves were generally similar for each

Table 4.10 Effect of temperature on uptake and incorporation of (Me-^{11}C) choline in different cultivars.

Temp.(°C)		MNH-93	Qalandri	Rehmani	S-12
25	Uptake	112.3±19.2	105.2±10.4	73.7±4.4	63.3±2.3
	Incorp.	23.1±1.60	19.4±2.7	16.7±0.5	14.7±2.8
	I/U ratio	0.21±0.03	0.19±0.02	0.23±0.02	0.24±0.04
30	Uptake	143.0±12.0	136.4±23.5	89.2±26.5	110.4±6.7
	Incorp.	19.4±1.3	16.0±1.9	17.9±4.7	14.8±1.1
	I/U ratio	0.14±0.02	0.12±0.01	0.20±0.02	0.13±0.01
35	Uptake	136.6±15.9	143.8±45.5	138.4±26.1	89.7±17.4
	Incorp.	18.9±0.9	13.4±2.4	15.2±2.7	11.8±0.8
	I/U ratio	0.14±0.01	0.10±0.02	0.11±0.01	0.14±0.01
40	Uptake	182.3±5.3	88.6±9.3	145.5±32.0	106.6±1.2
	Incorp.	14.8±1.2	6.3±1.1	11.3±2.5	8.3±0.9
	I/U ratio	0.08±0.01	0.07±0.02	0.08±0.01	0.08±0.00
45	Uptake	136.6±7.5	137.7±20.6	78.1±17.1	86.3±4.1
	Incorp.	8.6±0.3	7.4±1.6	5.3±0.5	4.7±0.2
	I/U ratio	0.06±0.00	0.05±0.01	0.07±0.01	0.05±0.00
50	Uptake	125.8±18.3	82.3±4.2	103.0±18.0	74.0±18.3
	Incorp.	2.8±0.7	2.1±0.1	2.9±0.4	2.3±0.08
	I/U ratio	0.022±0.00	0.024±0.00	0.03±0.00	0.03±0.01

Each value is the mean uptake and incorporation (dpm x 10^{-3}(25 roots)$^{-1}$) ±sd from 3 to 5 experiments.

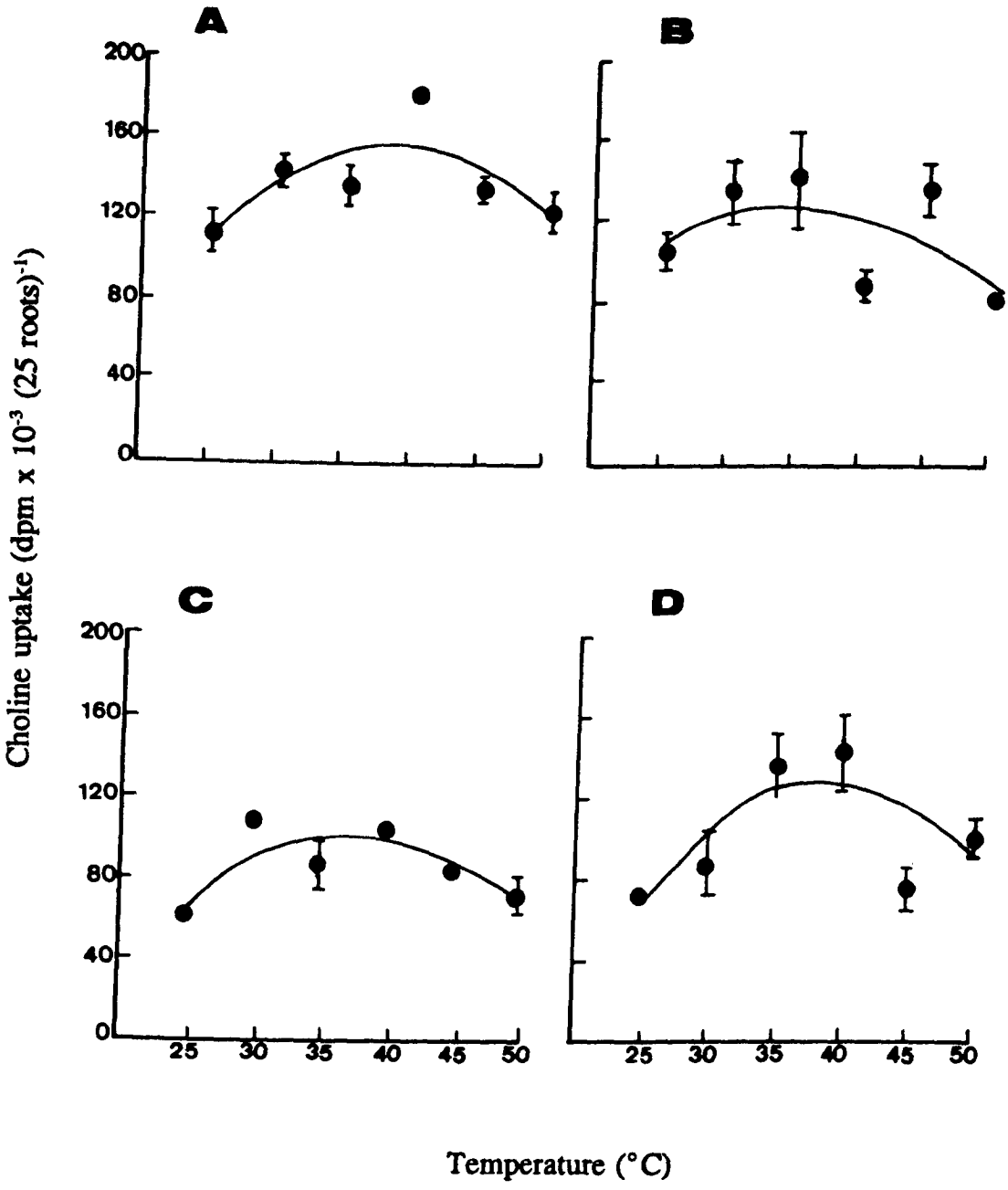


Figure 4.9 Effect of temperature on uptake of (Me-^{14}C) choline in various cotton cultivars. (A) MNH-93, (B) Qalandari, (C) S-12, (D) Rehmani. Vertical bars indicate standard error values.

cultivar. They show that, at temperatures up to about 40°C, uptake appears to increase, while above 40°C the uptake of (Me-^{14}C) choline declined. The effects are not large, however, and there is a noticeable scatter of the plotted points.

At 25°C (control), the different cultivars also showed different incorporation activities. The incorporations of (Me-^{14}C) choline into phosphatidyl choline in the cultivars MNH-93, Qalandari, Rehmani and S-12 were 23062, 19434, 16720 and 14707 dpm (25 roots)$^{-1}$ respectively (Table 4.10). The incorporation was adversely affected at 35°C and above in MNH-93 and Qalandari, while in the cultivars Rehmani and S-12 the incorporation was affected only at 40°C and above. At 45°C, the incorporation in all the four cultivars was strongly affected. Above this temperature, the effect of heat stress on all the cultivars was large and very similar. At 50°C, incorporation was reduced by more than 80% in all the cultivars. (Figure 4.10).

A statistical analysis of the data in Table 4.10 reveals that the incorporation in MNH-93 and Qalandari was not significantly affected at 30°C ($P>0.09$), but it was affected at 35°C ($P<0.05$) and more strongly at 40 to 50°C ($P<0.005$). In S-12 and Rehmani, incorporation was not significantly affected at either 30 or 35°C ($P>0.24$). In both of these cultivars, it was significantly affected at 40°C ($P<0.05$) and at 45 or 50 ($P<0.001$).

Figure 4.10 shows the effect of heat stress on (Me-^{14}C) choline incorporation in the four cotton cultivars in graphical form. Despite the above statistical analysis, it is difficult to see real differences between the various cultivars. To overcome this problem, a decision was taken to calculate TII-50 values (Temperature Inhibiting Incorporation by 50%; cf. TIG-50 value in Section 3.3.1).

The TII-50 values were calculated as follows: On each graph, a

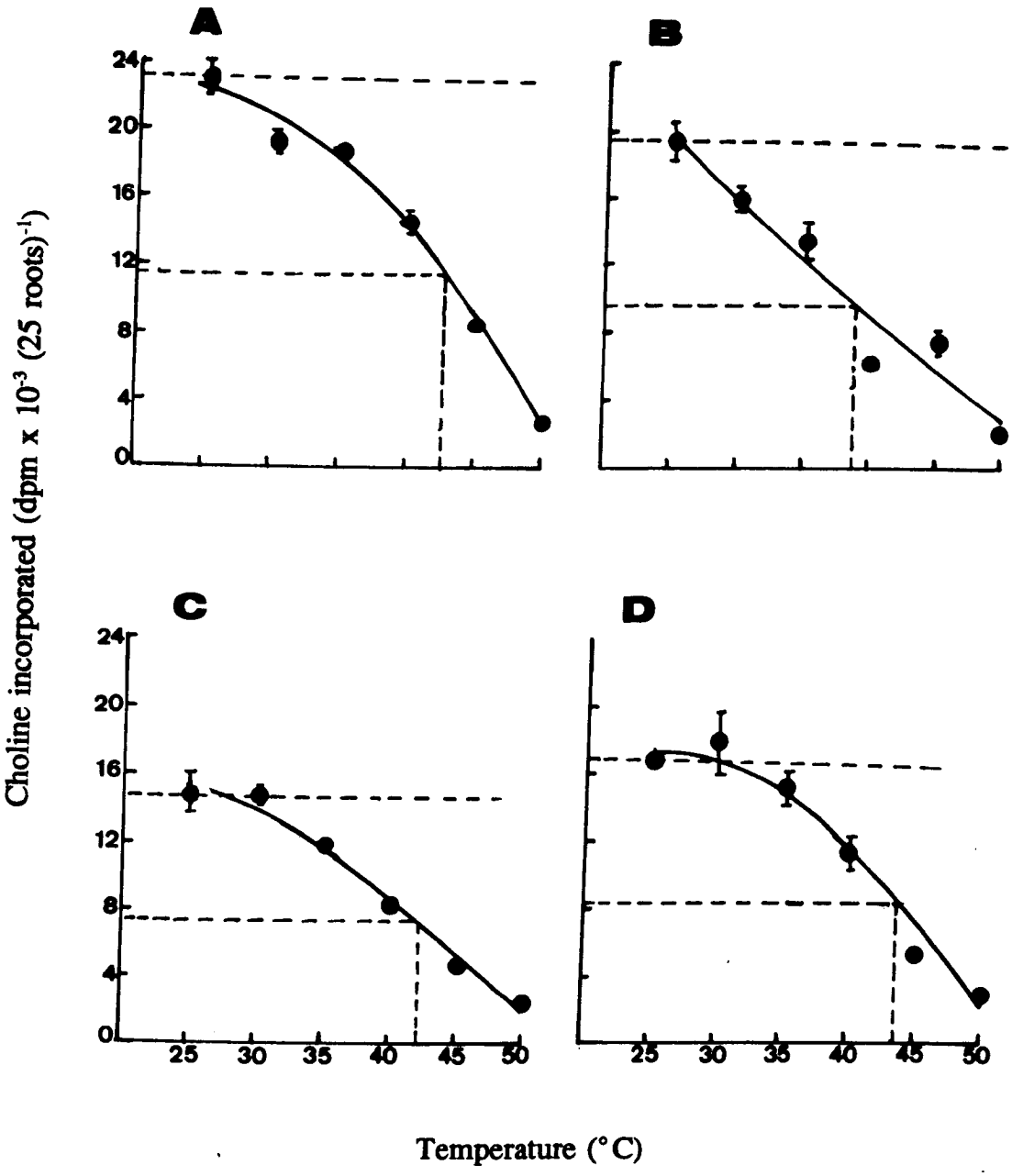


Figure 4.10 Effect of temperature on incorporation of (Me-^{14}C) choline in various cotton cultivars. (A) MNH-93, (B) Qalandari, (C) S-12, (D) Rehmani. Vertical bars indicate standard error values.

Table 4.11 Temperature at which (Me-^{14}C) incorporation is inhibited by 50 percent (TII-50) in various cultivars of cotton

Cultivar	Incorporation TII-50	I/U ratio TII-50
MNH-93	43.0	37.5
Qalandari	39.0	35.2
Rehmani	43.7	36.6
S-12	42.4	35.0

The TII-50 values (°C) are calculated from Figure 3.10.

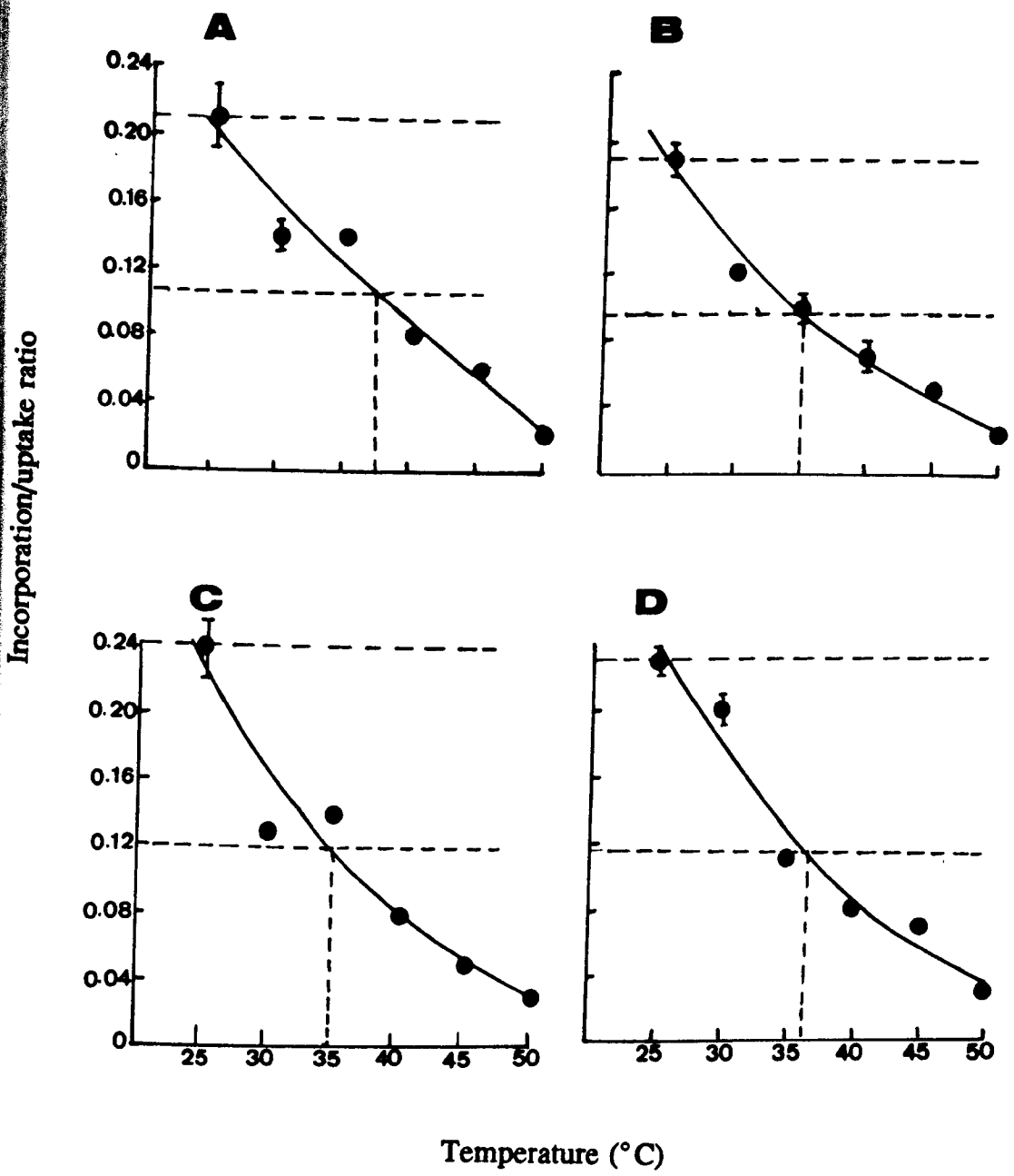


Figure 4.11 Effect of temperature on (Me-^{14}C) choline incorporation/uptake ratio in various cotton cultivars. (A) MNH-93, (B) Qalandari, (C) S-12, (D) Rehmani. Vertical bars indicate standard error values.

horizontal line was drawn corresponding to the control incorporation of (Me-^{14}C) choline at 25°C. Then, a second horizontal line was drawn half way between the base line (x-axis) and the 25°C control line. Where this line cut the plotted curve, a vertical line was dropped to the x axis. The point where this vertical line intercepts the x axis is the temperature at which (Me-^{14}C) choline incorporation is inhibited by 50%. This is defined as the TII-50 value. The TII-50 values for MNH-93, S-12, Rehmani and Qalandari were 43.0, 42.4, 43.7 and 39.0 respectively (Table 4.11). It is quite obvious that there are only small differences between these values for the four cultivars. Qalandari showed somewhat less tolerance to high temperature than other cultivars.

When the incorporation:uptake ratios were calculated, the effects of temperature on uptake could be seen more clearly (Table 4.10 and Figure 4.11) and it became obvious that all the cultivars were similarly affected by high temperature. As the temperature increased, the incorporation:uptake ratio decreased. The TII-50 values of ratios were also calculated. The TII-50 values for MNH-93, S-12, Rehmani and Qalandari were 37.5, 35.0, 36.6 and 35.2 respectively (Table 4.11).

4.4 Discussion

All germinating seeds most probably have similar patterns of developing phospholipid levels. In the case of cotton seeds germinating at 25°C, the pattern was very similar to that for wheat seedlings observed by McDonnell (1980). Thus, the phospholipid levels in the roots increase 5-fold from 24 hour to 72 hour, while the wheat seedlings showed a 7-fold increase over a similar period. This sharp increase presumably arises due to synthesis of new membranes

associated with cell enlargement and cell division. El-Nockrashy *et al.* (1974) reported that, as cotton seed germination proceeds, there was a continuous increase in the percentage of total phospholipids.

In the first experiment on MNH-93, the difference in phospholipid levels between the first, second and third quartiles became apparent at 60 hours and were even greater at 72 hours. The first quartile roots accumulated more phospholipids than either the second or the third quartile, and the second quartile accumulated more phospholipids than the third quartile. These results suggest a direct correlation relationship between the growth of roots and the development of phospholipids-containing membranes. It further indicates that the second and third quartiles have less membranes than the first quartile. This is presumably due to lower rates of protein and mRNA synthesis which result in slower rates of cell enlargement and cell division.

An attempt was made to present the results for total phospholipids per gram fresh weight or per gram dry weight. When this was done, however, very scattered results were obtained which were difficult to interpret. This was probably due to effect on all the three parameters of fresh weight, dry weight and total phospholipid levels. Therefore, it was decided to present all the results per 25 roots.

In contrast to the results of phospholipids, when the results of (Me-^{14}C) choline uptake and incorporation were calculated and plotted either as per gram fresh weight or per gram dry weight, these results showed similar pictures to the per 25 roots data. To make uniform pattern presentation, however, these results are also presented on a per 25 roots basis.

Severe heat shock to the cotton seedling roots resulted in a

loss of fresh weight, dry weight and total phospholipids, possibly due to effects on membrane integrity. Raison *et al.* (1980) has reported that membranes are primary sites for high temperature injury. Tischner *et al.* (1978) has also reported that phospholipids levels in *Synchronous chlorella* are reduced by heat shock.

From these results (Figures 4.1 to 4.3), two effects could be observed due to heat stress. The first effect was one in which the growth of seedlings was slowed down by treatment at 35 and 40°C resulting in the reduction of fresh and dry weight gains. The situation for phospholipid level was similar. The second effect was one in which an actual loss of fresh weight, dry weight and total phospholipids was observed following heat shock at 42.5 and 45°C. This was probably due to tissue damage including damage to the membranes. Quinn (1988) has previously observed that high temperature is damaging to membrane structure.

Another interesting feature of the MNH-93 results for fresh weight, dry weight and phospholipid levels was that the second quartile was apparently less tolerant than the first quartile to the treatment at 35°C. Furthermore, the third quartile appeared to be generally less tolerant than the second quartile. The effects of higher temperature also had a similar effect and we can assume that the third quartile is less tolerant than the second and first quartiles and the second is less tolerant than the first quartile in this respect. When these results were analysed by the student's T-tests, however, all of the differences proved to be not significant. It is interesting, nevertheless, that other workers have reported results indicating such relationships. Sethar (1993), also working on cotton seedling roots, reported that high temperature caused more electrolyte leakage from shorter roots than longer roots and that

their distal halves leaked more than proximal halves. Similarly with regard to cold shock, Dexter *et al.* (1932) reported that alfalfa roots exposed to 5°C showed greater electrolyte loss. They further added that longer roots seem to be distinctly more cold tolerant than the smaller roots as measured by electrolyte leakage.

The lipid contents of the cotton roots will only be decreased if the rate of break down is greater than the rate of synthesis of new lipids, combined with the ability of the cell to incorporate the new lipids into membranes. Benzioni and Itai (1973) reported that damage to the membranes will affect the activities of membrane-bound enzymes as well as compartmentation of metabolites in the tissue. The latter effect may cause a rise in catabolic activity. In addition to this, they stated that the changes in membrane integrity caused by heat treatment could be at least partly due to the effect of heat on membrane biosynthesis.

From earlier studies, it has been reported that phosphatidyl choline is the major phospholipid of non-chloroplast membranes in higher plants (Harwood, 1980; Ansel and Spanner, 1982). It has also been shown that (Me-^{14}C) choline is incorporated exclusively into phosphatidyl choline (Kagawa *et al.*, 1973; Varty, 1975; Mirbahar, 1981). It was for this reason that the uptake of (Me-^{14}C) choline into cotton roots and its incorporation into phosphatidyl choline was measured in the present study. It is interesting in this context that Yarden and Mayer (1981) observed that during the rapid increase in membrane phospholipids in pea seedlings (*P. sativum* and *P.elatius*), free choline content also increased very rapidly. Increases in the choline content of seeds have also been reported in germinating wheat by Chittenden *et al.* (1978).

It was quite clear from the present results that the effect of

heat shock on (Me-^{14}C) choline incorporation into phosphatidyl choline was similar in both the first and third quartiles of the MNH-93 seedlings.

The results tabulated in Table 4.10 suggest that temperature above 35°C had significant effect on (Me-^{14}C) choline uptake and incorporation. Thus, as the temperature increased above 35°C, the rate of synthesis of phosphatidyl choline decreased. At 50°C, however, more than 80% of the phosphatidyl choline synthesis was inhibited in all cultivars. There have been only a few studies of the effects of high temperature on lipids, but the literature available on the subject supports our observations. Thus, it has been reported that high temperature inhibits the synthesis of phosphatidyl choline. Benzioni and Itai (1973); Ordin *et al.* (1974); Tischner *et al.* (1978) reported that reduction in tobacco, *Phaseoulus vulgaris* and *Synchronous chlorella,* phospholipids biosynthesis occurred at high temperatures. Lin (1990) reported that exposure of *Phaseoulus vulgaris* seeds to 100% relative humidity at 45°C for 1-4 days reduced the total phospholipid level. She further added that reduction in phospholipids is due primarily to degradation of phosphatidyl choline.

The present effects of high temperature could be due to the direct effect of temperature on the biosynthetic enzyme systems involved. Membrane-bound enzymes might be expected to be especially sensitive to temperature change due to their dependency on the correct degree of fluidity in the membrane. Lin *et al.* (1984) reported that high temperature reduces the rate of normal protein synthesis and induces the synthesis of heat-shock proteins. Key *et al.* (1981) found that soybean seedlings shifted from a normal growth temperature of 28°C up to 40°C similarly showed dramatic changes in protein synthesis including the synthesis of heat-shock proteins.

A statistical analysis of the raw data in Table 4.10 indicated that MNH-93 and Qalandari are less heat tolerant than S-12 and Rehmani with respect to phosphatidyl choline synthesis. From the TII-50 values for the four cultivars, however, it appears that there is no real difference between the three cultivars MNH-93, Rehmani and S-12. Qalandari appeared to be more sensitive to heat stress than the other cultivars however. It showed a 3°C difference in TII-50 compared with the other three cultivars. When TII-50 values for incorporation/uptake ratios were determined, however, the 3°C difference of Qalandari disappeared.

It may be concluded from this experiment that there is no real difference between the four cotton cultivars with respect to their heat tolerance.

CHAPTER FIVE

Chapter Five

Effects of Heat Shock on Fatty Acid Composition

5.1 Introduction

Under normal conditions, the polar part of the lipid molecule is localised at the outer faces of biological membranes, with the fatty acyl 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). In most plants, the five major fatty acids, i.e. palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), account for 95-98% of the total fatty acids.

Temperature has been identified as an important environmental factor affecting the fatty acid composition in seeds (Tremolieres *et al.*, 1978), roots (Willemot, 1975; Simolenska and Kuiper, 1977) and leaves (Wilson and Crawford, 1974; St. John and Christiansen, 1976). Several workers have observed that the proportions of these fatty acids are strongly influenced by high temperatures (Slack and Roughan, 1978; Wolf *et al.*, 1982; 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.

The membrane lipids maintain their fluidity under normal growing conditions for the functioning of membrane-bound enzymes such as ATPase (Grisham and Barnett, 1973). The change in the degree of unsaturation of the lipids of organisms grown at different temperatures is associated with this property of membrane fluidity (Raison *et al.*, 1980; Harwood, 1983). This in turn directly affects the activity of fatty acyl desaturase enzymes (Brenner, 1984). Changes to lower temperature can cause phase transition from fluid to solid state in the membrane lipids, which would induce a conformational change in the enzyme and affect its activation energy (Lyons, 1973). Harris and James (1969a and b) suggested that temperature could also influence the rate of fatty acid desaturation through an effect on oxygen solubility in the membrane lipid phase. Desaturase mechanisms leading to linoleic and linolenic acid formation were found to be temperature-dependant in cell cultures of *Catharanthus roseus G. Don, Glycine max* (L.) *Merr.* and *Nicotiana tobacum* L. and the optimum temperatures varied with the cell lines (MacCarthy and Stumpf, 1980).

Kuiper (1984) reported that changes in membrane lipid composition may also be attributed to stress-induced degradation processes. Enzymes such as phospholipase and lipoxygenase may be involved in these degradation processes. Mishra and Singhal (1992) stated that 8-10 day old wheat seedlings exposed to temperatures of 35-45°C and high light intensity showed an increase in the peroxidation of thylakoid lipids. They further reported that the lipid peroxidation leads to the oxidative degradation of polyunsaturated fatty acyl residues in the thylakoid lipids.

In our own earlier experiments (Chapter 4) there was evidence

that 2 hours heat-shock at 40°C reduced total phospholipid levels and also reduced the synthesis of the phosphatidyl choline. 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.

5.2 Methods

5.2.1 Determination of fatty acid composition

In these experiments, seeds of two cultivars MNH-93 and S-12, were used from the 1992-93 harvests. Details of the procedures employed are described in the Material and Methods chapter. Briefly, 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 (Section 2.7). 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 (Section 2.6) were harvested and their roots were isolated from the cotyledons using a scalpel. The root lipids were then extracted using hot water-saturated butanol (Section 2.11). After extraction, the crude lipid was purified by chromatography on columns of Sephadex G-25 (Section 2.12). Free fatty acids and esterified fatty acids were separated from the purified lipid fraction (Section 2.13) and the esterified fatty acids were further separated into neutral lipid, glycolipid and phospholipid fractions by chromatography on columns of silicic acid (Section 2.14). Finally, fatty acid methyl esters were prepared from the various fractions and analysed by gas-liquid chromatography (Section 2.17).

Each experiment was duplicated and each fatty acid analysis

within each experiment was also duplicated. The values present in these experiments are means ±sd from the four individual values.

The resulting data were analysed statistically as described in Section 2.18. All the graphs were plotted using a personal computer with the Quattro Pro 3 (Borland International Inc.).

5.3 Results

In addition to the expected fatty acid methyl esters, a few low molecular weight peaks were found in the chromatograms from some tissues, especially in neutral lipids and glycolipids (see Figure 2.2). The appearance of some of the peaks was spasmodic, however, and they are therefore not included in our calculations. On the other hand, 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.

5.3.1 Effect of heat shock on fatty acid composition in the cultivar S-12

The results from this experiment are shown in Tables 5.1 and 5.2 and in Figures 5.1 to 5.5.

The levels of all fatty acids in the various fractions are presented in Table 5.1 and in Figure 5.1. The total amount of esterified plus free fatty acids at 48 hour germination was 1970 µg (25 roots)$^{-1}$, which increased slightly in the 72 hour control to 2110 µg (25 roots)$^{-1}$. In the seedlings heat shocked for 2 hours there was a significant increase ($P=0.02$) to 2200 µg (25 roots)$^{-1}$, but after recovery the level fell again to below the 72 hours control. The difference between the level at the end of the recovery and the 72 hour control value was near to the significance level ($P=0.06$).

A marked increase in neutral lipid fatty acids was found during germination from 48 to 72 hours. The increase was from 348 to 439 µg (25 roots)$^{-1}$. However, because the latter value had a large standard deviation, the significance of the increase could not be proved ($P=0.12$). Seedlings heat shocked for 2 hours at 40°C showed a significant increase ($P=0.04$) in the neutral lipid fatty acids to 650 µg (25 roots)$^{-1}$. At the end of the recovery period following heat shock, the level had declined again to 445 µg (25 roots)$^{-1}$.

During germination between 48 and 72 hours, the glycolipid fatty acids apparently increased from 75 to 107 µg (25 roots)$^{-1}$. The increase was not statistically significant, however ($P=0.28$). Seedlings heat shocked for 2 hours did not show any significant increase in this fraction. Moreover, at the end of the recovery following heat shock, the glycolipid fatty acids had decreased compared with the 72 hour control value. Again, the decrease was not significant ($P=0.18$).

The amount of phospholipid fatty acids remained unchanged

Table 5.1 Effects of heat shock on the esterified and free fatty acids in the cultivar S-12

Lipid fraction	Total germ. time (h)	Fatty acid content µg (25 roots)$^{-1}$
Neutral lipids	48h control	348 ±82
	72h control	439 ±29
	After heat shock	650 ±180
	After recovery	445 ±92
Glycolipids	48h control	75 ±16
	72h control	107 ±44
	After heat shock	84 ±8
	After recovery	66 ±12
Phospholipids	48h control	1520 ±104
	72h control	1505 ±198
	After heat shock	1440 ±257
	After recovery	1405 ±76
Free fatty acids	48h control	27 ±6
	72h control	62 ±2
	After heat shock	25 ±2
	After recovery	22 ±3
Combined acyl-Lipids	48h control	1940 ±60
	72h control	2050 ±150
	After heat shock	2170 ±120
	After recovery	1920 ±40
Total lipids	48h control	1970 ±60
	72h control	2110 ±150
	After heat shock	2200 ±130
	After recovery	1940 ±30

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

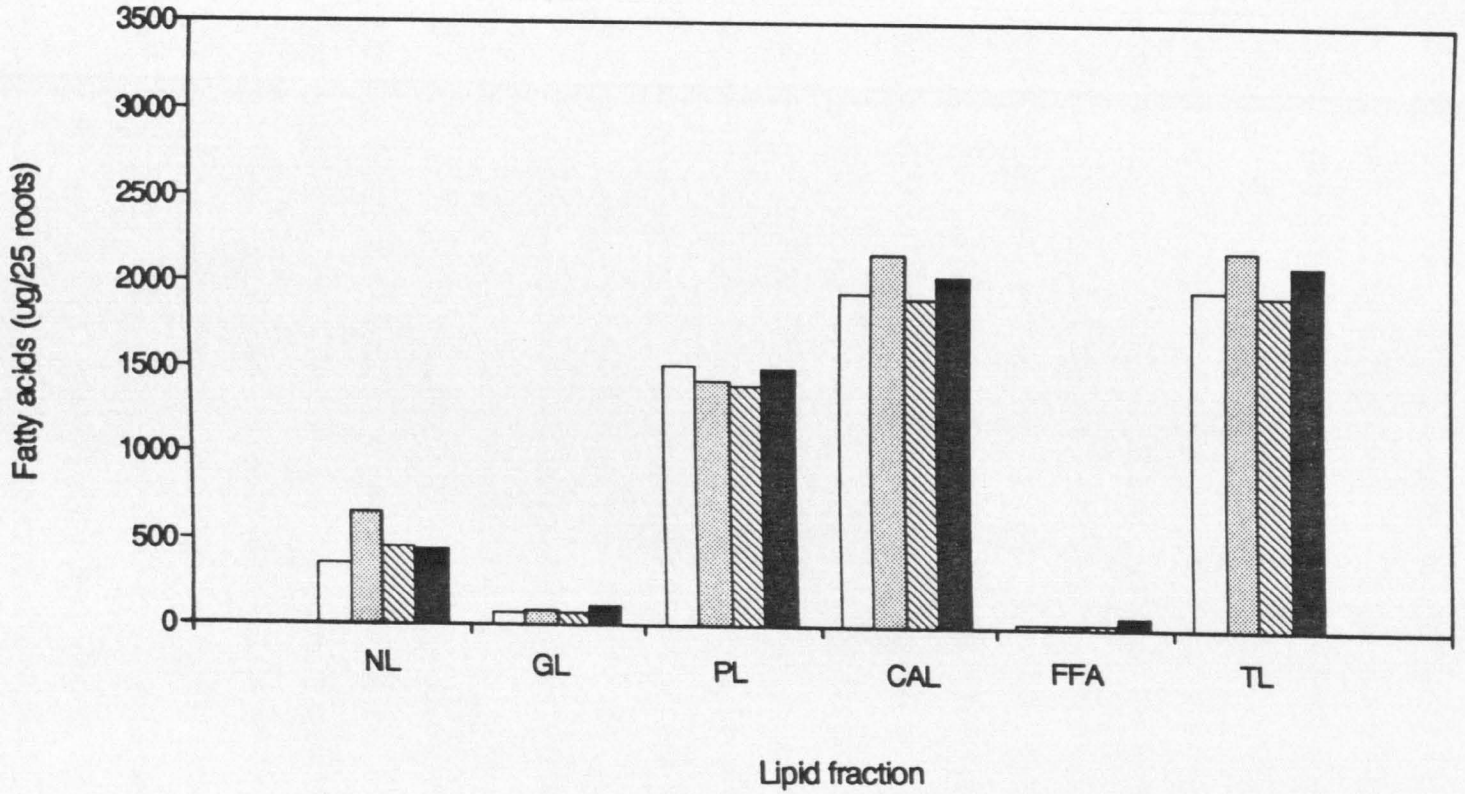


Figure 5.1 Effects of heat shock on total fatty acids in various lipid fractions in cultivar S-12. ▭, 48h control; ■, 72h control; ▨ ,after heat shock; ▨, after recovery. NL, neutral lipid; GL, glycolipid; PL, phospholipid; CAL, combined acyl lipids ; FFA, free fatty acids; TL, total lipids.

between 48 and 72 hours germination at a value of about 1500 µg (25 roots)$^{-1}$. Seedlings heat shocked for 2 hours showed an insignificant reduction in the value to 1440 µg (25 roots)$^{-1}$ ($P=0.63$). At the end of the recovery period, the value had not changed.

Table 5.1 also contains data for the total acyl lipids (neutral lipid + glycolipid + phospholipid). The combined fatty acids in these fractions changed very little under the various treatments. Thus, the values for the 48 hour control was 1940 µg (25 roots)$^{-1}$ increasing to 2050 µg (25 roots)$^{-1}$ in the 72 hour control. The level following heat shock was 2170 µg (25 roots)$^{-1}$ and following the recovery period it was 1920 µg (25 roots)$^{-1}$. Heat shock thus apparently caused a small but significant increase (12%) in the value ($P=0.02$) which was not maintained during the subsequent recovery period.

In the free fatty acid fraction, the fatty acid level increased from 27 µg (25 roots)$^{-1}$ at 48 hours to 62 µg (25 roots)$^{-1}$ at 72 hours ($P<0.01$). Heat shock for 2 hours did not have 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 Table 5.2 and Figure 5.2. 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% linoleic 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 linolenic acid increased to 12.2% at the expense of linoleic acid ($P<0.04$). Since there was an increase in the amount of neutral lipid during this period (Figure 5.1), the increase in the linolenic acid was not quantitatively accounted for by a decrease in the linoleic acid. Seedlings heat shocked for 2 hours

Table 5.2 Effect of heat-shock on the percentage fatty acid compositions in the cultivar S-12

Lipid fraction	Germination time (hours)	Fatty acids					
		Unknown	C16:0	C18:0	C18:1	C18:2	C18:3
Neutral lipids	48h control	2.9±0.6	24.4±1.6	8.8±0.9	15.8±1.4	37.7±0.8	10.3±2.6
	72h control	2.2±0.3	23.9±1.4	8.9±1.3	16.9±0.6	34.9±1.7	12.2±2.8
	After heat-shock	2.8±0.6	20.2±1.5	7.4±0.9	16.8±0.3	38.4±1.5	14.4±1.4
	After recovery	16.0±4.5	22.1±0.5	7.2±1.5	14.7±1.0	32.0±1.4	8.2±0.7
Glycolipids	48h control	1.3±0.2	24.9±1.2	7.9±1.3	15.1±1.2	23.1±2.6	27.7±3.9
	72h control	0.5±(tr)	25.4±1.3	4.5±1.5	11.1±1.0	21.9±4.4	37.0±6.7
	After heat-shock	0.6±0.1	33.9±2.3	4.7±0.7	11.0±2.3	21.4±2.0	28.5±1.5
	After recovery	1.7±0.4	40.9±4.1	5.8±0.8	9.9±0.5	15.6±0.9	26.1±2.8
Phospholipids	48h control	0.6±(tr)	32.7±1.4	2.8±0.3	4.9±0.2	39.2±1.3	19.9±0.7
	72h control	0.6±(tr)	31.8±0.5	3.1±0.5	5.0±0.5	39.5±1.0	20.2±0.7
	After heat-shock	0.6±(tr)	35.6±1.9	3.6±0.8	5.8±0.8	35.8±1.3	18.7±1.4
	After recovery	0.6±(tr)	34.1±1.4	3.2±0.4	5.2±0.1	38.2±1.4	18.1±0.5
Free fatty acids	48h control	2.8±0.4	36.7±3.2	13.1±2.5	11.5±2.2	25.2±2.9	10.7±1.7
	72h control	1.4±0.3	31.2±1.4	6.7±0.2	9.9±0.9	33.0±3.2	17.8±4.0
	After heat-shock	2.9±1.5	30.4±3.8	6.6±1.8	12.7±3.9	35.8±3.0	11.6±3.3
	After recovery	3.5±0.8	28.6±2.5	10.2±1.5	21.4±3.6	26.8±3.6	9.7±2.0

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

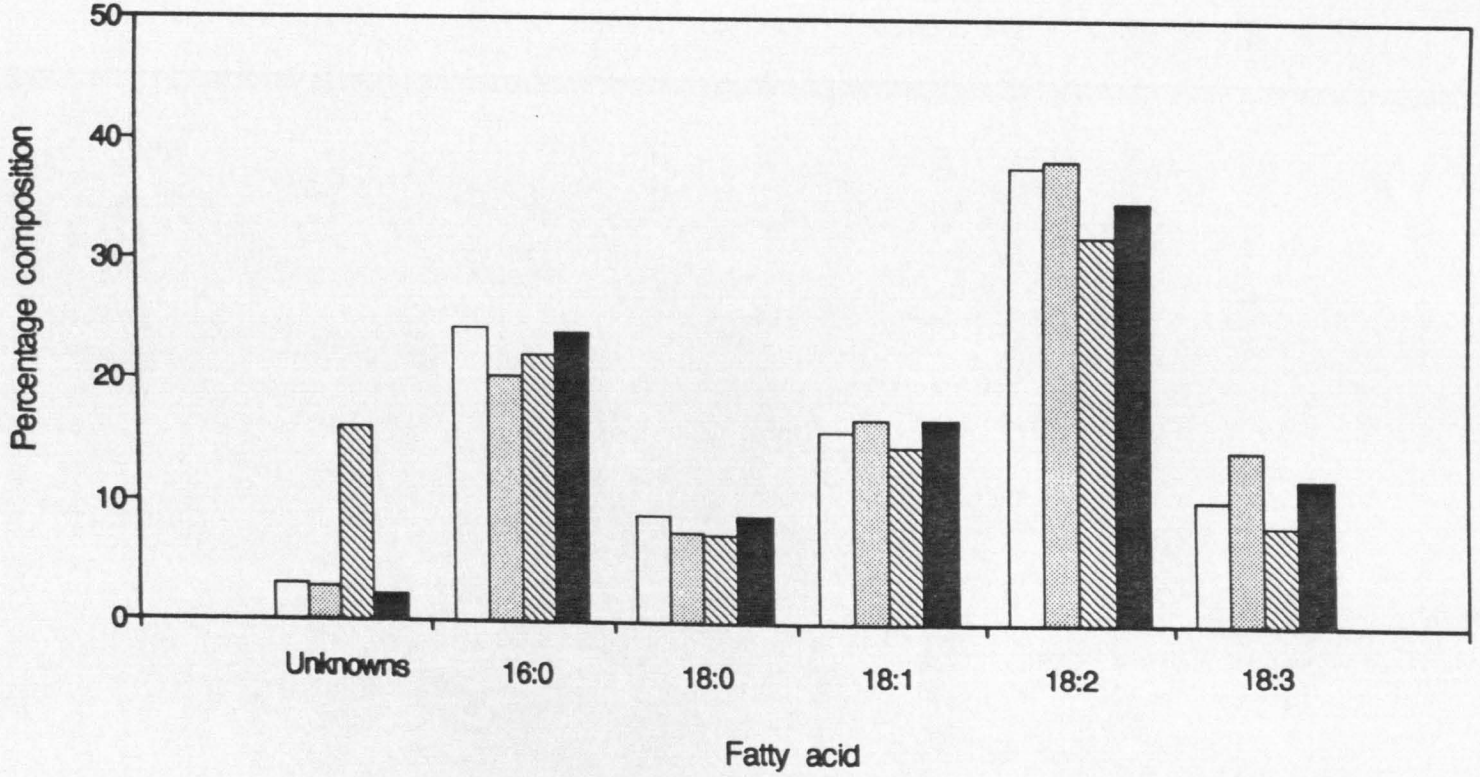


Figure 5.2 Effect of heat shock on fatty acid composition in the neutral lipid fraction in cultivar S-12. ▭, 48h control; ■, 72h control; ▨, after heat shock; ▧, after recovery.

showed increases in the proportions of unsaturated fatty acids, especially that of linolenic acid, at the expenses of the two saturated fatty acids. At the end of the recovery period following heat shock, the proportions of the unsaturated fatty acids had declined again to levels below those in either the 48 hour or the 72 hour controls. A small increase was also observed during the recovery period in the proportion of palmitic acid and a significant increase (from 2.8 to 16.0%) occurred in the set of unknown low molecular weight compounds ($P<0.001$).

The results for the glycolipid fraction are shown in Table 5.2 and in Figure 5.3. 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 proportions were observed. The largest increase appeared to be in the proportion of linolenic acid from 27.7 to 37.0% at the significant decreases in stearic and oleic acids ($P<0.03$). The change in linolenic acid was statistically not significant however ($P=0.08$). The relative amounts of palmitic and linoleic acids remained constant. Seedlings heat shocked for 2 hours showed a sharp increase in palmitic acid from 24.9 to 33.9% and decreases in stearic, and oleic acids. All of these changes were statistically significant ($P<0.03$). At the end of the recovery period, a further significant increase ($P<0.04$) in the percentage of palmitic acid to 40.9% occurred at the expenses of the unsaturated fatty acids especially linoleic acid. The proportion of the latter fell from 21.4 to 15.6% ($P=0.03$).

The results for the phospholipid fractions are shown in Table 5.2 and in Figure 5.4. The proportions of fatty acids in this fraction did not show any significant changes between 48 and 72 hours ($P>0.4$).

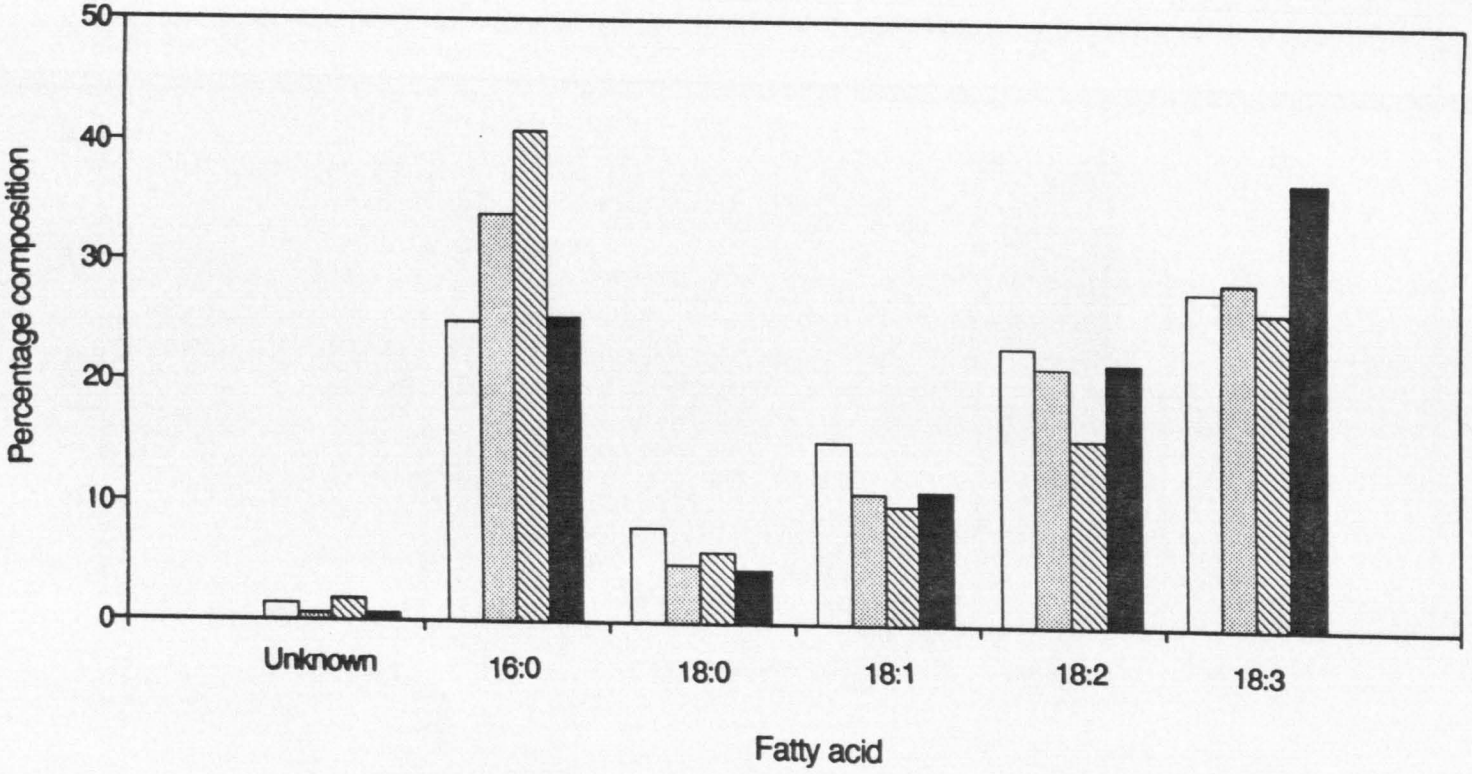


Figure 5.3 Effect of heat shock on fatty acid composition in the glycolipid fraction in cultivar S-12. ▭, 48h control; ■, 72h control; ▩, after heat shock; ▨, after recovery.

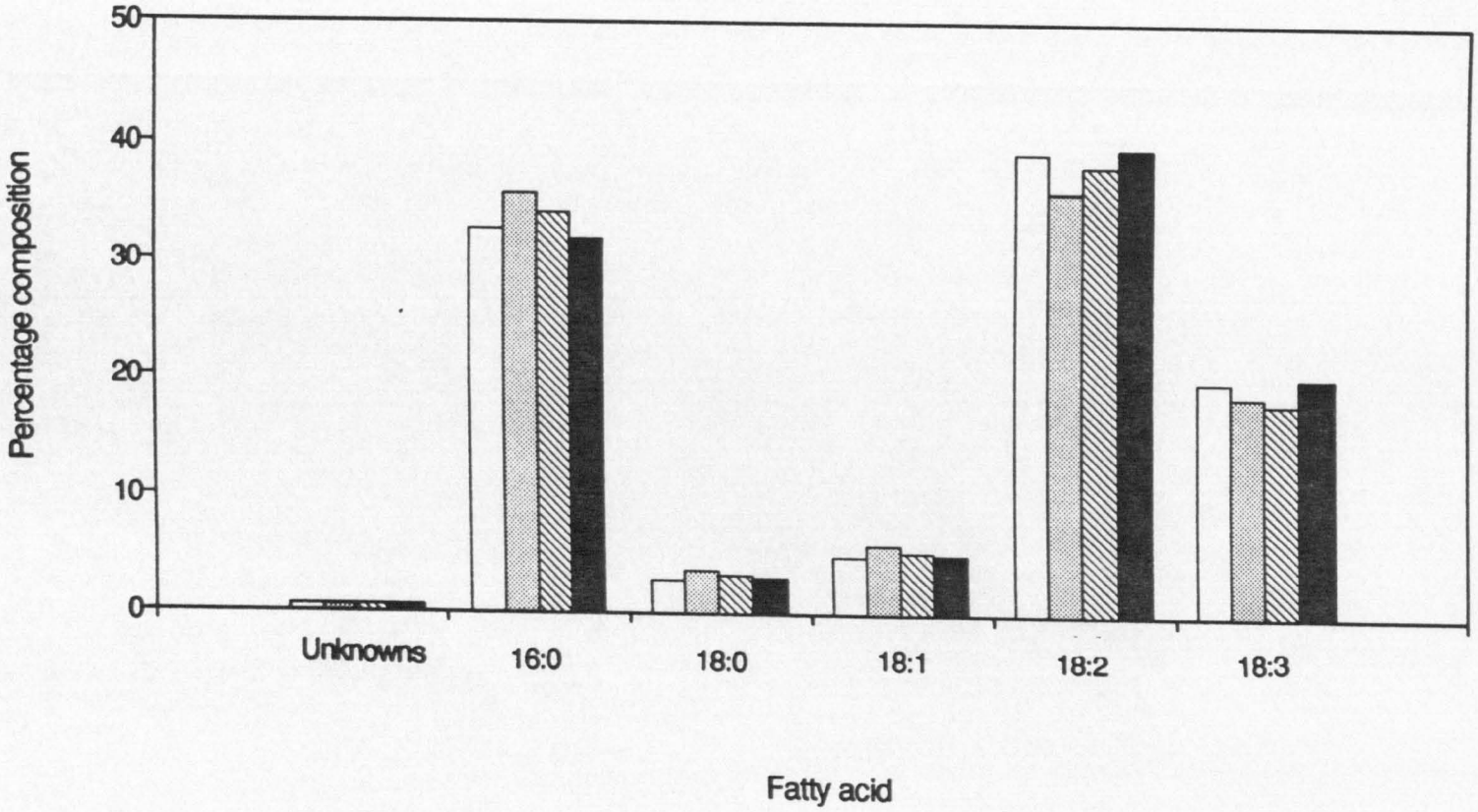


Figure 5.4 Effect of heat shock on fatty acid composition in the phospholipid fraction in cultivar S-12. ☐, 48h control; ■, 72h control; ▨, after heat shock; ▨, after recovery.

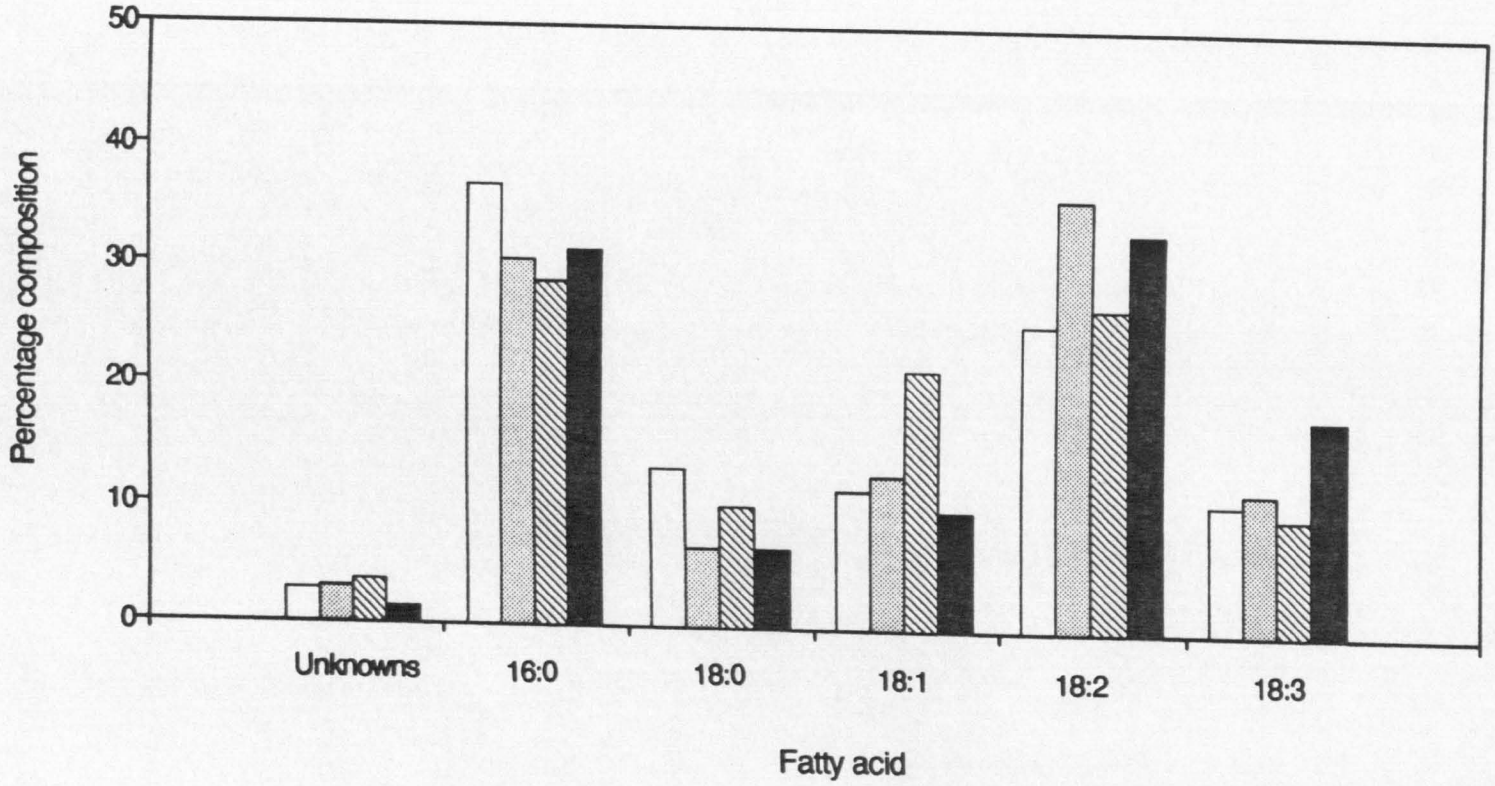


Figure 5.5 Effect of heat shock on fatty acid composition in the free fatty acids in cultivar S-12. ▭, 48h control; ■, 72h control; ▨, after heat shock; ▨, after recovery.

In seedlings heat shocked for 2 hours, there was a small and statistically insignificant increase in the proportion of palmitic acid (P=0.07) at the significant expense of linoleic acid (P=0.02). The proportions of stearic and linolenic acids did not show any significant change (P>0.16). The proportion of oleic acid were significantly changed during this period (P=0.02). At the end of the recovery, the amount of linoleic acid recovered up to the control value.

The results for the free fatty acid fraction are shown in Table 5.2 and in Figure 5.5. In the roots of seeds germinated for 48 hours, the proportion of the fatty acids were 36.7% palmitic acid, 13.1% stearic acid, 11.5% oleic acid, 25.2% linoleic acid, 10.7% linolenic acid and 2.8% unknowns. During the next 24 hours germination, considerable changes in these proportions were observed. The most significant increases were observed in the proportions of linoleic acid from 25.2 to 33.0% and linolenic acid from 10.7 to 17.8% at the expenses of palmitic and stearic acids (P<0.03). Seedlings heat shocked for 2 hours showed a significant increase in linoleic acid from 25.2 to 35.8% (P=0.005) and decreases in palmitic and stearic acids. The change in stearic acid was significant (P=0.01). At the end of the recovery period, significant increases in the percentages of stearic and oleic acids, to 10.2 and 21.4% respectively (P<0.03), occurred at the expenses of the unsaturated fatty acids, especially linoleic acid which decreased from 35.8 to 26.8% (P=0.02).

5.3.2 Effect of heat shock on fatty acid composition in the cultivar MNH-93

The results from this experiment are shown in Tables 5.3 and 5.4 and in Figures 5.6 to 5.10.

The fatty acids levels in the various fractions are presented in Table 5.3 and in Figure 5.6. The total amount of esterified plus free fatty acids at 48 hour germination was 2270 µg (25 roots)$^{-1}$ which increased significantly in the 72 hour control to 3330 µg (25 roots)$^{-1}$ ($P<0.001$). In the seedlings heat shocked for 2 hours there was a small increase (11%) in the value to 2510 µg (25 roots)$^{-1}$ and after recovery the level increases further up to 3070 µg (25 roots)$^{-1}$ ($P<0.04$).

During germination from 48 to 72 hours, an insignificant increase in neutral lipid fatty acids was found ($P=0.14$). The apparent increase was from 537 to 718 µg (25 roots)$^{-1}$. Seedlings heat shocked for 2 hours also showed an apparent increase to 749 µg (25 roots)$^{-1}$. At the end of the recovery period following heat shock, the level had declined to 581 µg (25 roots)$^{-1}$. Because of the large standard deviation values, these changes were also statistically insignificant ($P>0.36$).

The glycolipid fatty acids also increased significantly from 52 to 123 µg (25 roots)$^{-1}$ during germination between 48 and 72 hours ($P=0.01$). Seedlings heat shocked for 2 hours showed a significant increase from 52 to 139 µg (25 roots)$^{-1}$ ($P=0.01$). After recovery following heat shock, the glycolipid fatty acids underwent a further small increase from 139 to 154 µg (25 roots)$^{-1}$.

A relatively large and significant increase in the amount of phospholipid fatty acids was found during germination from 48 to 72 hours ($P<0.001$). The increase was from 1655 to 2453 µg (25 roots)$^{-1}$. Seedlings heat shocked for 2 hours showed a small reduction in the value from 1655 to 1580 µg (25 roots)$^{-1}$. At the end of the recovery period, the value had increased again to a value of 2254 µg (25 roots)$^{-1}$. This value is near to the 72 hour control value and the difference between them is statistically insignificant ($P=0.23$).

Table 5.3 Effects of heat shock on the esterified and free fatty acids in the cultivar MNH-93

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

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

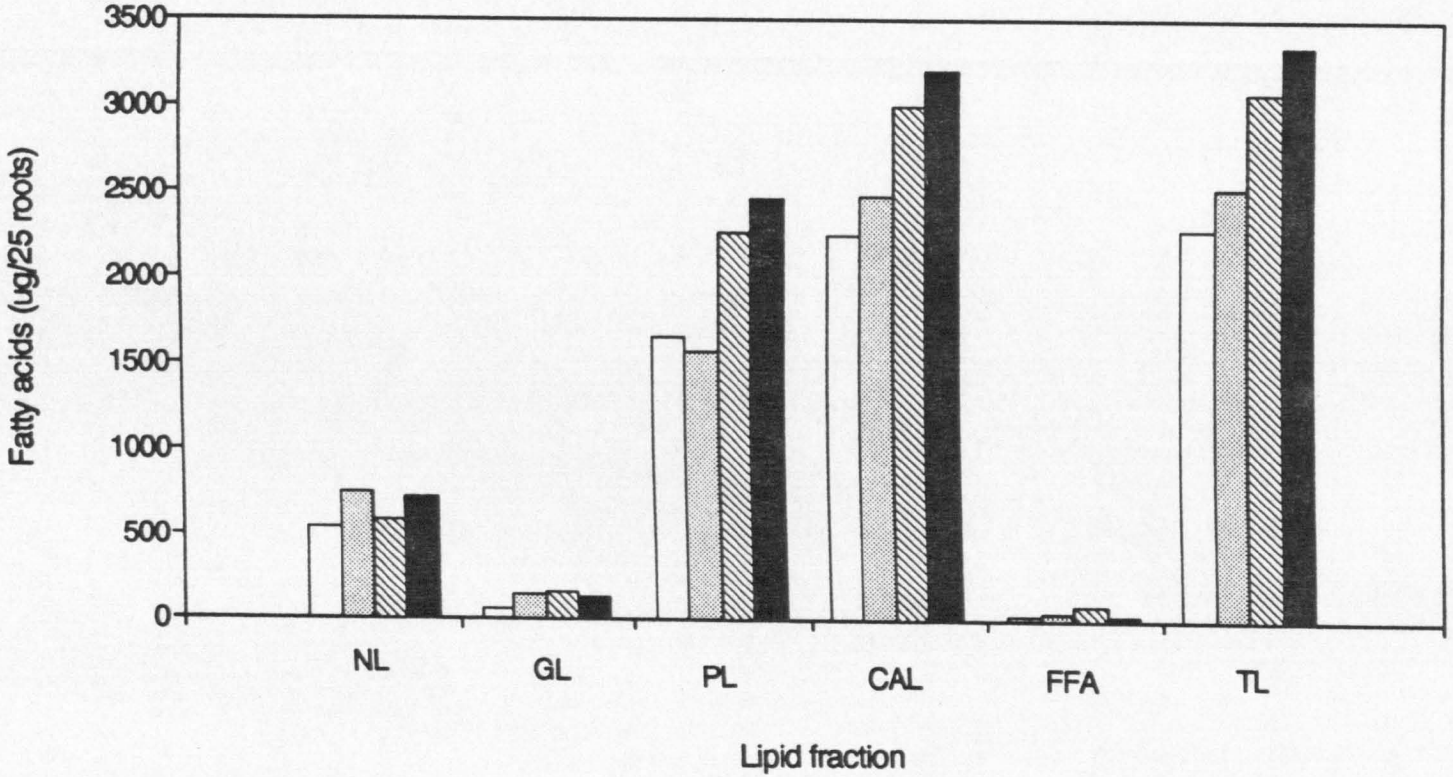


Figure 5.6 Effects of heat shock on total fatty acids in various lipid fractions in cultivar MNH-93. ▭, 48h control; ■, 72h control; ▨, after heat shock; ▨, after recovery. NL, neutral lipid; GL, glycolipid; PL, phospholipid; CAL, combined acyl lipids ; FFA, free fatty acids; TL, total lipids.

Table 5.3 also contains the data for the total acyl lipids (neutral lipid + glycolipid + phospholipid). The combined fatty acids in these fractions changed significantly under the various treatments. Thus, the value for the 48 hour control was 2240 µg (25 roots)$^{-1}$ increasing to 3200 µg (25 roots)$^{-1}$ in the 72 hour control (P=0.001). The level following heat shock was 2470 µg (25 roots)$^{-1}$ and following the recovery period it was 2990 µg (25 roots)$^{-1}$. Thus, heat shock still allowed a small but insignificant increase in the value (P>0.46), but this rate of increase was not maintained during the subsequent recovery period (cf 72 hour control).

In the free fatty acid fraction, the fatty acid level increased slightly from 25 µg (25 roots)$^{-1}$ at 48 hours to 32 µg (25 roots)$^{-1}$ at 72 hours. Seedlings heat shocked for 2 hours showed an increase from 25 to 40 µg (25 roots)$^{-1}$, but the increase was statistically insignificant (P=0.06). At the end of the recovery, the level showed a further increase to 78 µg (25 roots)$^{-1}$ (P<0.001).

The ratios of the individual fatty acids in the neutral lipid fraction are shown in Table 5.4 and in Figure 5.7. In roots germinated for 48 hours, the fatty acid composition of this fraction was 27.5% palmitic acid, 6.2% stearic acid, 20.3% oleic acid, 40.4% linoleic acid, 3.9% linolenic acid and 2.3% unknowns. During the next 24 hours of germination, there were only limited changes in these proportions. In particular, the proportion of linolenic acid increased significantly (P=0.003) to 9.1% at the expense of palmitic acid (P=0.023). Since there was an increase in neutral lipid during this period (Table 5.3), the increase in the linolenic acid was not quantitatively accounted for by a decrease in the palmitic acid. Seedlings heat shocked for 2 hours showed increases in the proportions of palmitic and linoleic acids at the expense of oleic and linolenic

Table 5.4 Effect of heat-shock on the percentage fatty acid compositions in the cultivar MNH-93

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 control	1.8±(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

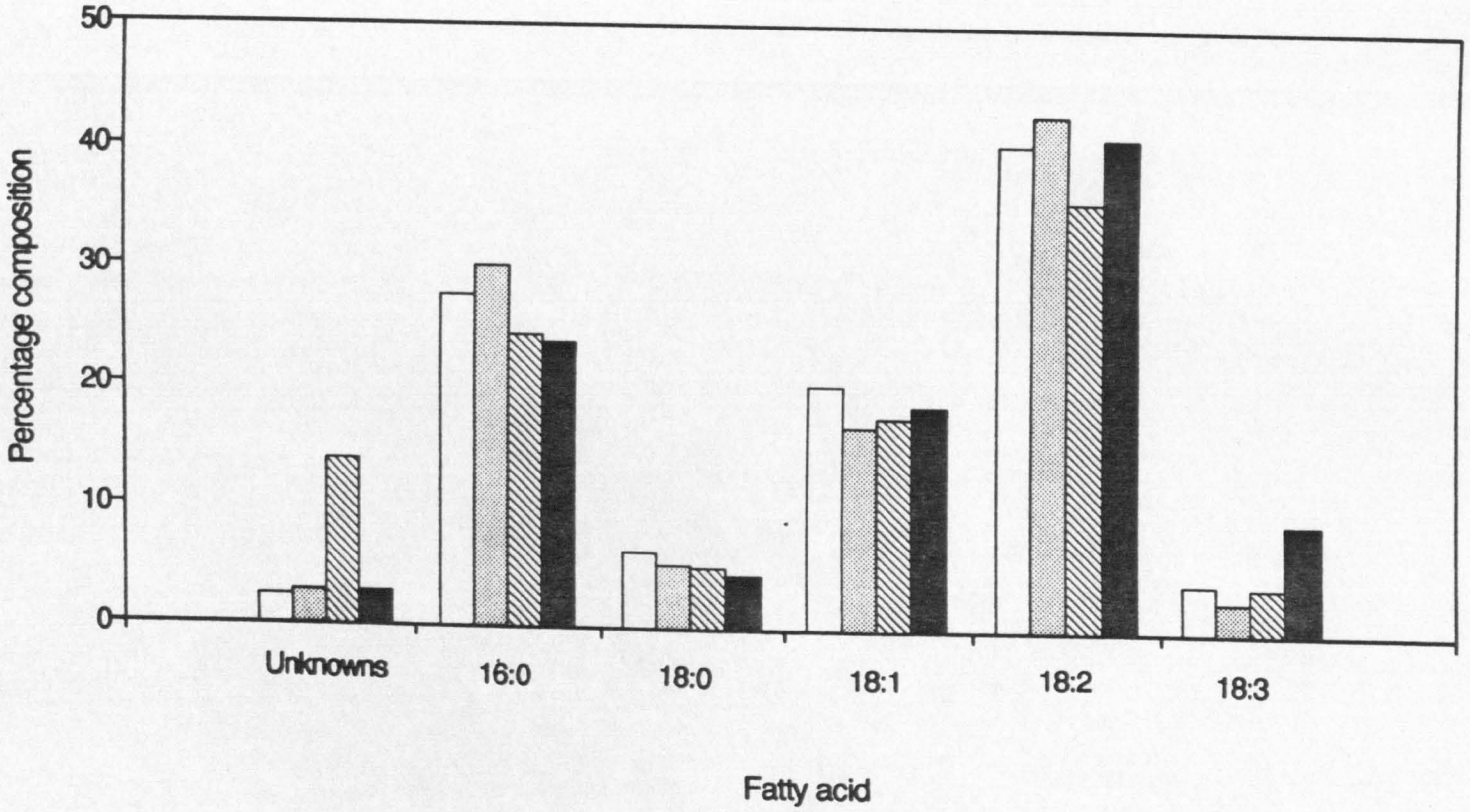


Figure 5.7 Effect of heat shock on fatty acid composition in the neutral lipid fraction in cultivar MNH-93. ▭,48h control; ▬, 72h control; ▩, after heat shock; ▨, after recovery.

acids. The latter decreased significantly (P=0.03). At the end of the recovery period following heat shock, the greater change was noted in the set of unknown low molecular weight compounds which increased from 2.7 to 13.7% (P=<0.01) at the expenses of palmitic and linoleic acids. The proportions of palmitic and linoleic acids were statistically insignificant compared with 72 hour control (P=0.06), but the proportion of linolenic acid was significantly decreased compared with 72 hour control (P=0.003).

The results for the glycolipid fraction are shown in Table 5.4 and in Figure 5.8. In the roots of seeds germinated for 48 hours, the proportions of the fatty acids were 36.8% palmitic acid, 3.9% stearic acid, 18.2% oleic acid, 25.3% linoleic acid and 15.7% linolenic acid. During the next 24 hours of germination, considerable changes in these proportions were observed. Marked increases took place in the proportions of stearic acid from 3.9 to 6.7% and linolenic acid from 15.7 to 35.0% at the expenses of palmitic, oleic and linoleic acids. All of these changes were statistically significant (P<0.04) except that for linoleic acid (P=0.40). Seedlings heat shocked for 2 hours showed a small and insignificant increase in palmitic acid from 36.8 to 42.3% (P=0.33) and also in the set of unknowns from zero to 2.6%. There was a decrease in the proportion of oleic acid. At the end of the recovery period, an increase in the proportion of linolenic acid from 15.8 to 22.1% (P=0.05) was found at the expense of a similar decrease in the combined proportion of palmitic and linoleic acids.

The results for the phospholipid fractions are shown in Table 5.4 and in Figure 5.9. In the roots germinated for 48 hours, the proportions of the fatty acids were 34.1% palmitic acid, 3.0% stearic acid, 5.8% oleic acid, 40.1% linoleic acid, 16.6% linolenic acid and 0.5% unknowns. These values underwent only small changes between 48

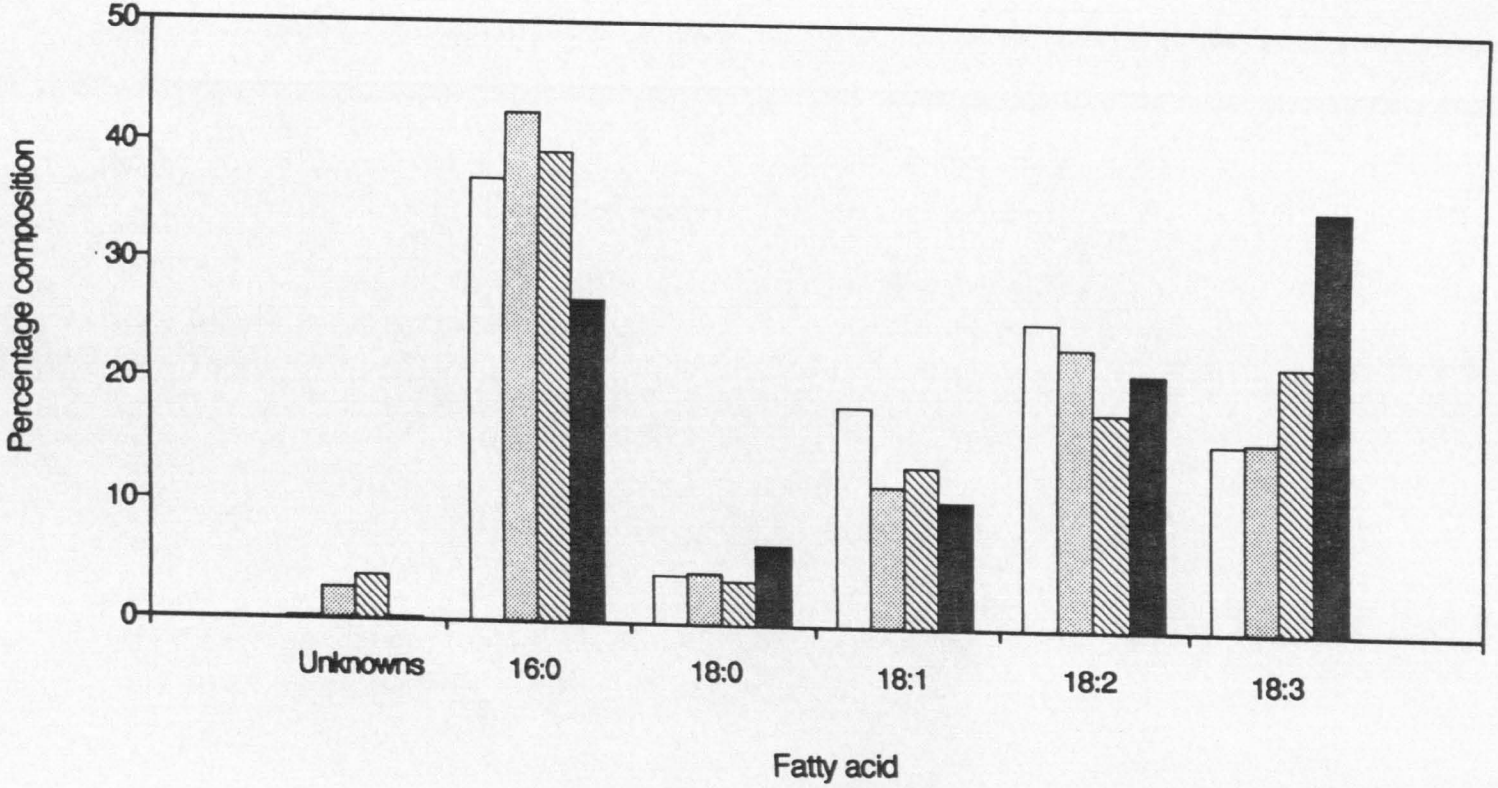


Figure 5.8 Effect of heat shock on fatty acid composition in the glycolipid fraction in cultivar MNH-93. ▭,48h control; ■, 72h control; ▨, after heat shock; ▧, after recovery.

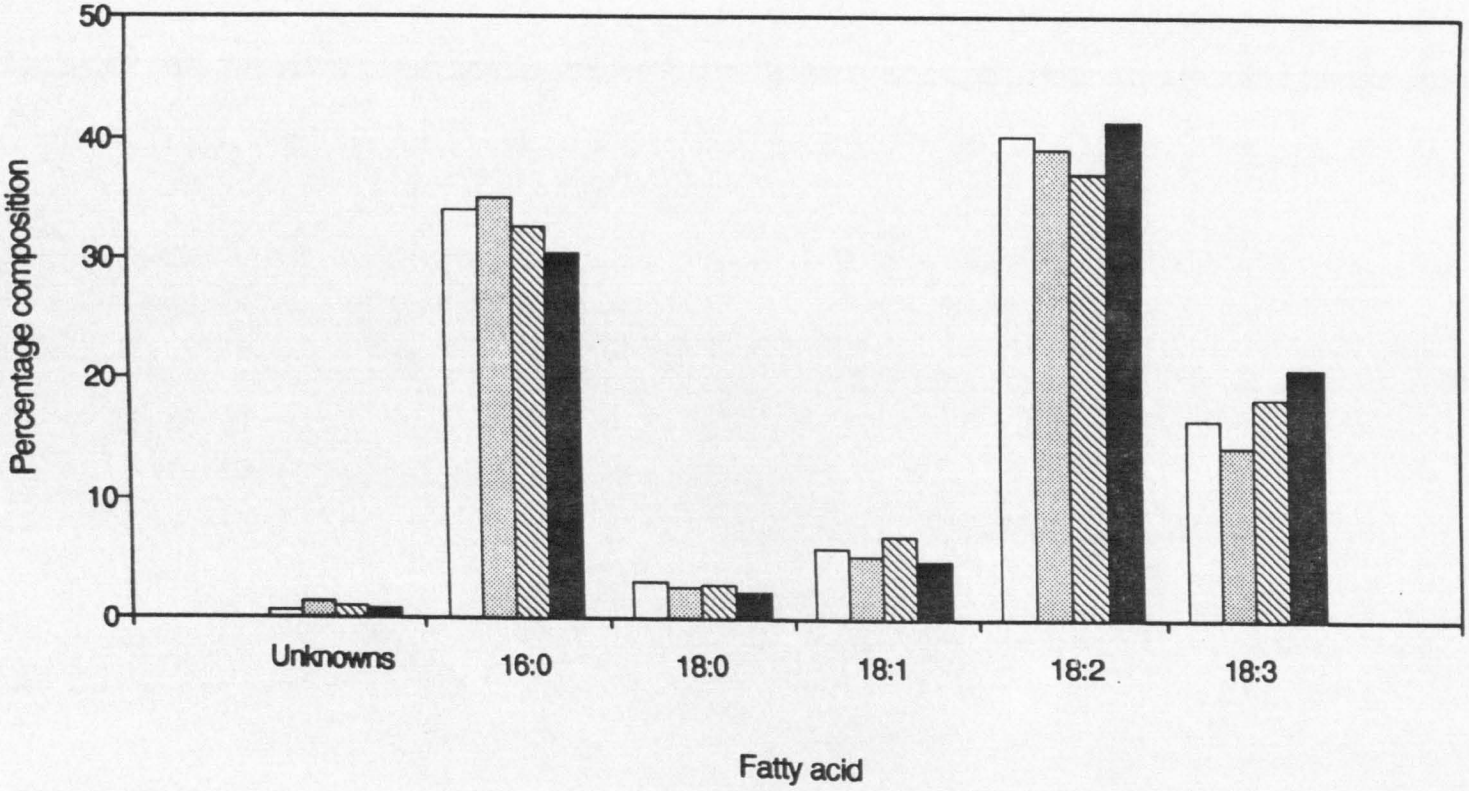


Figure 5.9 Effect of heat shock on fatty acid composition in the phospholipid fraction in cultivar MNH-93. ▭ ,48h control; ■ , 72h control; ▭ , after heat shock; ▨ , after recovery.

and 72 hours. The largest changes were in the proportion of linolenic acid, which increased from 16.6 to 20.8% at the expense of palmitic acid. Both of the changes were significant ($P<0.04$). In seedlings heat shocked for 2 hours, there were few significant changes in these proportions. A small increase was observed in the percentage of palmitic acid at the significant expense of linolenic acid ($P=0.02$). At the end of the recovery period, the amount of linolenic acid increased again to the control value and a small decreases was found in the palmitic and linoleic acids. However, all of these values were insignificantly different when compared with the 72 hour controls ($P>0.08$).

The results for the free fatty acid fraction are shown in Table 5.4 and in Figure 5.10. In the roots of seeds germinated for 48 hours, the proportions of the fatty acids were 48.7% palmitic acid, 5.3% stearic acid, 14.5% oleic acid, 24.8% linoleic acid, 5.9% linolenic acid and 1.8% unknowns. During the next 24 hours germination, the proportions of the unsaturated fatty acids (linoleic and linolenic acids) increased significantly ($P<0.001$). The largest increase was noted in the proportion of the linoleic acid from 24.8 to 43.8% with a significant decrease ($P<0.03$) in the proportion of palmitic acid. A small decrease in the proportion of oleic acid was also observed. At this stage, the unknown low molecular weight compounds had disappeared. Seedlings heat shocked for 2 hours showed a significant increase in linoleic acid from 24.8 to 40.2% ($P=0.01$) and a corresponding decreases in palmitic and oleic acids. During the recovery period, the proportion of palmitic acid increased ($P<0.001$) and the percentages of oleic and linoleic acids decreased compared with 72 hour controls. The proportion of linolenic acid recovered up to corresponding control.

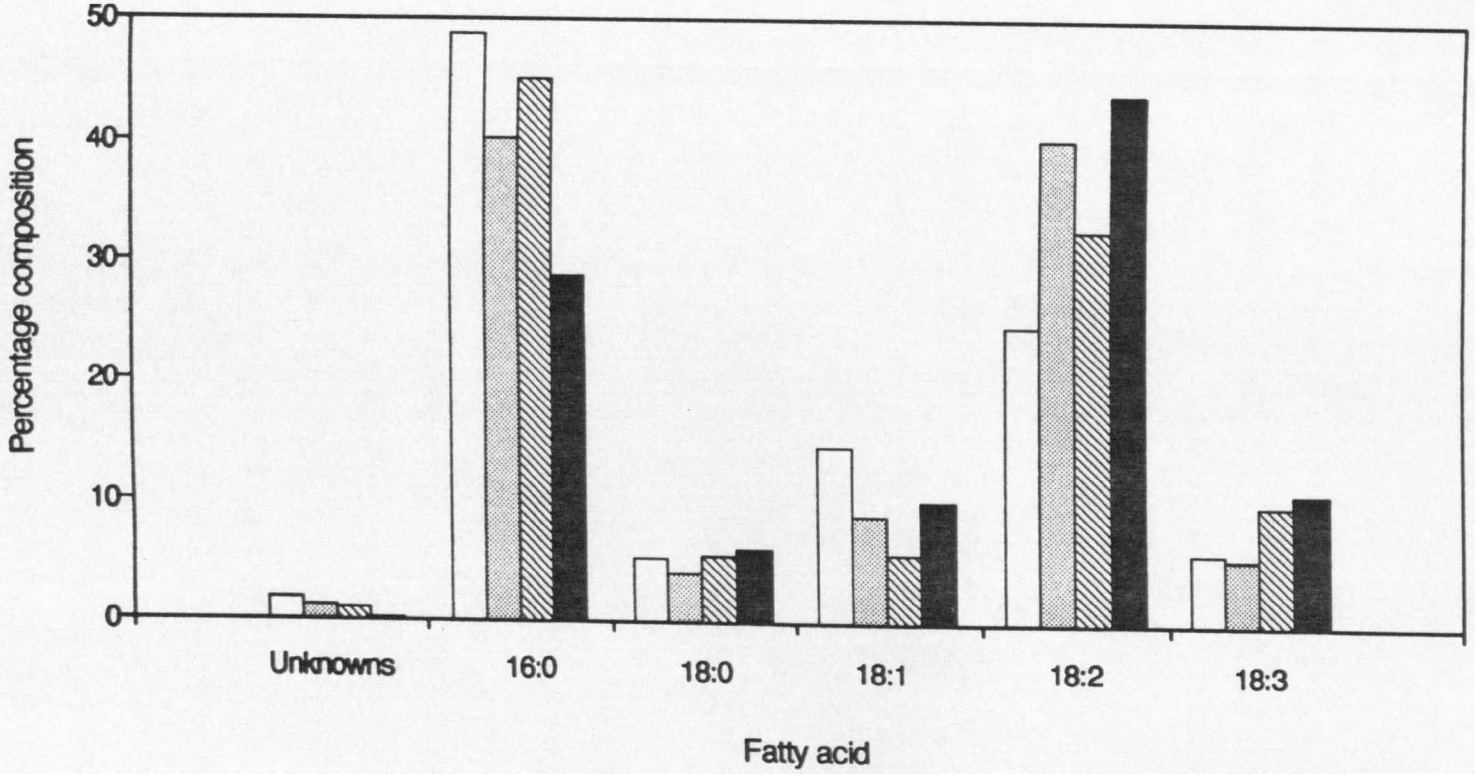


Figure 5.10 Effect of heat shock on fatty acid composition in the free fatty acids in cultivar MNH-93. ☐ ,48h control; ■, 72h control; ▨, after heat shock; ▨ , after recovery.

5.4 Discussion

It is unfortunate that, when statistical analyses (Student T-test) were done, some of the large experimental changes were found to be insignificant due to large standard deviation values. For example, the increase in neutral lipid fatty acids due to heat shock was found to be significant in the cultivar S-12, but not in MNH-93. Despite the statistics, however, the differences may be real in both instances. In any future extension of the present studies, it will be necessary to increase the number of replicate experiments and analyses.

Analysis of the fatty acids from cotton roots following heat shock show 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).

The present results indicate that the total amount of esterified and free fatty acids increased with increasing germination time. This increase was greater in MNH-93 than in the cultivar S-12. These increases were 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 in both cultivars, with the exception of the phospholipids in S-12. The greater increase of fatty acids in the phospholipid fraction in MNH-93 may be due to the formation of more membrane in this cultivar than S-12. The fresh weight values for the roots used in this experiment confirms this; the fresh weight of the MNH-93 increased from 1.8g at 48 hours to 3.3g at 72 hours, while in

the cultivar S-12 the fresh weight increased only from 2.0g at 48 hours to 2.5g at 72 hours.

Heat shock caused increases in the levels of the neutral lipid fatty acids and decreases in the levels of phospholipid fatty acids in both cultivars. 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. After recovery from heat shock, the levels of fatty acids in the neutral lipid, glycolipid and phospholipid fractions recovered towards their 72 hour control levels in the cultivar S-12. In the cultivar MNH-93, 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 in this cultivar. Such increased levels of free fatty acids might cause the disruption of membrane structures and decrease the thermostability of membranes. Weiss (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.

In S-12, there was an increase in the total acyl lipids as well as in the total lipids but the picture was dominated by phospholipid which were present in the greatest amount. In the case of the MNH-93, the picture is quite clear and shows the greater increase in the total acyl lipids as well as in the total lipids. The main reason for this is that MNH-93 contains greater amount of all lipid classes.

The results of phospholipid fraction in the cultivar MNH-93 are in agreement with the results obtained in the Chapter 4 (see Section 4.3.2.). These results suggest that heat shock at 40°C had no significant effect on phospholipid fatty acid composition or total phospholipid 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. If this is true, then the plastid membranes of cultivar S-12 are somewhat less sensitive to heat stress than those of MNH-93. The proportions of the unsaturated fatty acids in the phospholipid fraction, also showed reductions in the both cultivars. These reductions were greater in the cultivar MNH-93 than in cultivar S-12. In this respect, the cultivar S-12 once again showed the greater tolerance to heat stress.

CHAPTER SIX

Chapter Six

Effects of Waterlogging on Root Development

6.1 Introduction

Because of the failure to find significant differences between the Pakistan cotton cultivars with respect to heat stress, it was decided to extend the present study to include another abiotic stress (waterlogging) experienced by the crop. It was hoped that a greater degree of genetic variability might be found with respect to this stress.

The period between planting and emergence is a critical time in the development of the cotton plant. Exposure of seedlings to excess water causes reduction in oxygen supply to the roots (Orchard and So, 1985) and can bring adverse changes in physiological and biochemical functions. In Pakistan, the sowing season for cotton extends from the end of April to the end of June depending upon the locality. Soil waterlogging after planting is not uncommon as a result of heavy rainfall, overflowing of river banks and sometimes over-irrigation at this time. This results in the submergence of plants in compacted soils. The ultimate result is poor seedling growth and poor yield. Moreover, damage to seedlings during this stage of growth can affect subsequent productivity.

Woodstock and Taylorson (1981) reported that as little as 1 hour submergence of soybean seedlings can be severely injurious. Hodgson and Chan (1982) and Woodstock *et al.* (1985) have reported that even shorter periods of waterlogging reduce plant productivity. Cotton also appears to be very sensitive to this form of stress (Huck, 1970; Hocking *et al.*, 1985 and 1987).

In normal conditions, oxygen diffuses through the air spaces of

the soil to satisfy the oxygen demand of roots. Waterlogging prevents this diffusion and causes the depletion of oxygen at the roots (Stolzy *et al.*, 1961; Turner *et al.*, 1983). The lack of oxygen also starts a sequence of chemical and biochemical reduction reactions, producing compounds that may be injurious to root metabolism (Drew, 1983; Jackson and Drew, 1984). Root growth generally decreases with decreased oxygen content in the soil (Letey *et al.*, 1961; Stolzy *et al.*, 1961). Tackett and Pearson (1964) and Hopkins and Patrick (1969) working on sudangrass and cotton (*Gossypium hirsutum*) concluded that soil oxygen levels below 10% suppress the penetration of roots into the soil. Letey *et al.* (1962) and Meek *et al.* (1980) stated that the overall growth of cotton is reduced by poor aeration and results in plant with reduced height, dry matter and fruiting parts (Hodgson, 1982).

The root tip is more sensitive to low oxygen tension than the other parts of the root. Anaya (1972) reported that elongation of wheat roots was 25, 32 and 36 centimetres at oxygen levels of 2, 6 and 10%, respectively, after 21 days exposure. Cannon (1925) stated that plant roots need 8 to 10% oxygen for fast growth. In addition to this, he further added that roots of many species can make limited growth with 2% oxygen or less. Initiation of new roots requires about 12% oxygen however (Boynton *et al.*, 1938) and formation of new roots is reduced when the oxygen concentration drops below 15%. The root tip is the major site for the synthesis of gibberellins (Jones and Phillips, 1966) and cytokinins (Itai and Vaadia, 1965). During waterlogging, when this region of the root is subjected to anaerobic conditions, their synthesis and translocation will be reduced. In sunflower, for example, synthesis and translocation of cytokinin is reduced by waterlogging (Burrows and Carr, 1969). Even short-term waterlogging

increases the production of ethylene and abscisic acid (Hocking *et al.*, 1985). Increasing concentrations of abscisic acid leads to stomatal closure and it may be a contributory cause of reduced growth in waterlogged conditions (Milborrow, 1974).

Lack of oxygen is undoubtedly the trigger for changes in the metabolism of the roots (Drew and Sisworo, 1979). For example, anaerobiosis in roots has been shown to increase the activities of four enzymes, alcohol dehydrogenase, pyruvate decarboxylase, lactate dehydrogenase and malic enzyme in maize seedlings (Vantoi *et al.*, 1987). The dependence upon anaerobic respiration also leads to insufficient ATP for growth and maintenance. Anaerobic respiration in roots produces compounds such as ethanol that are potentially harmful when accumulated in large concentrations. Accumulation of ethanol resulting from increased glycolysis can readily lead to membrane disruption by lipid solubilization and this will inactivate mitochondrial enzyme activity and further increase the preponderance of glycolytic activity. Leakage of cell contents will quickly follow, and the exudation of soluble metabolites promotes the growth of soil pathogens which then assail the roots and eventually cause death.

There are several other reports regarding the growth of cotton and waterlogging under field and laboratory conditions which show that nutrient uptake mechanisms are impaired (Hocking *et al.*, 1985, 1987; Soomro and Waring, 1987). Both groups of workers specified that short-term waterlogging reduces the uptake of phosphorus, nitrogen, potassium and calcium. Hocking *et al.* (1985) also reported that waterlogging increased the accumulation of proline in shoots.

The main objective of present experiments was to quantify and compare the effects of waterlogging on root growth in various cultivars of cotton (*Gossypium hirsutum*).

6.2 Methods

6.2.1 Plant material

In these experiments, seeds of four cultivars were used: Qalandari from the 1989-90 harvest and MNH-93, S-12 and Rehmani from the 1991-92 harvest.

6.2.2 Application of waterlogging

The detailed procedures for these experiments are described in the Material and Methods chapter. Briefly, seedlings that had been germinated for 48 hours at 25°C were waterlogged for 6, 12 or 24 hours (Section 2.8). The seedlings were waterlogged either in non-aerated or in aerated water. After the waterlogging treatment, they were drained and returned to the incubator at 25°C for recovery. The recovery period was adjusted so that the total experimental period was 72, 96 or 120 hours for each waterlogging treatment. Controls consisted of seeds germinated for up to 120 hours at 25°C (ie without waterlogging). At the end of the experimental treatments, seedlings were harvested, their roots were detached from their cotyledons using a scalpel and the length of each root was measured using a ruler. For fresh weight determination, the isolated roots from batches of seedlings were weighed on a five-place analytical balance. The roots were then dried for 48 hours in an oven at 70°C and re-weighed to determine their dry weights.

The resulting data were analysed statistically as described in Section 2.18. All graphs were plotted using a personal computer with the Systat/Sygraph (Smooth=short) software programme.

6.3 Results

6.3.1 Oxygen levels during waterlogging

The concentration of oxygen in the submerging water was measured before and at the end of each waterlogging treatment. The results are presented in Table 6.1 and in Figure 6.1. It is clear that, after placing the seedlings in the water, the concentration of oxygen declined with increasing duration of waterlogging treatment. Thus, after 6 hour treatment the seedlings of S-12 consumed most oxygen (76%) while the other cultivars consumed about 50% of the available oxygen. At the end of the 24 hour waterlogging treatment, the cultivar S-12 still showed the greatest consumption of oxygen (97%) together with MNH-93 and Qalandari (95%). Rehmani had consumed 90% of the available oxygen.

6.3.2 Effect of waterlogging on root growth

The results for these experiments are presented in Table 6.2 and in Figure 6.2. They show that the patterns of root growth in the controls at 25°C were similar for all the cultivars. There were small quantitative differences however. At 48 hour, for example, Qalandari showed the greatest root growth and the lowest root growth was observed in Rehmani. After 120 hours germination, Qalandari once again had the greatest root growth but the lowest root growth was now found in the cultivar S-12.

Seedlings subjected to waterlogging showed marked reductions in their root growth. The effect of waterlogging on each cultivar was variable. After 6 hour waterlogging, the root growth (between 48 and 120 hours) was reduced by 33, 54, 60 and 69%, respectively, in S-12, Rehmani, MNH-93 and Qalandari compared with their controls. Waterlogging for 12 hours caused greater inhibition so that more than

Table 6.1 Oxygen levels during waterlogging

Stress period (h)	Rehmani	Qalandari	S-12	MNH-93
0	8.20	8.20	8.20	8.20
3	6.00	5.80	5.80	6.40
6	3.60	4.20	2.00	3.90
12	1.00	0.70	0.70	0.70
24	0.60	0.40	0.25	0.45

Each value is the mean oxygen concentration (mg l^{-1}) from 3 to 5 experiments.

Table 6.2 Effect of waterlogging on root growth of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	24	12 ±6	--	--	--
	48	47 ±15	--	--	--
	72	75 ±22	53 ±15	46 ±16	43 ±14
	96	94 ±30	54 ±18	58 ±18	43 ±16
	120	111 ±35	67 ±23	64 ±19	49 ±17
S-12	24	11 ±5	--	--	--
	48	46 ±15	--	--	--
	72	58 ±19	46 ±15	49 ±15	45 ±13
	96	89 ±29	70 ±18	54 ±17	55 ±18
	120	89 ±35	75 ±24	55 ±19	54 ±19
MNH-93	24	9 ±4	--	--	--
	48	43 ±14	--	--	--
	72	64 ±27	50 ±17	48 ±16	42 ±15
	96	90 ±35	63 ±21	52 ±19	44 ±17
	120	108 ±37	69 ±22	58 ±17	53 ±15
Rehmani	24	5 ±4	--	--	--
	48	35 ±14	--	--	--
	72	59 ±22	49 ±17	45 ±13	36 ±14
	96	105 ±29	56 ±19	46 ±18	37 ±14
	120	105 ±30	67 ±25	52 ±19	41 ±15

Each value is the mean length (mm) ±sd from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

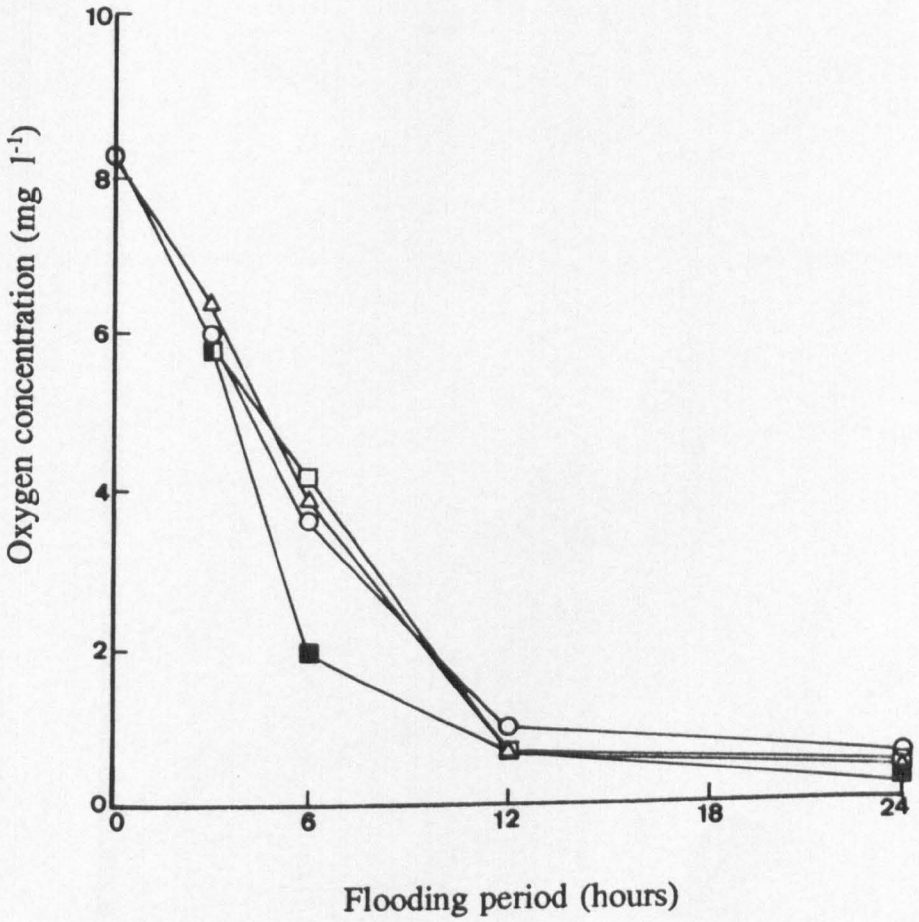


Figure 6.1 Levels of oxygen during waterlogging. □, Qalandari; ○,Rehmani; ■, S-12; △,MNH-93.

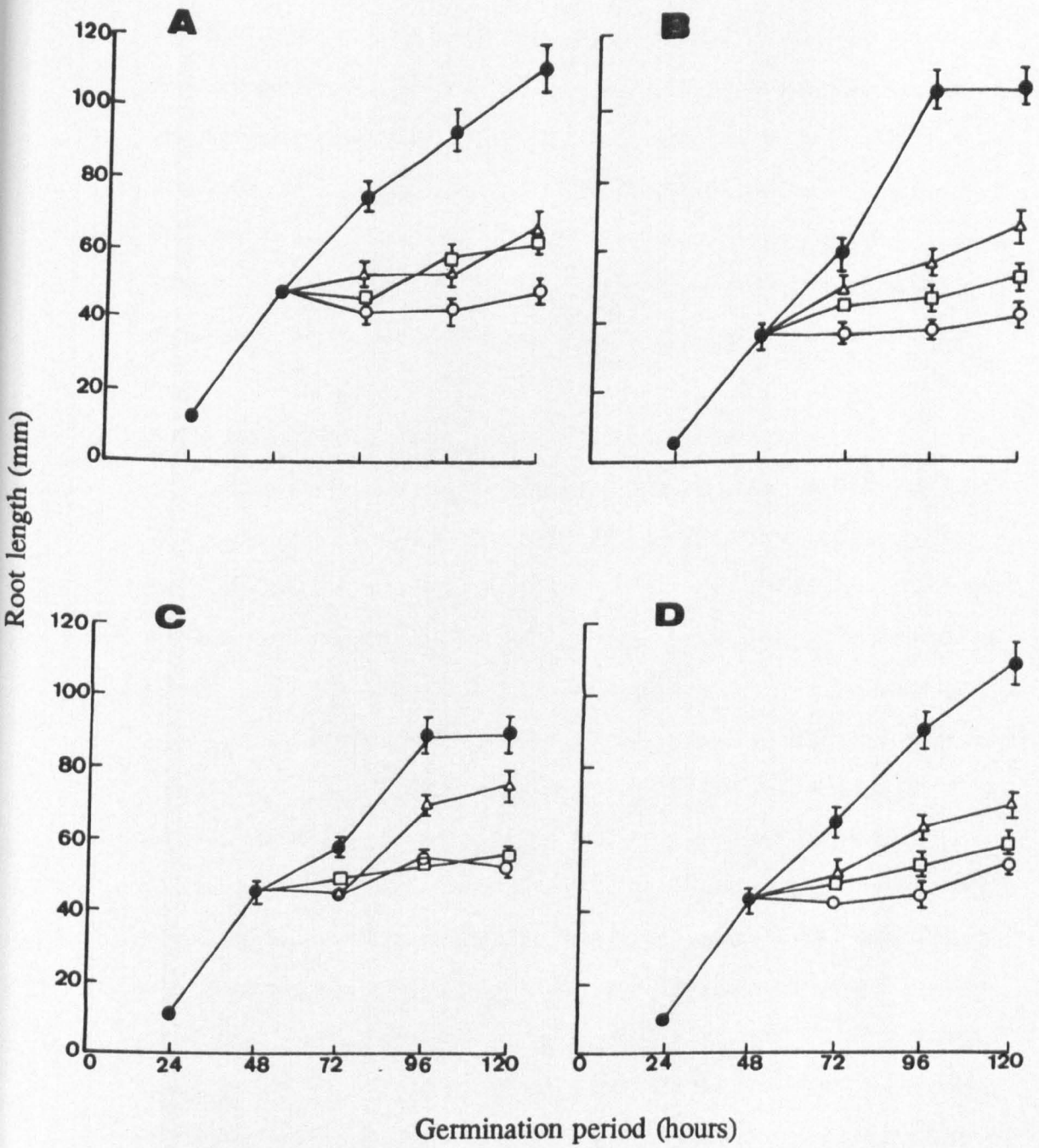


Figure 6.2 Effect of waterlogging on root growth in various cotton cultivars. (A) Qalandari, (B) Rehmani, (C) S-12, (D) MNH-93. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment. Vertical bars indicate standard error values.

70% of control growth had been lost at 120 hours in all the cultivars. In this case, the increases in length between 48 and 120 hours were reduced by 79, 76, 80 and 73%, respectively, in S-12, Rehmani, MNH-93 and Qalandari compared with their controls. The 24 hour waterlogging treatment had an even greater effect so that root growth was almost stopped in all cultivars. The best growth (19% of control) in this situation was found in S-12. The greatest inhibition was observed in Qalandari which showed a growth of only 3% compared with its control.

6.3.3 Effects of waterlogging on fresh and dry weights

The results for this experiment are presented in Table 6.3 and in Figure 6.3. They show that the pattern for fresh weight gains in the controls at 25°C were similar for all the cultivars. The fresh weights of roots in all the cultivars increased with increasing germination time between 48 and 120 hours and there were only small quantitative differences between the cultivars. At 120 hour, MNH-93 showed the highest fresh weight gain and the lowest fresh weight gain was observed in S-12. Waterlogging for 6 hour showed variable effects in the different cultivars. After 6 hour waterlogging, the fresh weight gain (between 48 and 120 hours) was reduced by only 13% in S-12 compared with its control. The other cultivars showed greater reductions; their fresh weight gains were reduced by 34, 39 and 43%, respectively, in the cultivars Qalandari, Rehmani and MNH-93 compared with their controls. Waterlogging for 12 hours had a greater effect. In this case, the fresh weight gains between 48 and 120 hours were reduced by 55-60% depending on the cultivar. As expected, the 24 hour waterlogging treatment had an even greater effect. After this treatment, the gain in fresh weight was actually negative in some cases (see Figure 6.3 B and D).

Table 6.3 Effect of waterlogging on root fresh weight gain of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	48	82	--	--	--
	72	130	115	93	92
	96	193	135	125	81
	120	235	183	150	93
S-12	48	84	--	--	--
	72	118	90	108	93
	96	201	170	123	101
	120	228	209	145	109
MNH-93	48	78	--	--	--
	72	116	101	86	64
	96	147	149	109	61
	120	240	170	145	86
Rehmani	48	71	--	--	--
	72	107	92	94	74
	96	218	131	99	61
	120	232	170	133	75

Each value is the mean weight (mg $(root)^{-1}$) from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

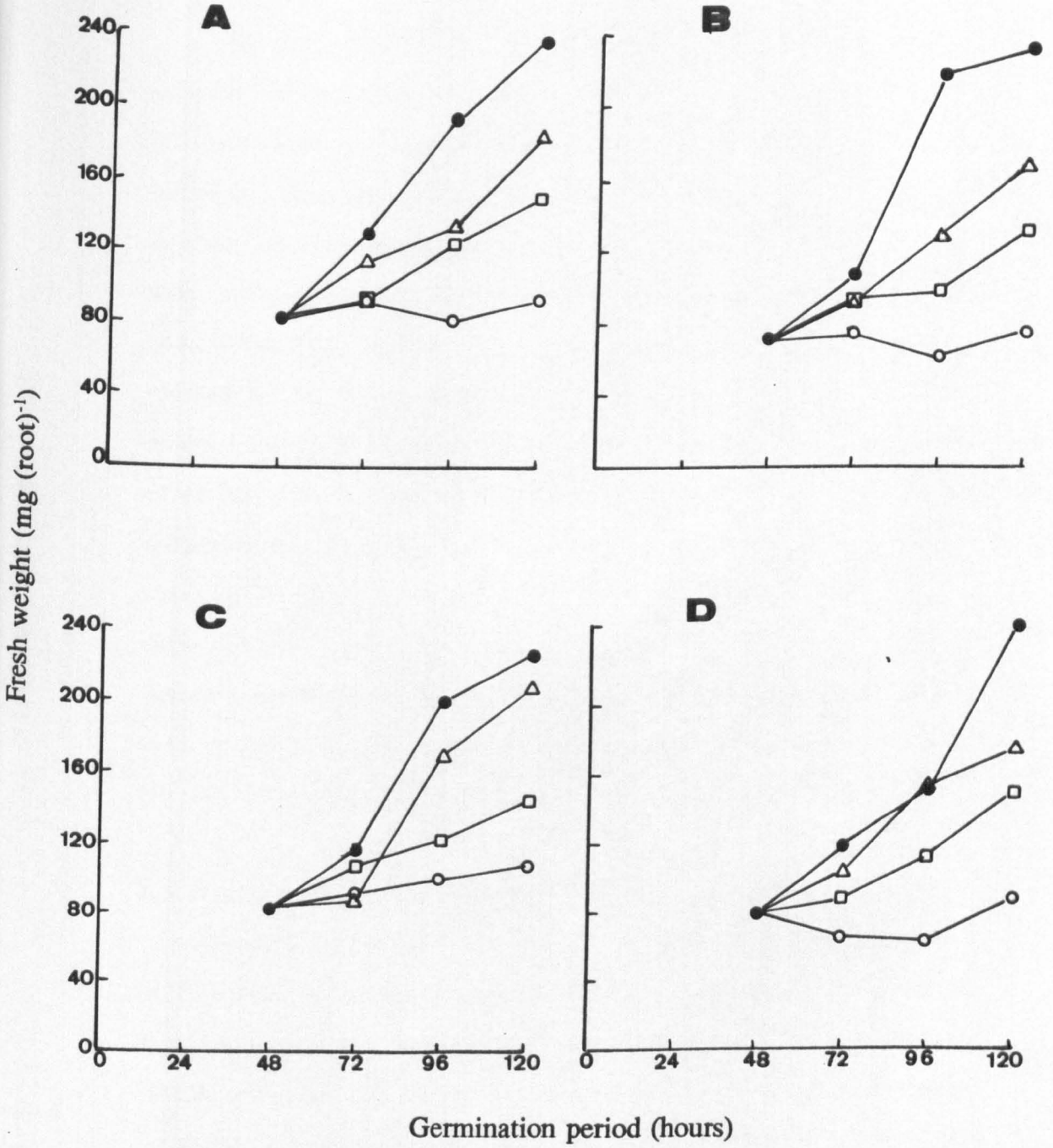


Figure 6.3 Effect of waterlogging on root fresh weight gain in various cotton cultivars. (A) Qalandari, (B) Rehmani, (C) S-12, (D) MNH-93. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

The dry weight results are presented in Table 6.4 and in Figure 6.4. The dry weight patterns for the roots of waterlogged seedlings in all cultivars are qualitatively very similar to the fresh weight patterns. Some significant quantitative differences were observed however. The dry weight of S-12 was not greatly affected by the 6 hour waterlogging treatment, while other cultivars showed significant reductions. Their gains in dry weight between 48 and 120 hours were reduced by 42, 54 and 30%, respectively, in Qalandari, MNH-93 and Rehmani. After a 12 hour waterlogging treatment, the gain in fresh weight between 48 and 120 hours was reduced by 63, 59, 72 and 69%, respectively, in S-12, Rehmani, MNH-93 and Qalandari compared with their controls. After the 24 hour waterlogging treatment, the accumulation of dry weight was severely affected. This treatment actually caused reductions in dry weight in all the cultivars (Figure 6.4). Qalandari, S-12 and MNH-93 actually showed some recovery towards the end of the recovery period.

6.3.4 Effect of aerated waterlogging on root growth

Table 6.5 and Figure 6.5 contain the results of this experiment. In this case, only two cultivars, S-12 and Qalandari, were studied. They were the cultivars that suffered the least and the greatest damage, respectively, under the non-aerated waterlogging treatment. Seedlings subjected to 6 hour waterlogging treatment showed reductions in their root growth. Thus, the increase in root length between 48 and 120 hours was reduced by 12 and 17%, respectively, in S-12 and Qalandari compared with their controls. The 12 hour waterlogging treatment caused significant inhibition, so that the increase in length between 48 and 120 hours was reduced by 42 and 33%, respectively, in S-12 and Qalandari compared with their controls.

Table 6.4 Effect of waterlogging on root dry weight gain of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	48	5.6	--	--	--
	72	8.1	6.3	5.0	4.3
	96	11.7	7.4	6.9	3.8
	120	12.7	9.7	7.8	4.7
S-12	48	5.3	--	--	--
	72	6.9	5.0	5.5	4.2
	96	11.1	9.6	6.6	4.7
	120	12.3	12.3	7.9	5.3
MNH-93	48	5.7	--	--	--
	72	7.7	5.9	5.2	3.1
	96	8.3	7.9	6.2	3.0
	120	13.7	9.4	7.9	4.4
Rehmani	48	4.4	--	--	--
	72	6.8	5.1	4.8	3.5
	96	11.5	7.2	5.7	3.2
	120	11.8	9.6	7.4	3.5

Each value is the mean weight (mg $(root)^{-1}$) from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

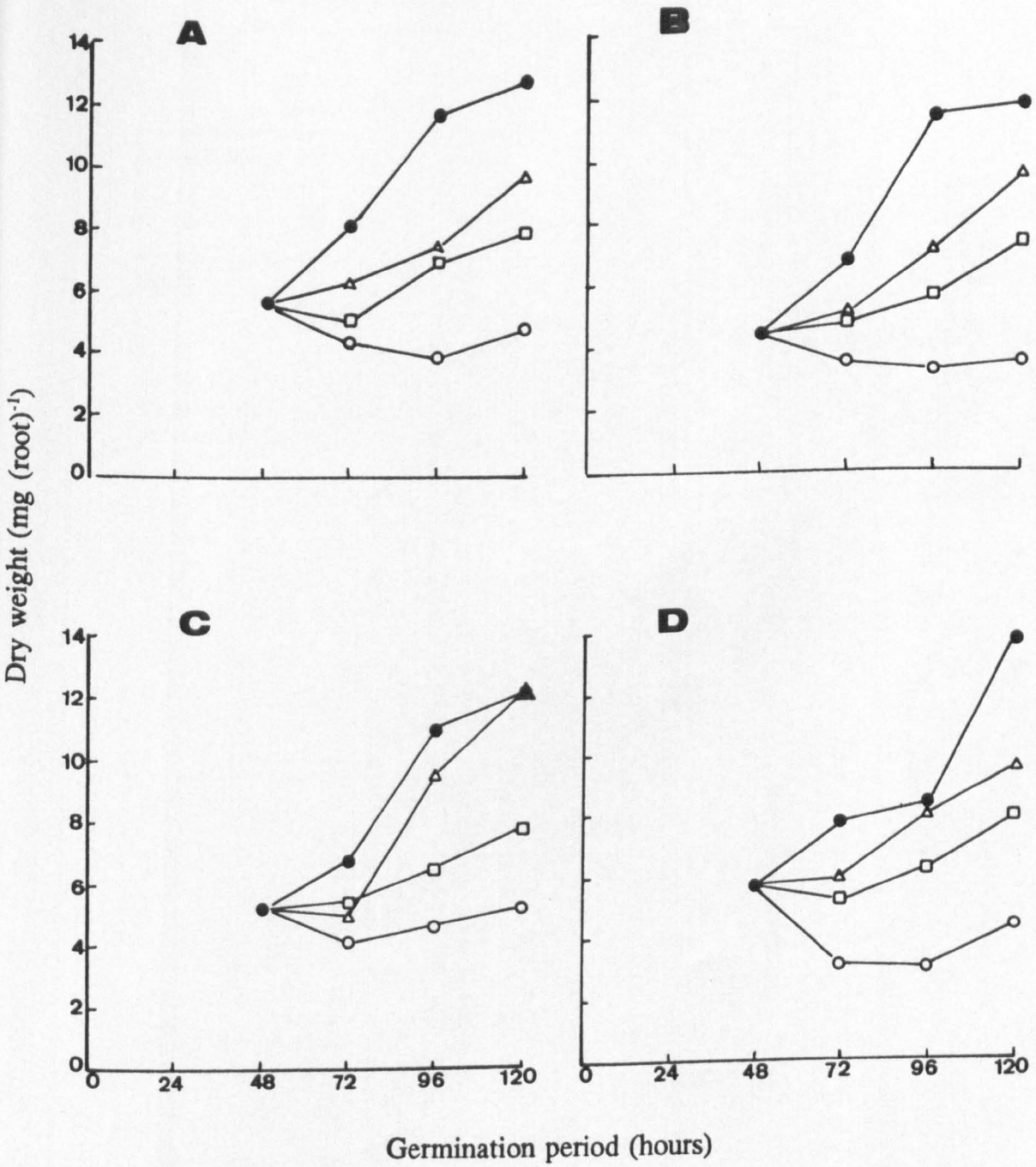


Figure 6.4 Effect of waterlogging on root dry weight gain in various cotton cultivars. (A) Qalandari, (B) Rehmani, (C) S-12, (D) MNH-93. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

Table 6.5 Effect of aerated waterlogging on root growth of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	48	47 ±15	--	--	--
	72	75 ±22	61 ±17	55 ±16	70 ±25
	96	94 ±30	82 ±27	76 ±23	74 ±26
	120	111 ±35	100 ±33	90 ±29	77 ±27
S-12	48	46 ±15	--	--	--
	72	58 ±19	61 ±18	50 ±17	58 ±20
	96	89 ±29	79 ±26	69 ±22	66 ±22
	120	89 ±35	84 ±26	71 ±23	70 ±23

Each value is the mean length (mm) ±sd from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

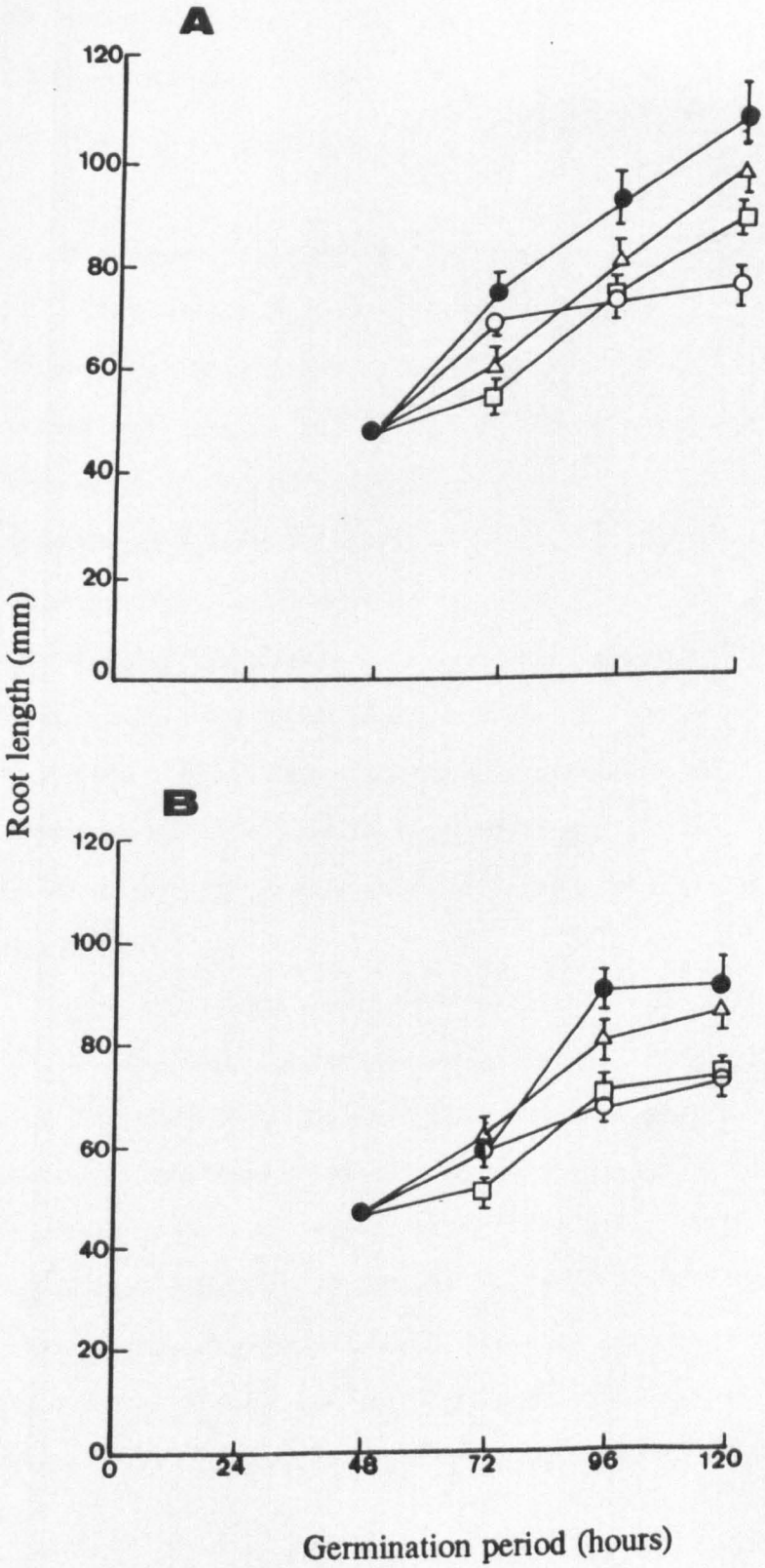


Figure 6.5 Effect of aerated waterlogging on root growth in two cotton cultivars. (A) Qalandari, (B) S-12. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.
Vertical bars indicate standard error values.

Seedlings waterlogged for 24 hour showed an even greater effect so that the increase in root length between 48 and 120 hours was reduced by 44 and 53%, respectively, in S-12 and Qalandari.

6.3.5 Effects of aerated waterlogging on fresh and dry weights

The results for fresh weight are presented in Table 6.6 and in Figure 6.6. These results imply that the 6 hour waterlogging treatment caused only small reductions in fresh weight gains. After this treatment, the fresh weight gain at 120 hours was not significantly affected in Qalandari, which showed only 5% reduction compared to its control. There was a greater reduction of 17% in the case of S-12. Waterlogging for 12 hour also caused reductions in fresh weight gain. These reductions were greater than in the 6 hour waterlogging treatment. The 24 hour waterlogging treatment had an even greater effect so that the gain in fresh weight up to 120 hours was reduced by 55 and 45%, respectively, in S-12 and Qalandari compared with their controls.

The dry weight results are presented in Table 6.7 and in Figure 6.7. The dry weight patterns for the roots of waterlogged seedlings in both cultivars were qualitatively very similar to the fresh weight patterns. Dry weight in the cultivar Qalandari was not affected following a 6 hour waterlogging treatment, while S-12 showed a significant reduction of 20%. In the 24 hour waterlogging treatment, however, dry weight was greatly affected. In this case, the gain in dry weight between 48 and 120 hours was reduced by 70 and 56%, respectively, in S-12 and Qalandari compared with their controls.

Table 6.6 Effect of aerated waterlogging on root fresh weight gain of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	48	82	--	--	--
	72	130	121	118	139
	96	193	172	149	164
	120	235	228	218	166
S-12	48	84	--	--	--
	72	118	124	100	118
	96	201	168	146	133
	120	228	204	169	149

Each value is the mean weight (mg $(root)^{-1}$) from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

Table 6.7 Effect of aerated waterlogging on root dry weight gain of various cotton cultivars

Cultivar	Total germ. period (h)	Control	Period of waterlogging (h)		
			6	12	24
Qalandari	48	5.6	--	--	--
	72	8.1	6.5	6.2	7.1
	96	11.7	9.7	8.7	8.2
	120	12.7	12.7	12.1	8.7
S-12	48	5.3	--	--	--
	72	6.9	6.8	5.3	5.7
	96	11.1	9.7	7.9	6.9
	120	12.3	10.9	8.6	7.4

Each value is the mean weight (mg $(root)^{-1}$) from 150-200 seedlings.
Controls: Seeds germinated at 25°C without waterlogging treatment.

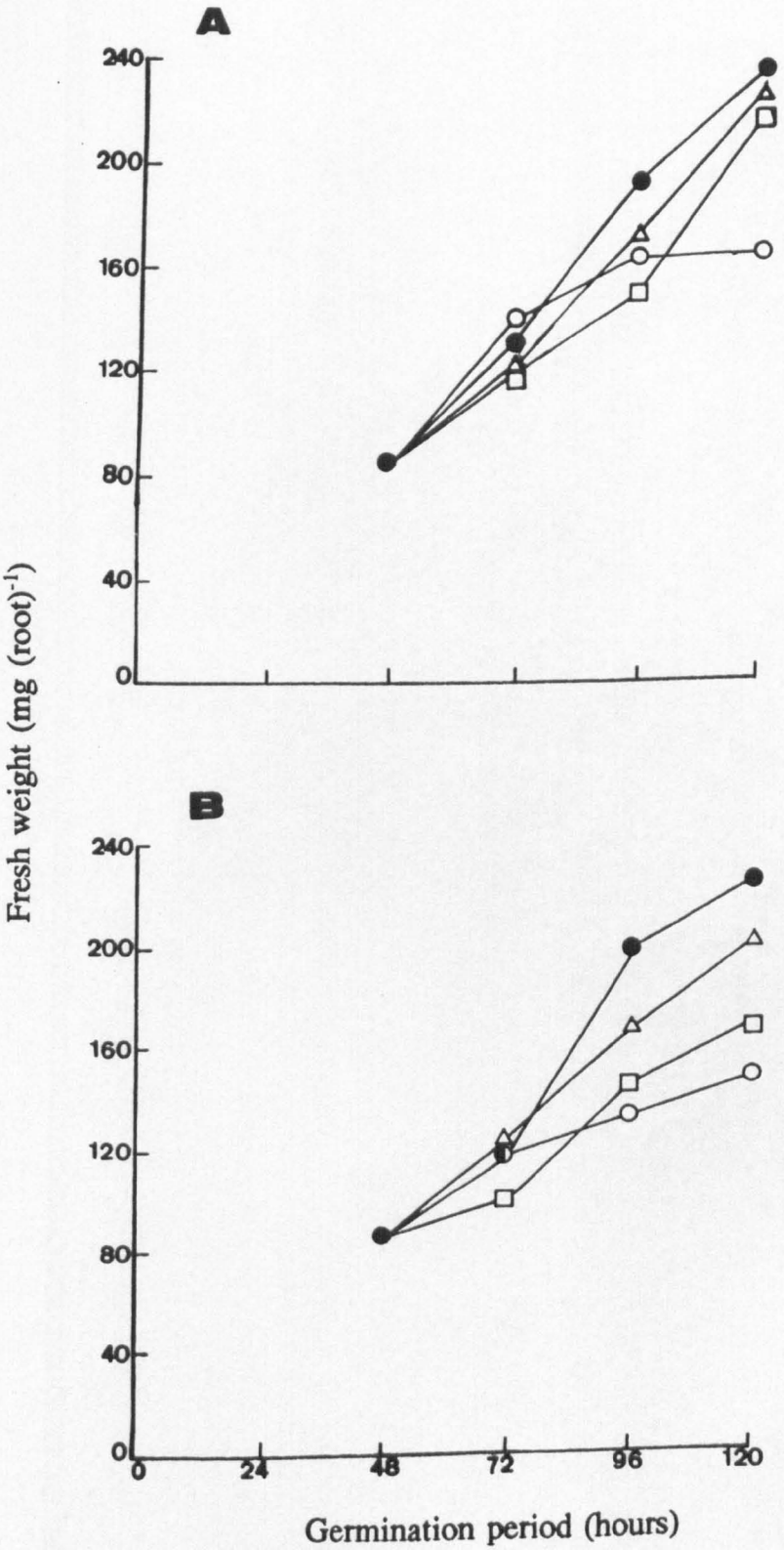


Figure 6.6 Effect of aerated waterlogging on root fresh weight gain in two cotton cultivars. (A) Qalandari, (B) S-12. ●, control; △, 6h treatment; □, 12h treatment; ○,24h treatment.

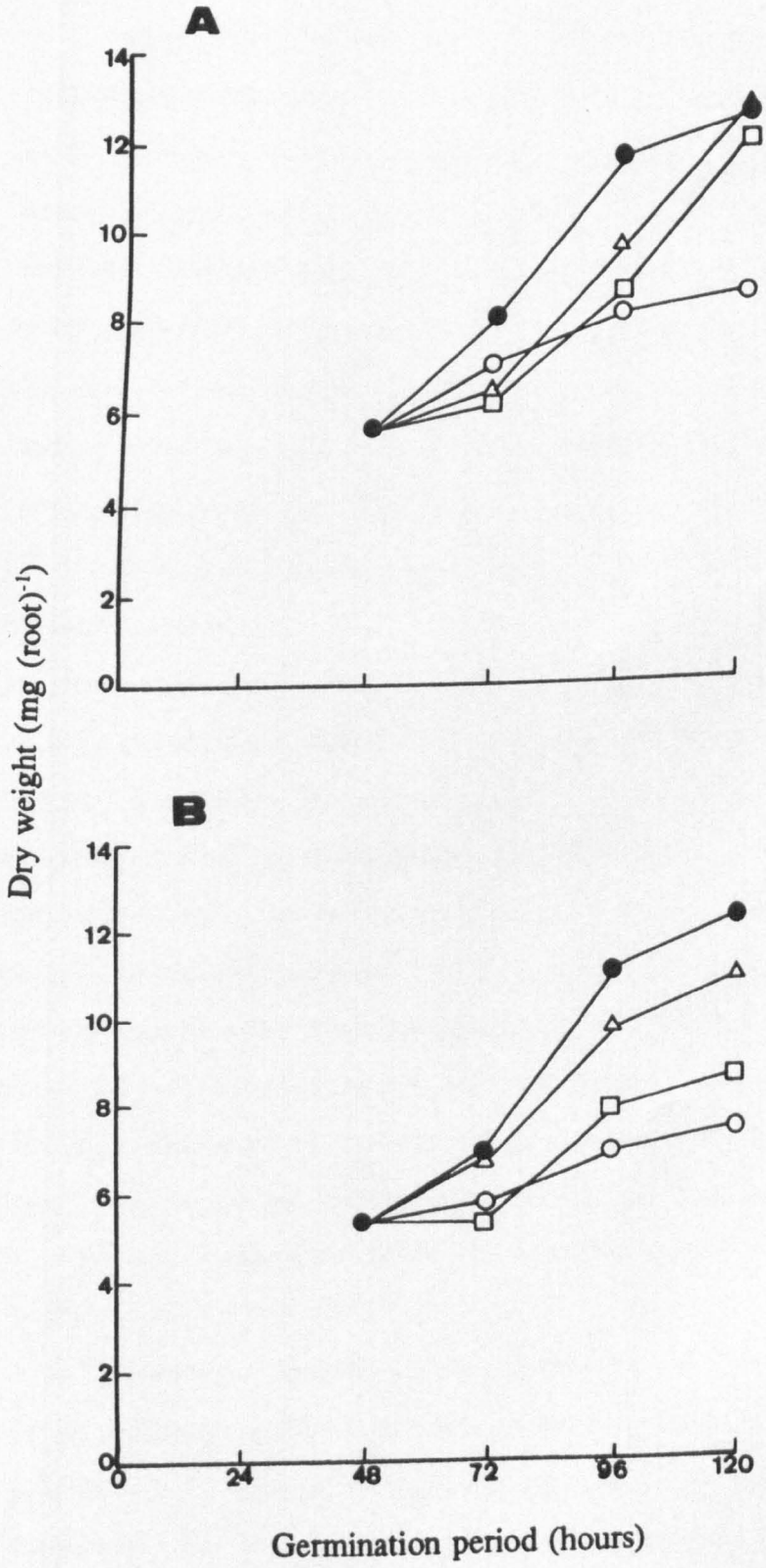


Figure 6.7 Effect of aerated waterlogging on dry weight gain of two cotton cultivars. (A) Qalandari, (B) S-12. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

6.4 Discussion

Under normal germination conditions, root length increases with increasing germination time. In the case of cotton seeds germinated at 25°C, the root length progressively increased during the first 96 hours of germination. After this time, MNH-93 and the Qalandari achieved further growth while the other two cultivars (Rehmani and S-12) showed no further increase. Similar results have been reported for root growth patterns in wheat, maize and soybean (Mengal and Barber, 1974; Sivakumar *et al.*, 1977; Gregory, 1978). Interestingly, roots of Qalandari had the fastest root growth during the early stages up to 48 hours, whereas the slowest root growth was observed in Rehmani. After 120 hours germination, Qalandari again showed the greatest root growth while the slowest growth was observed in S-12. Dalians (1982) reported that the rapidity with which the cotton seed radicle emerges can provide a measure of the vigour of the seed and may predict seedling performance. Kreig and Carrol (1978) also stated that growth rate in cotton seedlings is closely associated to the quantity of storage material and the density of the seed. This implies that the particular seed lot of Qalandari used in this experiment shows greater vigour than the other cultivars. A slightly different situation was found in the heat-shock studies reported in chapter 3. There, MNH-93 had the fastest growth rate, at least during the first 48 hours of germination. Different seed lots had been used for that study.

The results reported in this section suggest that waterlogging has a profound effect on root growth in the different cotton cultivars. It was generally noticed that during waterlogging treatments, the growth of roots is strongly suppressed. This was presumably due to the decline in oxygen concentration. It has been

reported by others that poor aeration limits plant growth, and especially root growth, if the oxygen supply to the root system is inadequate for respiration. Letey *et al.* (1962); Huck (1970) and Meek *et al.* (1980) reported that even short periods of anaerobic conditions in the soil can irreparably damage the root system of many plants including cotton.

Seedlings that had been waterlogged for 6 hour were significantly affected, although the effect was quantitatively different for the different cultivars; S-12 showed the greatest tolerance while Qalandari showed the least tolerance. The 12 hour waterlogging treatment caused significant reductions in root growth and generally the root tips became dark yellow and they were obviously damaged. Nevertheless, a small amount of growth was observed during recovery. Morriset (1978) reported that, under poor aeration, root tip may be damaged, so that when aeration is restored, growth can only be continued from the tip regions proximal to the rest of the stem. Qalandari unexpectedly showed slightly greater tolerance to this waterlogging treatment than the other cultivars but this difference was very small. The 24 hour waterlogging treatment stopped root growth and this was followed by a breakdown of root tissue. This treatment caused great damage to almost all the seedlings (Plate 6.1 to 6.4). The damage was more clearly visible on the seedlings of MNH-93 and Rehmani. Their distal halves were more brownish than the seedlings of S-12 and Qalandari. There were also shrivelling of the distal halves of the roots, especially the tips; this was severe in all the cultivars. These results are in agreement with other investigators who have reported that waterlogging significantly damages roots (Meyer *et al.*, 1987; Salam and Scott, 1987).

In the cases of the fresh and dry weights, the results showed

Plate 6.1 Effects of waterlogging on the roots of MNH-93. A, 48 hour control; B, 6 hour waterlogging followed by 66 hour recovery; C, 12 hour waterlogging followed by 60 hour recovery; D, 24 hour waterlogging followed by 48 hour recovery; E, 120 hour control.

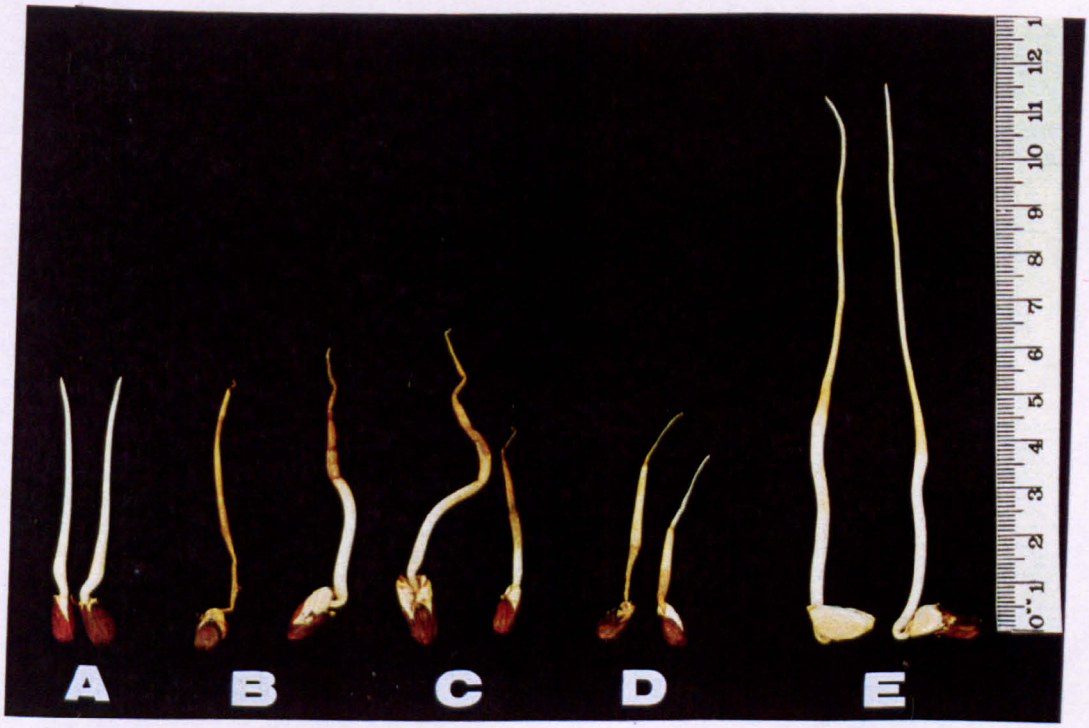


Plate 6.2 Effects of waterlogging on the roots of Rehmani. A, 48 hour control; B, 6 hour waterlogging followed by 66 hour recovery; C, 12 hour waterlogging followed by 60 hour recovery; D, 24 hour waterlogging followed by 48 hour recovery; E, 120 hour control.

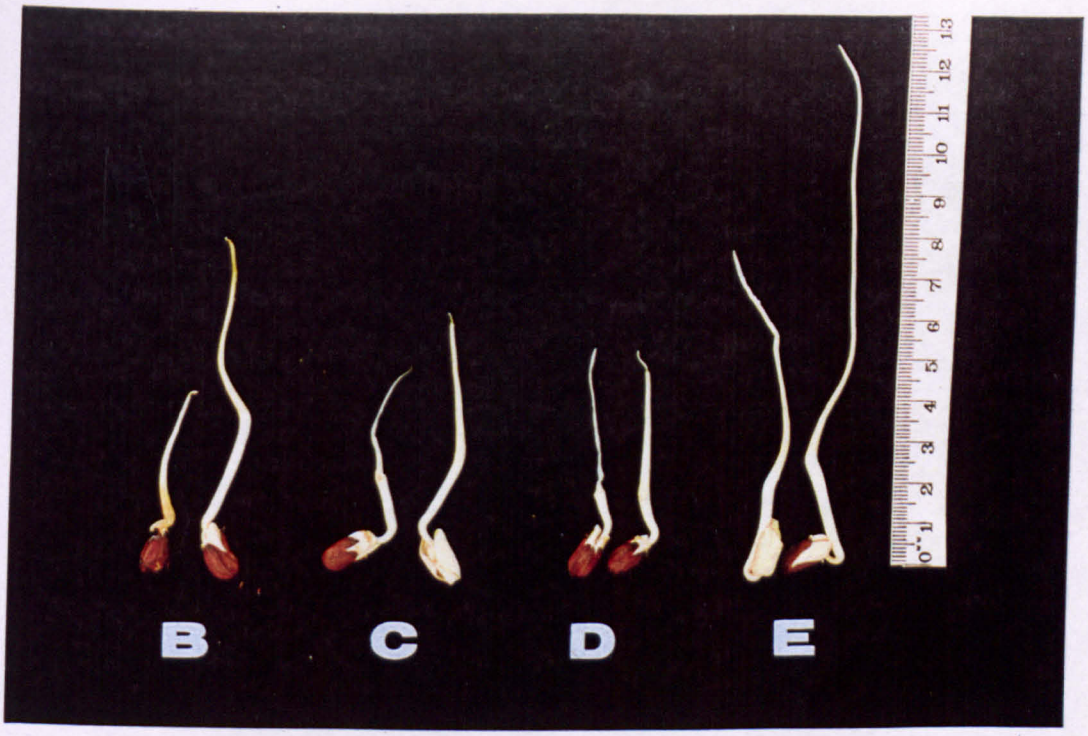


Plate 6.3 Effects of waterlogging on the roots of S-12. B, 6 hour waterlogging followed by 66 hour recovery; C, 12 hour waterlogging followed by 60 hour recovery; D, 24 hour waterlogging followed by 48 hour recovery; E, 120 hour control.

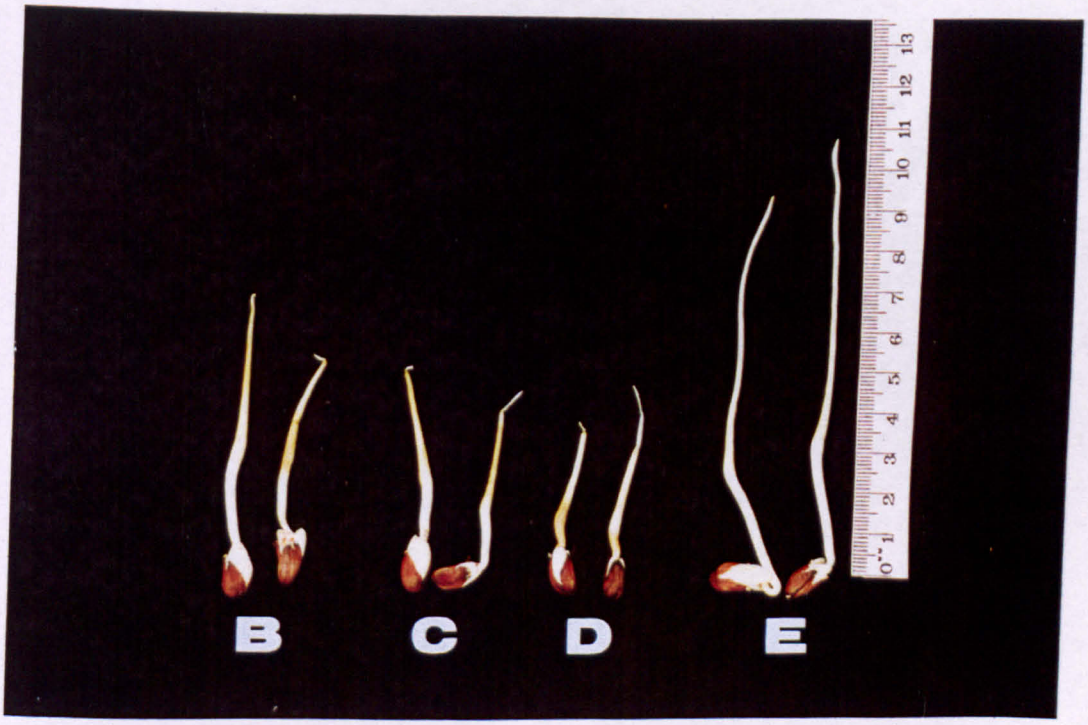


Plate 6.4 Effects of waterlogging on the roots of Qalandari. B, 6 hour waterlogging followed by 66 hour recovery; C, 12 hour waterlogging followed by 60 hour recovery; D, 24 hour waterlogging followed by 48 hour recovery; E, 120 hour control.

a slightly different pattern compared with that seen for the root length data. Seedlings waterlogged for 6 and 12 hours showed less inhibition of fresh and dry weight gains compared with the effect on root length. This suggests that despite the reduction in root growth, these roots could still accumulate water and tissue dry mass. Following 6 hour waterlogging, the cultivar S-12 showed greatest tolerance, while MNH-93 showed the least tolerance. The 12 hour waterlogging treatment showed no apparent difference between the cultivars. In the seedlings waterlogged for 24 hours, however, the fresh weight changes were actually negative in some cases. There was, therefore, a loss of water and/or tissue material compared with the controls. Sena-Gomes and Kozlowski (1980a) reported that flooding completely stopped root growth in *Pinus halepensis* seedlings. They further reported that, after 40 days of flooding, the dry weight of the root system was lower than before flooding was initiated. This was presumably due to severe damage and even the death of cells leading to loss of turgor and shrivelling of the tissues. There are also several other reports which support our findings that longer periods of waterlogging reduce dry weight accumulation (Trought and Drew, 1980 a and c; Krishnamorthy *et al.*, 1987; Meyer *et al.*, 1987; Del-Rosario and Fajardo, 1991). In the present work, it was found that S-12 was relatively more tolerant of waterlogging than Qalandari under all the treatments employed.

Seedlings waterlogged in aerated water also suffered reductions in root growth in both cultivars studied. These reductions were lower than those seen in the waterlogging treatment without aeration. It was noted that, during aerated waterlogging treatment, root growth continued at a significant rate. In this experiment, it was also observed that, during recovery following each waterlogging treatment,

the roots appeared to be quite healthy and no apparent damage was seen. The upper half of the roots become pale yellow and the junction point between hypocotyl and the roots was swollen however. The reduction in the root growth seen in these experiments might be due to the presence of a layer of relatively unaerated water around the roots (water which does not mix with the bulk water). Karim *et al.* (1986) reported that root growth was curtailed in the presence of excess water, possibly due to imbalance in the seedling's metabolism. This in turn might have produced compounds that are inhibitors of root growth.

It was also noted from these results that there is no correlation between vigour and waterlogging. Qalandari showed the faster root growth under non-waterlogging conditions, but it was the most severely affected by waterlogging.

It may be concluded from these experiments that anaerobic waterlogging for longer than 6 hour irreparably damages the seedlings of all the cultivars. In the 6 hour waterlogging treatment, S-12 was found to be relatively more tolerant and Qalandari was the most susceptible among the cultivars studied. In the experiment in which seedlings were waterlogged in an aerated water, the best root growth was observed in S-12 while the fresh and dry weights accumulation was highest in Qalandari.

CHAPTER SEVEN

Chapter Seven

Effects of waterlogging on respiration rate

7.1 Introduction

Respiration is the process of gaseous exchange (O_2 taken up and CO_2 given out) associated with the oxidative catabolism of organic substrates. This produces carbon skeletons for biosynthetic purposes and energy for ion transport and assimilation, growth and cellular maintenance processes. Rumpho and Kennedy (1981) stated that higher plants have an absolute requirement for oxygen for the maintenance of metabolism and growth.

Under normal conditions in well-drained soils, the aerobic respiration of plant roots and soil microorganisms requires typically 5 and 24g oxygen for each square metre of land surface per day during the growing season (Russel, 1973). During flooding, the soil pore space is totally water filled and gaseous exchange between soil and atmosphere is virtually eliminated. Due to the low diffusivity in water, the oxygenated zone at the soil surface may extend to a depth of only a few millimeters. Depending upon the soil temperature and tissue respiration rates, therefore, the dissolved oxygen in the soil water will be exhausted in only hours or days and the soil will become anaerobic.

Waterlogging creates an anaerobic environment and this in turn brings changes in metabolism from aerobic to anaerobic pathways. This results in reduced energy yield, accumulation of toxic compounds and rapid depletion of organic substrates (Ueda and Tsuji, 1971; Gupta, 1977b). The adverse effects of root anaerobiosis in mesophyte plants can be primarily attributed to the deficiency of molecular oxygen which prevents normal aerobic metabolism. Lee and Lee (1991) and Del-Rosario

and Fajardo (1991) reported that waterlogging reduces the respiration rate of apple, soybean, mungbean, tomatoes, cotton, pumpkins and rice seedling roots. Mohanty *et al.* (1993) also suggested that rates of respiration (O_2 uptake and CO_2 production) and fermentation (CO_2 production alone) all declined with time in anoxia. It has been reported that waterlogging increases the activity of alcohol dehydrogenase *in tomato roots* (Wignarajah and Greenway 1976; Del-Rosario and Fajardo, 1991), while Vantoi *et al.* (1987) reported that waterlogging increases the activities of four enzymes, namely alcohol dehydrogenase, lactate dehydrogenase, pyruvate decarboxylase and malic enzymes, in maize seeds. Crawford (1969) showed that oxygen deficiency induced the synthesis of the glycolytic enzymes as well as ethanol accumulation only in the roots of flood intolerant species.

Glycolysis and the pentose phosphate pathway play important roles in the germination process (Botha and Small, 1985). In contrast, it was found that gluconeogenesis before radicle protrusion is slight but rapidly increased following the initiation of radicle growth (Becker *et al.*, 1978; Botha and Small, 1985). It is known that anaerobic stress inhibits gluconeogenesis and lipid breakdown in seeds (Slack *et al.*, 1977; Knowles and Kennedy, 1984). In general, it appears that lipid rich seeds are less capable of maintaining ATP levels under anoxia than starchy seeds (Raymond *et al.*, 1985) and the oily seeds are more sensitive to inhibition of germination under restricted oxygen supply than starchy seeds (Al-Ani *et al.*, 1985).

Some of the available literature on anerobiosis is quite complex and puzzling. Oliveira (1977) and Vartapetian *et al.* (1977 and 1978c) reported that, when the roots of non-wetland species were excised and subjected to anaerobic conditions under laboratory conditions, their mitochondria show abnormal ultrastructure (irregular

shape and dilated cristae) after several hours followed by generalized degeneration within 24-48 hours. No such type of changes were observed in well-aerated roots. Oliveira (1977) further reported that when anaerobic roots were returned to air before the cristae degenerated, the initial structure is restored and respiratory activity partially regained. Morisset (1973) revealed that, under anaerobic conditions, mitochondria of lateral-root meristems show less structural degeneration than the mitochondria of apical meristems.

Biochemical studies on rice coleoptiles grown under anaerobic conditions have shown that their mitochondria contain the entire respiratory chain, but the amount of cytochromes present is 2 to 3 times less than in the mitochondria of coleoptiles grown in air (Vartapetian *et al.*, 1975). Furthermore, the anaerobic mitochondria oxidize substrates of the Krebs cycle and NADH 3 to 7 times more slowly than aerobic mitochondria do. They are, however, able to carry out some oxidative phosphorylation.

Electron microscope studies by Vartapetian *et al.* (1971) showed that the cells of rice coleoptiles grown under anaerobic conditions contain all the organelles typical for aerobic coleoptiles including mitochondria. Such mitochondria are larger in size (1.5 to 2.0 µm in diameter) than the mitochondria of the aerobic coleoptile cells (0.5 to 0.6 µm). They were irregularly shaped and the matrix was clarified. These mitochondria have a low number of cristae and the intracristal space was dilated.

Ultra-structural studies on intact root cells of 8-10 day and 24-30 day cotton plants (Vartapetian *et al.*, 1978c) showed that 48 and 72 hours of root anaerobiosis at 25°C causes no pathological changes in the mitochondria or in other cell organelles. However, a majority of the mitochondria in the meristem cells and the extension zone have

a parallel arrangement of cristae, which could be indicative of the cells suffering from deficiency of molecular oxygen and adapting to the oxygen deficiency.

Because of the effects reported above, it was decided to measure the respiration rate and quantify the effect of waterlogging in seedling root tips. In our earlier experiment (Chapter 6) it was found that Qalandari was most sensitive and S-12 was the least sensitive cultivar with respect to waterlogging. It was therefore decided to use these two cultivars.

7.2 Methods

In this study, the seeds of two cultivars, Qalandari and S-12, were used from the 1991-92 harvest. The detailed procedures employed are described in the Material and Methods Chapter. Briefly, cotton seedlings that had been germinated for 48 hours at 25°C were waterlogged for 3, 6, 12 or 24 hours (Section 2.8). After the waterlogging treatment, they were drained and returned to the incubator at 25°C for recovery. The recovery period was adjusted so that the total experimental period was 72, 96 or 120 hours for each waterlogging treatment. Controls consisted of seeds germinated for up to 120 hours at 25°C in the same incubator. At the end of the experimental treatments, seedlings were harvested and sorted into quartiles according to their root length (Section 2.6). The seedlings of the first and the third quartiles were taken for analyses in these experiments. From each quartile, the five best seedlings were selected and their 5mm root tips were isolated using a scalpel. The tips were immediately transferred to the sample compartment of the oxygen electrode for determination of respiration rate (Section 2.9). Tissue fresh weight was determined, after measurement of the respiration

rate. For this, the root tips were taken out of the oxygen electrode, damp dried and weighed on a five-place analytical balance. In cases where root tips were damaged by waterlogging, their fresh weights were determined in separate experiments. Finally, the root tips were dried for 48 hours in an oven at 70°C and re-weighed to determine their dry weights.

The resulting data were analysed statistically as described in the Section 2.18. All graphs were plotted using a personal computer with Systat/Sygraph (Smooth=short) software programme.

7.3 Results

The results for the two cultivars are reported separately.

7.3.1 Effects of waterlogging on fresh weight, dry weight and respiration rate in the cultivar Qalandari

The results are shown in Tables 7.1 to 7.3 and in Figures 7.1 and 7.2. In seeds germinated at 25°C (control), root tip fresh weight and dry weight decreased with increasing germination time. In each case, the curves followed a similar pattern. The respiratory activities of the tips also followed a similar decreasing course. Moreover, the general patterns were qualitatively and quantitatively very similar for both quartiles. For example, in the first quartile, the root tip fresh weight decreased from 24.6 mg $(5 \text{ tips})^{-1}$ at 24 hour to 8.7 mg $(5 \text{ tips})^{-1}$ at 120 hours, while in the third quartile the fresh weight decreased from 25.6 to 9.1 mg $(5 \text{ tips})^{-1}$ over a similar period. The changes were highly significant statistically ($P<0.03$). The rate of respiration in the first quartile also significantly decreased from 17.6 nmoles min^{-1} $(5 \text{ tips})^{-1}$ at 24 hours to 4.0 nmoles min^{-1} $(5 \text{ tips})^{-1}$ at 120 hours ($P<0.01$), while in the third quartile the rate decreased from 16.8 to 3.6 nmoles min^{-1} $(5 \text{ tips})^{-1}$ ($P<0.01$).

Seedlings that had been waterlogged for 3 to 24 hours showed no significant changes in fresh weight in both quartiles during the waterlogging treatments ($P>0.22$) (Figures 7.1A and 7.2A). During recovery following 6 hour waterlogging treatment, the fresh weight was also not affected and even showed small but insignificant increases in both quartiles at 120 hour compared with the 120 hour controls ($P>0.28$). At the end of the recovery period following 12 hour treatment, the fresh weight was reduced from 8.7 to 6.9 mg $(5 \text{ tips})^{-1}$ in the first quartile and from 9.1 to 6.1 mg $(5 \text{ tips})^{-1}$ in the third quartile. These reductions were statistically insignificant however ($P>0.22$). During recovery following 24 hour waterlogging there were similar decreases. In this case, the fresh weight at 120 hour was reduced significantly in the third quartile compared with its 120 hour control ($P=0.04$).

The dry weight patterns following waterlogging were very similar to those seen for fresh weights (Figures 7.1B and 7.2B). In this case, seedlings waterlogged for 6 hours also showed small increases during the recovery period. Waterlogging for 12 hours had no significant effect on both quartiles ($P>0.21$). At the end of the recovery following 24 hour waterlogging treatment, the dry weight was significantly decreased from 0.60 to 0.37 mg $(5 \text{ tips})^{-1}$ in the first quartile and from 0.77 to 0.40 mg $(5 \text{ tips})^{-1}$ in the third quartile ($P<0.04$).

Waterlogging for 3 hours sharply reduced the respiration rates in both quartiles. The rate at the end of the waterlogging treatment was reduced from 11.6 to 5.6 nmoles min^{-1} $(5 \text{ tips})^{-1}$ in the first quartile and from 11.4 to 5.6 nmoles min^{-1} $(5 \text{ tips})^{-1}$ in the third quartile. The 6 hour waterlogging treatment caused similar reductions; in this case, they were reduced to 5.0 nmoles min^{-1} $(5 \text{ tips})^{-1}$ in the

Table 7.1 Effect of waterlogging on fresh weight in the cotton cultivar Qalandari.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	24.6	3.6	2.0	25.6	0.5	0.3
	36	16.0	0.3	0.2	20.2	0.2	0.1
	48	12.7	1.6	0.8	13.7	1.5	0.8
	51	12.7	1.1	0.7	14.0	0.5	0.3
	54	11.9	0.6	0.4	13.5	1.3	0.6
	60	11.9	0.5	0.3	11.9	0.6	0.4
	72	10.6	0.4	0.2	10.6	0.1	0.1
	96	10.1	1.2	0.6	10.2	0.2	0.1
	120	8.7	1.8	0.9	9.1	0.8	0.4
3h	51	12.5	0.7	0.4	13.3	0.9	0.5
6h	54	12.1	0.8	0.4	12.5	1.0	0.5
	72	12.7	0.8	0.5	12.1	0.9	0.5
	96	9.9	1.8	0.9	11.1	0.2	0.1
	120	11.0	0.5	0.3	10.3	1.2	0.7
12h	60	11.2	0.7	0.4	12.3	1.2	0.7
	72	12.0	0.2	0.1	13.3	0.8	0.5
	96	6.3	1.4	0.8	7.5	0.9	0.5
	120	6.9	1.9	1.0	6.1	1.6	0.9
24h	72	9.4	1.1	0.6	10.8	1.3	0.8
	96	9.2	1.0	0.6	9.4	0.2	0.1
	120	6.1	0.8	0.5	3.9	0.7	0.4

All values are mean weight (mg (5 tips)$^{-1}$) ±sd and ±se from 3 to 5 experiments.

Table 7.2 Effect of waterlogging on dry weight in the cotton cultivar Qalandari.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	3.80	1.00	0.60	4.20	0.20	0.10
	36	1.73	0.05	0.03	2.00	0.08	0.05
	48	1.40	0.09	0.05	1.40	0.08	0.04
	51	1.35	0.08	0.04	1.50	0.07	0.04
	54	1.32	0.09	0.04	1.36	0.15	0.06
	60	1.20	0.08	0.04	1.20	0.08	0.04
	72	0.98	0.12	0.06	1.05	0.05	0.03
	96	0.88	0.08	0.04	0.90	0.18	0.09
	120	0.60	0.08	0.05	0.77	0.16	0.09
3h	51	1.56	0.10	0.04	1.60	0.06	0.03
6h	54	1.26	0.05	0.02	1.30	0.10	0.04
	72	1.32	0.08	0.04	1.28	0.08	0.04
	96	0.87	0.16	0.09	1.00	0.00	0.00
	120	1.00	0.08	0.04	0.97	0.05	0.03
12h	60	0.95	0.09	0.05	1.00	0.12	0.06
	72	1.10	0.00	0.00	1.13	0.08	0.04
	96	0.63	0.05	0.03	0.60	0.00	0.00
	120	0.63	0.12	0.07	0.73	0.09	0.05
24h	72	0.83	0.05	0.03	0.87	0.05	0.03
	96	0.70	0.16	0.09	0.77	0.17	0.09
	120	0.37	0.05	0.03	0.40	0.08	0.05

All values are mean weight (mg (5 tips)$^{-1}$) ±sd and ±se from 3 to 5 experiments.

Table 7.3 Effect of waterlogging on respiration rate in the cotton cultivar Qalandari.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	17.6	1.8	1.0	16.8	0.2	0.1
	36	13.2	1.0	0.6	13.2	0.2	0.1
	48	11.6	1.0	0.4	11.4	1.0	0.6
	51	11.0	0.8	0.4	10.6	1.2	0.8
	54	10.8	1.0	0.6	9.4	0.8	0.4
	60	8.8	1.4	0.8	7.4	1.2	0.8
	72	7.6	1.2	0.8	6.6	1.2	0.8
	96	5.4	0.6	0.4	5.2	0.4	0.2
	120	4.0	0.6	0.4	3.6	0.2	0.1
3h	51	5.6	0.6	0.4	5.6	1.6	1.0
6h	54	5.0	0.8	0.4	4.8	0.4	0.2
	72	6.8	1.8	1.0	5.8	1.4	0.8
	96	4.8	1.2	0.8	4.2	1.0	0.6
	120	4.6	0.6	0.4	3.8	0.4	0.2
12h	60	1.6	0.2	0.1	1.4	0.2	0.1
	72	4.6	1.2	0.8	3.8	0.6	0.4
	96	5.2	1.2	0.8	4.6	1.2	0.8
	120	4.8	0.8	0.4	3.8	0.8	0.4
24h	72	1.0	0.3	0.1	0.8	0.3	0.1
	96	3.6	0.8	0.4	3.4	1.0	0.6
	120	2.2	0.8	0.4	2.2	0.1	0.1

All values are mean (nmoles min^{-1} $(5\ tips)^{-1}$) ±sd and ±se from 3 to 5 experiments.

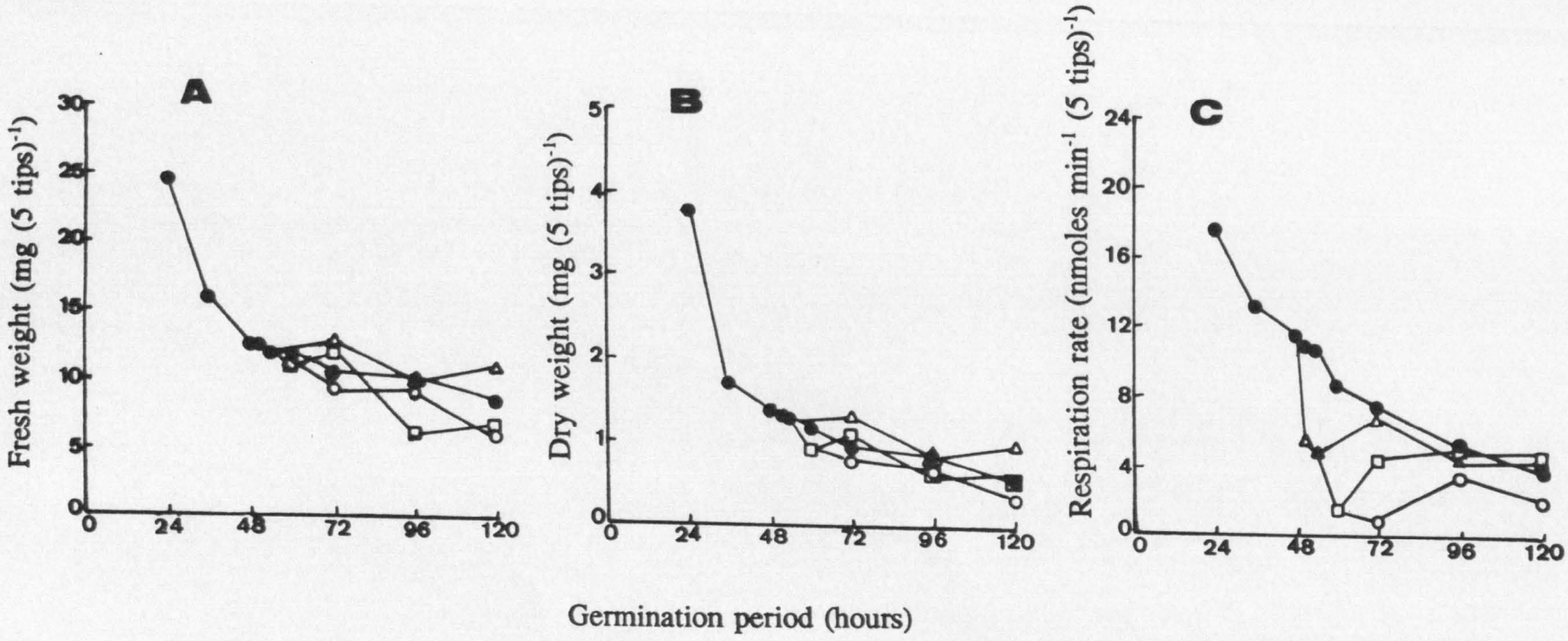


Figure 7.1 Effects of waterlogging on fresh weight, dry weight and respiration rate in the first quartile of Qalandari. (A) Fresh weight, (B) Dry weight, (C) Respiration rate. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

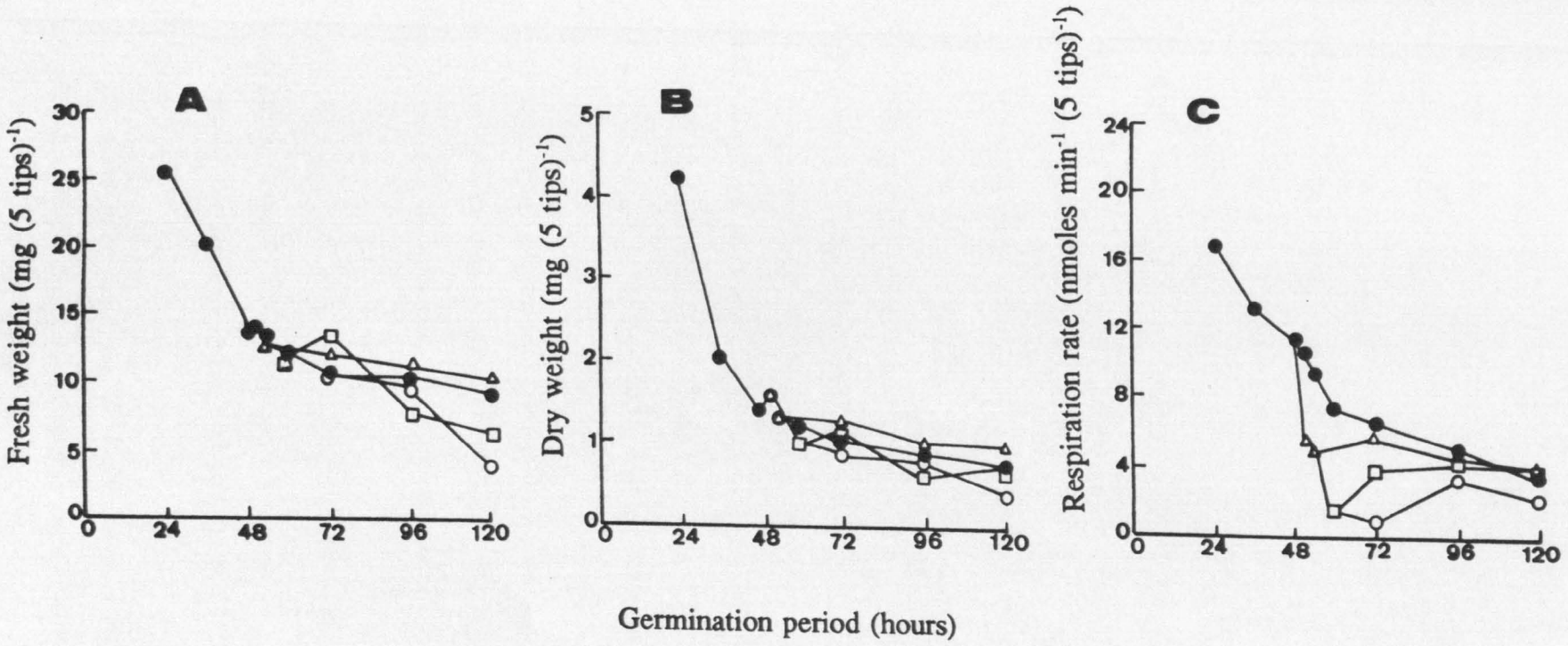


Figure 7.2 Effects of waterlogging on fresh weight, dry weight and respiration rate in the third quartile of Qalandari. (A) Fresh weight, (B) Dry weight, (C) Respiration rate. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

first quartile and to 4.8 nmoles min^{-1} (5 tips)$^{-1}$ in the third quartile. All of these reductions were highly significant ($P<0.001$). During recovery following the 6 hour waterlogging, the root tips rapidly recovered their respiratory activities. At 120 hours, they showed similar respiration rates in both quartiles compared with their controls ($P>0.7$) (see Figures 7.1C and 7.2C).

Seedlings waterlogged for 12 hours also showed large reductions in root tips respiration rates in both quartiles. These reductions were greater than for the 6 hour treatment, down to 1.6 nmoles min^{-1} (5 tips)$^{-1}$ in the first quartile and 1.4 nmoles min^{-1} (5 tips)$^{-1}$ in the third quartile. During recovery, the tips rapidly recovered their respiratory activities within 12 hours. At 120 hour, the rates for both quartiles were up to the control levels and there were no significant differences between them ($P>0.22$).

The 24 hour waterlogging treatment caused even greater reductions in the respiration. It decreased to 1.0 nmoles min^{-1} (5 tips)$^{-1}$ in the first quartile and to 0.8 nmoles min^{-1} (5 tips)$^{-1}$ in the third quartile. During the first 24 hours of recovery, respiration recovered to 3.6 and 3.4 nmoles min^{-1} (5 tips)$^{-1}$, respectively, in the first and third quartiles. The respiration rate then fell off again, however. At 120 hours, it was reduced by 45 and 39%, respectively, in the first and third quartiles compared with the controls ($P=0.04$).

7.3.2 Effects of waterlogging on fresh weight, dry weight and respiration rate in the cultivar S-12

These results are described in Tables 7.4 to 7.6 and in Figures 7.3 and 7.4.

In seeds germinated at 25°C (control), root tip fresh weight and dry weight decreased with increasing germination time. In each case,

the curves followed a similar pattern. The respiratory activities of the tips also followed a similar decreasing course (Figures 7.3 and 7.4). Moreover, the general patterns were qualitatively and quantitatively very similar for both quartiles. For example, in the first quartile, the root tip fresh weight decreased significantly from 23.7 mg (5 tips)$^{-1}$ at 24 hour to 8.7 mg (5 tips)$^{-1}$ at 120 hours, while in the third quartile the fresh weight decreased from 15.8 mg (5 tips)$^{-1}$ at 36 hour to 8.3 mg (5 tips)$^{-1}$ at 120 hours. The dry weight patterns were qualitatively very similar to those seen for fresh weight in both quartiles. The rate of respiration in the first quartile also decreased from 21.4 nmoles min^{-1} (5 tips)$^{-1}$ at 24 hours to 4.0 nmoles min^{-1} (5 tips)$^{-1}$ at 120 hours, while in the third quartile the rate decreased from 12.8 nmoles min^{-1} (5 tips)$^{-1}$ at 36 hours to 4.0 nmoles min^{-1} (5 tips)$^{-1}$. All of these changes were highly significant statistically ($P=0.01$ or less).

Seedlings waterlogged for 3 to 24 hours showed no significant changes in their fresh weight in either quartiles during the waterlogging (Figures 7.1A and 7.2A). During recovery following 6 hour waterlogging treatment, the fresh weight was also not affected and even showed small apparent increases in both quartiles at 120 hour ($P>0.18$). At the end of the recovery period following 12 hour treatment, the fresh weight was significantly reduced from 8.7 to 4.3 mg (5 tips)$^{-1}$ in the first quartile ($P=0.03$) and from 8.3 to 4.0 mg (5 tips)$^{-1}$ in the third quartile ($P=0.04$) compared with their 120 hour controls. During recovery following 24 hour waterlogging, there were similar decreases in fresh weights. These reductions were also statistically significant ($P<0.01$).

The dry weight patterns following waterlogging were very similar to those seen for fresh weights (Figures 7.3B and 7.4B). In this case,

Table 7.4 Effect of waterlogging on fresh weight in the cotton cultivar S-12.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	23.7	2.2	1.2	--	--	--
	36	13.4	1.9	1.0	15.8	0.9	0.6
	48	10.2	0.5	0.3	12.3	0.9	0.5
	51	9.8	0.3	0.2	11.5	0.6	0.2
	54	9.3	0.2	0.1	10.4	0.4	0.2
	60	9.0	1.0	0.6	10.2	0.9	0.6
	72	9.0	0.7	0.4	10.0	1.2	0.5
	96	9.0	0.7	0.4	9.3	1.6	0.8
	120	8.7	0.5	0.3	8.3	0.8	0.4
3h	51	9.8	0.5	0.3	10.9	0.1	0.1
6h	54	8.6	0.9	0.5	9.5	0.4	0.2
	72	9.0	0.4	0.2	10.0	0.4	0.2
	96	9.8	0.5	0.3	10.9	0.8	0.4
	120	9.5	0.7	0.4	9.8	0.9	0.6
12h	60	8.9	0.8	0.3	10.8	0.8	0.3
	72	8.6	0.5	0.3	10.9	0.7	0.4
	96	8.5	0.8	0.4	8.4	2.0	1.0
	120	4.3	1.2	0.7	4.0	0.6	0.4
24h	72	8.4	0.4	0.2	8.8	0.6	0.3
	96	8.2	0.7	0.4	8.2	0.6	0.3
	120	3.7	0.2	0.1	2.8	1.1	0.6

All values are mean weight (mg (5 tips)$^{-1}$) ±sd and ±se from 3 to 5 experiments.

Table 7.5 Effect of waterlogging on dry weight in the cotton cultivar S-12.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	3.90	0.30	0.20	--	--	--
	36	1.50	0.10	0.05	1.90	0.20	0.10
	48	1.50	0.05	0.03	1.60	0.05	0.03
	51	1.20	0.04	0.02	1.40	0.04	0.02
	54	1.10	0.08	0.04	1.20	0.05	0.03
	60	0.90	0.10	0.05	1.20	0.05	0.03
	72	1.00	0.00	0.00	1.10	0.04	0.02
	96	0.90	0.10	0.05	0.95	0.09	0.05
	120	0.63	0.05	0.03	0.63	0.05	0.03
3h	51	1.20	0.10	0.05	1.30	0.08	0.04
6h	54	0.98	0.04	0.02	1.08	0.08	0.04
	72	0.90	0.08	0.04	0.93	0.09	0.05
	96	0.76	0.05	0.03	0.80	0.00	0.00
	120	0.93	0.12	0.08	0.87	0.09	0.06
12h	60	0.88	0.10	0.05	1.10	0.20	0.10
	72	0.84	0.10	0.04	0.92	0.04	0.02
	96	0.53	0.09	0.05	0.70	0.08	0.05
	120	0.47	0.16	0.09	0.43	0.12	0.07
24h	72	0.80	0.00	0.00	0.85	0.05	0.03
	96	0.66	0.05	0.03	0.83	0.05	0.03
	120	0.47	0.16	0.07	0.37	0.09	0.06

All values are mean weight (mg (5 tips)$^{-1}$) ±sd and ±se from 3 to 5 experiments.

Table 7.6 Effect of waterlogging on respiration rate in the cotton cultivar S-12.

Treatment (h)	Total germ. period(h)	First quartile			Third quartile		
		mean	sd	se	mean	sd	se
Control	24	21.4	0.8	0.4	--	--	--
	36	12.0	0.2	0.1	12.8	0.8	0.4
	48	10.6	0.4	0.2	12.6	1.2	0.6
	51	9.4	1.2	0.7	10.6	2.0	0.8
	54	9.6	0.4	0.2	10.6	0.8	0.4
	60	9.4	0.2	0.1	9.4	0.8	0.4
	72	9.4	1.0	0.6	8.6	1.0	0.4
	96	6.4	1.0	0.6	5.4*	0.8	0.4
	120	4.0	0.2	0.1	4.0	0.2	0.1
3h	51	5.8	0.4	0.2	8.6	1.4	0.6
6h	54	5.8	0.4	0.2	6.6	0.8	0.4
	72	6.8	0.8	0.4	8.0	0.6	0.4
	96	5.6	0.6	0.4	5.8	0.1	0.0
	120	4.0	0.4	0.2	4.0	0.0	0.0
12h	60	2.0	0.4	0.2	2.6	0.4	0.2
	72	2.8	0.6	0.4	4.2	0.8	0.4
	96	5.0	0.6	0.4	5.2	0.4	0.2
	120	3.2	0.0	0.0	3.4	0.6	0.4
24h	72	2.0	0.4	0.2	1.8	0.4	0.2
	96	3.0	0.4	0.2	3.2	0.4	0.2
	120	2.4	0.2	0.1	2.0	0.4	0.2

All values are mean (nmoles min^{-1} $(5\ tips)^{-1}$) ±sd and ±se from 3 to 5 experiments.

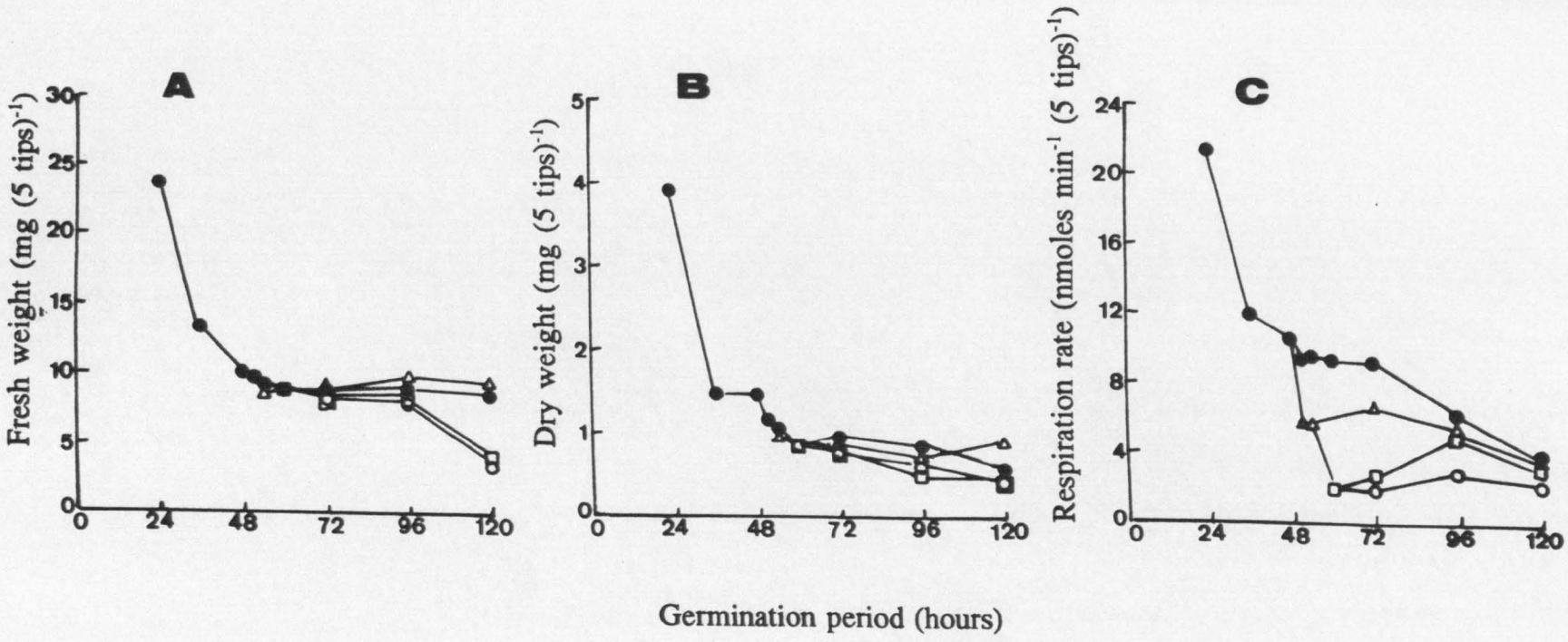


Figure 7.3 Effects of waterlogging on fresh weight, dry weight and respiration rate in the first quartile of S-12. (A) Fresh weight, (B) Dry weight, (C) Respiration rate. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

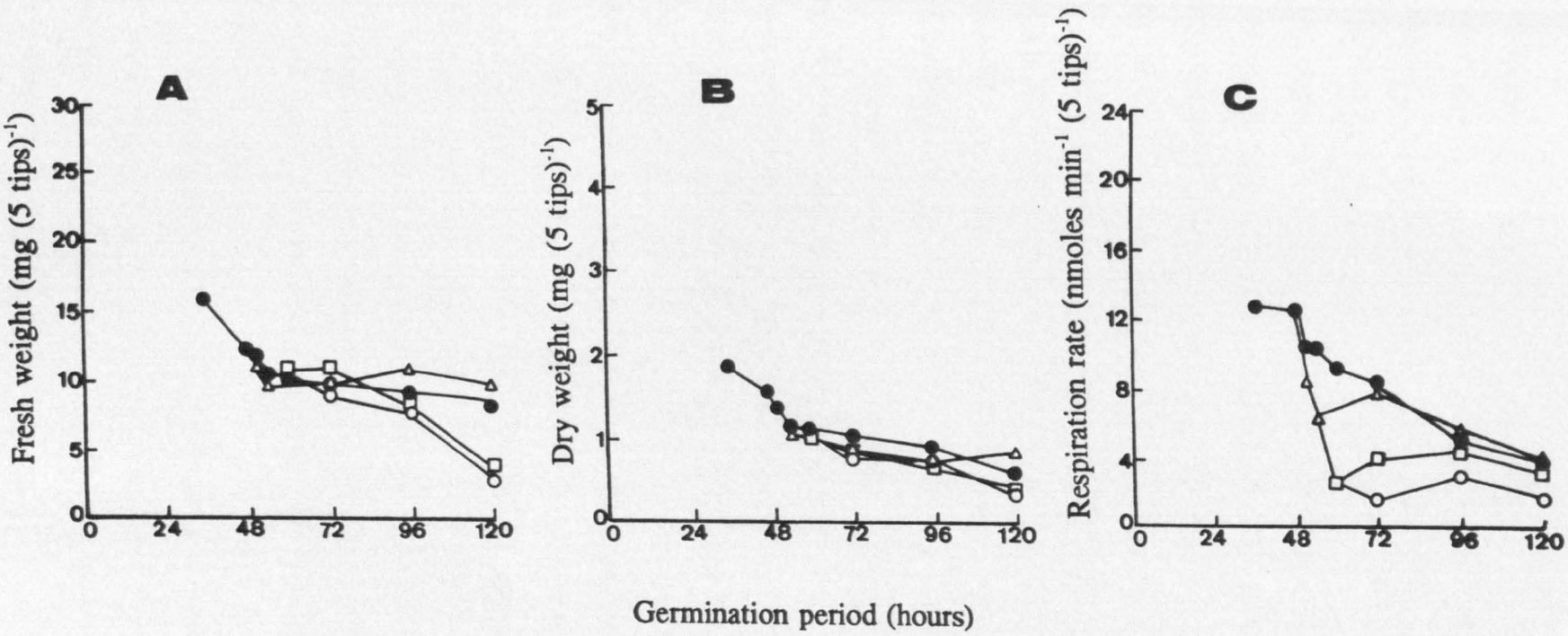


Figure 7.4 Effects of waterlogging on fresh weight, dry weight and respiration rate in the third quartile of S-12. (A) Fresh weight, (B) Dry weight, (C) Respiration rate. ●, control; △, 6h treatment; □, 12h treatment; ○, 24h treatment.

seedlings waterlogged for 6 hour showed significant increases at the end of the recovery period compared with the 120 hour control ($P<0.04$). Waterlogging for 12 hours caused apparent reductions during the recovery period and were reduced by 25 and 32%, respectively, in the first and the third quartiles. These reductions were statistically not significant, however ($P>0.10$). During recovery following 24 hour waterlogging treatment, the dry weight was decreased from 0.63 to 0.47 mg (5 tips)$^{-1}$ in the first quartile and from 0.63 to 0.37 mg (5 tips)$^{-1}$ in the third quartile. Due to large standard deviation in the first quartile values, the significance of the reduction could not be proved ($P=0.10$). However, in the third quartile, the dry weight showed a significant reduction ($P=0.02$).

Waterlogging for 3 hours sharply reduced the respiration rates in both quartiles. The rate was reduced from 10.6 to 5.8 nmoles min^{-1} (5 tips)$^{-1}$ in the first quartile and from 12.6 to 8.6 nmoles min^{-1} (5 tips)$^{-1}$ in the third quartile. The 6 hour waterlogging treatment caused similar reductions; in this case, they were reduced to 5.8 nmoles min^{-1} (5 tips)$^{-1}$ in the first quartile and to 6.6 nmoles min^{-1} (5 tips)$^{-1}$ in the third quartile. All of these reductions were statistically significant ($P<0.01$). During recovery following the 6 hour waterlogging, the root tips recovered their respiratory activities. The recovery was much slower than in Qalandari treated for a similar period. At 120 hours, they showed similar respiration rates in both quartiles compared with their controls (see Figures 7.3C and 7.4C).

Seedlings waterlogged for 12 hours also showed large reductions in respiration rates in both quartiles. These reductions were greater than for the 6 hour treatment, down to 2.0 nmoles min^{-1} (5 tips)$^{-1}$ in the first quartile and 2.6 nmoles min^{-1} (5 tips)$^{-1}$ in the third

quartile. During recovery, the root tips rapidly recovered their respiratory activities within 36 hours up to the 96 hour controls. At 120 hours, the respiratory activities of the root tips declined again in both quartiles. At this time, the respiration rate of root tips was reduced by 20 and 15%, respectively, in the first and the third quartiles compared with the 120 hour controls. In the first quartile, the reduction was significant ($P=0.01$), while the third quartile showed an insignificant reduction due to large standard deviation values ($P=0.40$).

The 24 hour waterlogging treatment caused even greater reductions in the root tips respiration. It decreased to 2.0 nmoles min^{-1} $(5\ tips)^{-1}$ in the first quartile and to 1.8 nmoles min^{-1} $(5\ tips)^{-1}$ in the third quartile. During the first 24 hours of recovery, the respiration recovered to 3.0 and 3.2 nmoles min^{-1} $(5\ tips)^{-1}$, respectively, in the first and third quartiles. The respiration rate then fell off again, however. At 120 hours, it was reduced by 40 and 50%, respectively, in the first and third quartiles compared with controls ($P<0.01$).

7.4 Discussion

The results presented in this chapter show that the fresh and dry weights of the root tips decrease with increasing germination time at 25°C. The decreases in fresh and dry weights were mainly a consequence of the growth of the roots; as roots grow longer they become thinner. The fresh and dry weights of Qalandari remained higher than in S-12. This suggests that root tips of Qalandari were thicker and healthier than those of S-12. Alternatively, they may have been thicker because they were shorter, that is less developed.

Immediately following waterlogging treatments, the root tips

showed no apparent changes in fresh or dry weights. This suggests that waterlogging does not cause extensive physical damage to the tips. During recovery following 6 hour waterlogging, the fresh and dry weights were also not affected. Waterlogging treatments of 12 hour or more caused damage, however. These treatments even caused weight reductions during the recovery periods in both cultivars. That is, there was actually a loss of tissue material. Meek and Stolzy (1978) reported that the root tip is more sensitive than the other parts of the root to low oxygen levels. Anaya (1972) found that root elongation in wheat was 25, 32 and 36 centimetres for oxygen levels of 2, 6 and 10%, respectively, after 21 days. Guyot and Prioul (1985); Orchard *et al.* (1985) working on sorghum, sunflower and wheat reported that waterlogging reduces the dry weight accumulation. Sena-Gomes and Kozlowski (1980a) reported that after 40 days of flooding of *Pinus halepensis* in the field, the dry weight of the root system was lower than before flooding was initiated. This was mainly due to damage to root tips and even cell death leading to a loss of turgor and shrivelling of the tissue. In the present study, the fresh and dry weights suggest that short term flooding did not affect the fresh and dry weights while 12 hours and more significantly damage the apical regions of roots. Only a small difference was found between two cultivars. Meek and Stolzy (1978) reported that roots grown in poor aeration are shorter and thicker than those grown with sufficient aeration.

In the case of respiration rate, the present results show that the rate of respiration decreases with increasing germination time and the pattern was very similar in the first and the third quartile. This presumably reflects the reducing tissue mass in the tip section. The respiration rate was significantly higher in the root tips of S-12

than in Qalandari at least at 24 hour germination. This was presumably related to the slower growth in S-12 compared with Qalandari.

An attempt was made to present the results for respiration per gram fresh weight or dry weight. When this was done, however, very scattered results were obtained which were difficult to interpret. This was mainly due to the effect of waterlogging on all three parameters of fresh weight, dry weight and respiration. Nevertheless, when the control data were calculated on either a per gram fresh weight or per gram dry weight basis, the results indicated that the rate of respiration increased with increasing germination time up to 54 hour in Qalandari and up to 72 hours in the cultivar S-12 then declined slowly. The greater per gram respiration rate in S-12 may be due to slower root growth than in Qalandari (see Chapter 6). Hanson *et al.* (1959) have reported that, during germination of corn seeds (Zea mays), the respiratory activity of the scutellum increases for 3-4 days, then declines. Opik (1965) also stated that oxygen uptake by the intact cotyledons of *Phaselous vulgaris* rises to a peak between the third and fifth days of germination. A vegetative tissue such as root might have been expected to continue respiration at a high level.

Waterlogging caused great reductions in the respiration rates in both cultivars. This was presumably due to the reduction in oxygen concentration during the waterlogging treatments. This in turn might have converted aerobic into anaerobic respiration. Crawford (1978) and Gupta (1977b) have pointed out that waterlogging causes a deficiency of oxygen that shifts respiratory metabolism from aerobic to anaerobic pathways. Crawford (1978) found that, in flood intolerant species, a reduction in soil aeration causes a rapid increase in glycolysis as well as a dramatic induction of alcohol dehydrogenase activity.

The reduction in respiration was greater in Qalandari than in

S-12 in both quartiles following 3, 6, 12 or 24 hours waterlogging. On the other hand, during recovery following 6 or 12 hours waterlogging Qalandari recovered better than S-12. This was probably due to greater damage to the seedlings of S-12 than Qalandari. Following 24 hour waterlogging, both cultivars showed a similar degree of damage in terms of respiration rate presumably due to severe damage to the root tips. It has been reported that rice coleoptiles, grown under anaerobic conditions for 6 days and then transferred into an ordinary atmosphere, quickly begin to absorb oxygen from the environment (Tsuji, 1972; Tsuji, 1973; Vartapetian *et al.*, 1973). Vartapetian *et al.* (1975) found that the neo-formation of respiratory enzymes begins only after transferring the anaerobic coleoptiles to aerobic conditions. Vartapetian *et al.* (1978c) reported that anaerobic mitochondria restore their ultrastructure within 1 to 3 hours after transfer of the anaerobic seedlings in to an ordinary atmosphere. In addition to that, they further explained that the activity of cytochrome oxidase reaches the level of aerobically grown coleoptiles within 18 hours.

It was also noted in the present experiment that, although waterlogging treatment for 12 hour or more caused visual damage to the root tips (they became brown), they could still respire. This suggests that mitochondria of cotton roots show great tolerance to waterlogging. Interestingly, Vartapetian *et al.* (1978c) reported that the mitochondria of cotton roots (relatively intolerant to flooding) maintained their ultrastructure longer under anoxia than those of rice (very tolerant to flooding).

CHAPTER EIGHT

Chapter Eight

Effects of Waterlogging on Fatty Acid Composition

8.1 Introduction

It has been pointed out in Chapter 5 that fatty acids are present in the structures of both storage lipids and membrane lipids. These fatty acids play an important role in the fluidity of the membranes (Section 5.1). Membrane lipids contain high levels of unsaturated fatty acids that create the proper environment for the proteins of the membrane (Lem *et al.*, 1980). In most plants, the five major fatty acids are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3).

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 an anaerobic atmosphere for the roots, which in turn will alter 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). Hiatt and Lowe (1967) reported 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 structure and functions (Quinn and Chapman, 1980). Hetherington *et al.* (1982) and 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 acid. They further added that anaerobiosis induced 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. Enzymes such as lipoxygenase, and phospholipase may be involved in these processes.

Little or no work has been done on the effects of waterlogging on fatty acid composition. More work has been done on the effects of drought on fatty acid composition. Liljenberg and Kates (1982), working on oat seedling roots, reported that water stress caused a reduction in membrane acyl lipids, as measured by the total fatty acid content. Similar results have also been reported by Pham-Thi *et al.* (1982 and 1987) who found alterations in the leaf membrane lipids of water-stressed cotton. In particular, a decrease of total fatty acids caused mainly by a decrease in linolenic acid was noted.

In our earlier experiments (see Chapter 6) it was observed that waterlogging caused significant reductions in root growth. The experiments described in this chapter were therefore carried out to observe the effect of waterlogging treatments on the fatty acid composition of cotton seedlings.

8.2 Methods

8.2.1 Determination of fatty acid composition

In these experiments, seeds of two cultivars, MNH-93 and S-12, were used from the 1992-93 harvests. Details of the procedures employed are described in the Material and Methods chapter. Briefly, cotton seedlings that had been germinated for 48 hours at 25°C were waterlogged for 6 hours (Section 2.8). After the waterlogging treatment, they were drained and returned to the incubator at 25°C for

recovery up to 72 hours. Controls consisted of seeds germinated for 48 or 72 hours at 25°C. At the end of the experimental treatments, the seedlings of the first quartile (Section 2.6) were harvested and their roots were isolated. The root lipids were then extracted using hot water-saturated butanol (Section 2.11). After extraction, the crude lipid was purified by chromatography on columns of Sephadex G-25 (Section 2.12). Free fatty acid and esterified fatty acid fractions were prepared from the purified lipid fraction (Section 2.13) and the esterified fatty acids were further separated into neutral lipid, glycolipid and phospholipid fractions (Section 2.14). Finally, fatty acid methyl esters were prepared from the various fractions and analysed by gas-liquid chromatography (Section 2.17).

Each experiment was duplicated and each fatty acid analysis within each experiment was also duplicated. The values presented in these experiments are the means ±sd from the four values.

The resulting data were analysed statistically as described in the Section 2.18. All the graphs were plotted using a personal computer with Quattro Pro 3 (Borland International Inc.).

8.3 Results

In control (non-waterlogged) roots, 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 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, linolenic acid was the major fatty acid in this fraction.

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 (see Figure 2.2). The appearance of some of these peaks was spasmodic, however, and they are therefore not included in our 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 times 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 they appeared to correspond to lauric acid (C12:0) and myristic acid (C14:0). They were tentatively identified as these fatty acids.

In addition to the low molecular-weight unknowns, a high molecular weight compound was observed as a large peak in chromatograms from waterlogged root tissues. Its appearance was regular and it occurred approximately 60 minutes later than the linolenic acid methyl ester peak. The peak disappeared again during recovery. Unfortunately, time did not permit us to identify this compound. Also, because it was difficult to quantify (the peak was very broad), data are not included in our results.

8.3.1 Effect of waterlogging on fatty acid composition in the cultivar S-12

The total levels of all fatty acids (esterified fatty acids plus free fatty acids) in the various fractions are presented in Table 8.1 and in Figure 8.1. The total amount of fatty acids at 48 hours germination was 1970 µg $(25\ roots)^{-1}$ which increased slightly

Table 8.1 Effects of waterlogging on the esterified and free fatty acids in the cultivar S-12

Lipid fraction	Total germ. time (h)	Fatty acid content µg (25 roots)$^{-1}$
Neutral lipids	48h control	348 ±82
	72h control	439 ±29
	After waterlogging	875 ±259
	After recovery	570 ±36
Glycolipids	48h control	75 ±16
	72h control	107 ±44
	After waterlogging	359 ±48
	After recovery	82 ±4
Phospholipids	48h control	1520 ±104
	72h control	1500 ±198
	After waterlogging	1610 ±170
	After recovery	1740 ±85
Free fatty acids	48h control	27 ±6
	72h control	62 ±2
	After waterlogging	23 ±4
	After recovery	23 ±3
Combined acyl-Lipids	48h control	1940 ±60
	72h control	2050 ±150
	After waterlogging	2840 ±160
	After recovery	2390 ±90
Total lipids	48h control	1970 ±60
	72h control	2110 ±150
	After waterlogging	2870 ±170
	After recovery	2420 ±90

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

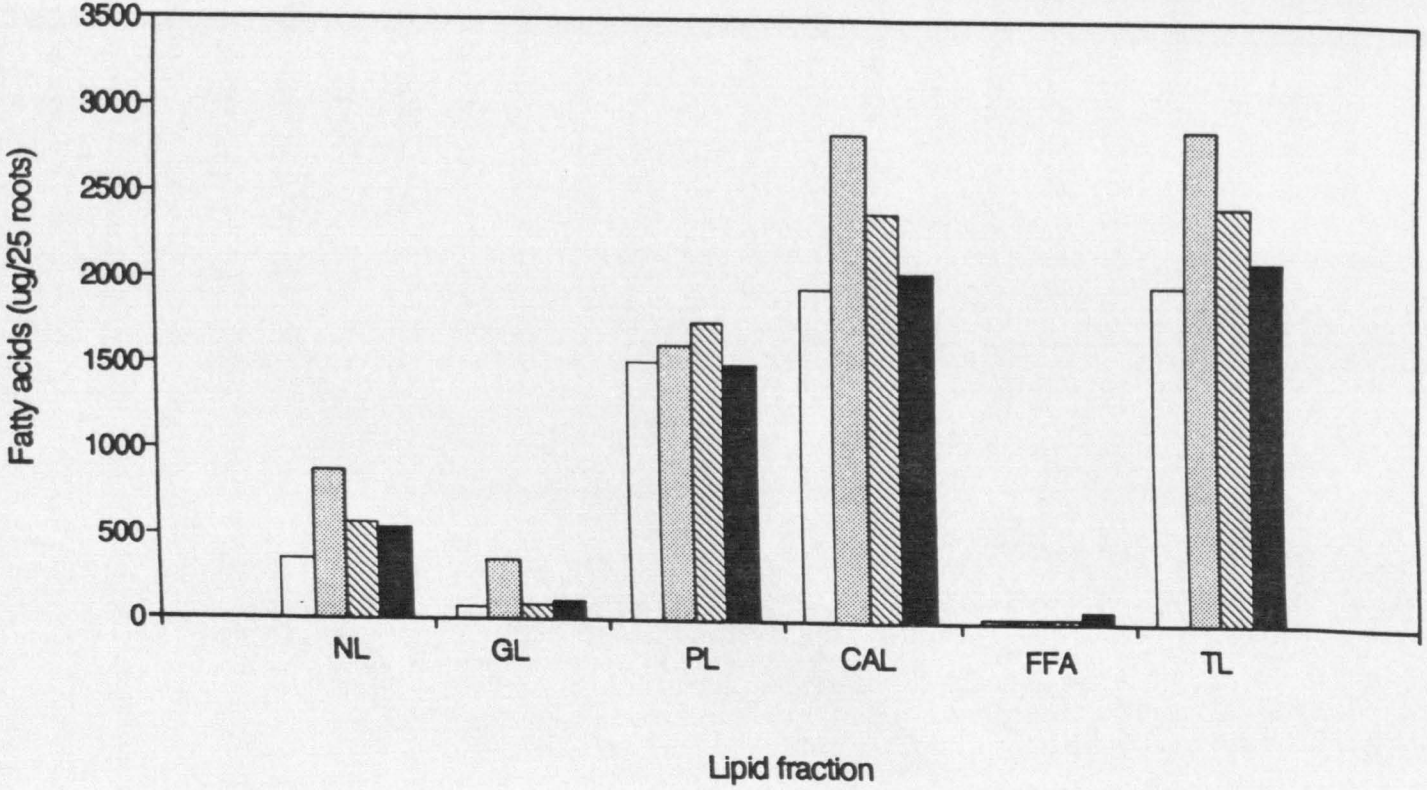


Figure 8.1 Effects of waterlogging on total fatty acids in various lipid fractions in cultivar S-12. ▭, 48h control; ■, 72h control; ▨, after waterlogging; ▧, after recovery. NL, neutral lipid; GL, glycolipid; PL, phospholipid; CAL, combined acyl lipids ; FFA, free fatty acids; TL, total lipids.

(insignificantly) in the 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 (P<0.001) increase 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 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 deviation, however, the significance of the change could not be proved (P=0.12). 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 fatty acids increased from 75 to 107 µg (25 roots)$^{-1}$. The increase was not significant (P=0.28). Seedlings waterlogged for 6 hours showed a significant increase in this fraction (P<0.001). 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 again to 82 µg (25 roots)$^{-1}$, a value not significant different from the 72 hour control (P=0.12).

The amount of phospholipid 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 in the value to 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 insignificant (P>0.10).

Table 8.1 and Figure 8.1 also contain data for the total acyl

lipids (neutral lipid + glycolipid + phospholipid). The combined fatty acids in these fractions changed under the various treatments. The value for the 48 hour control was 1940 µg (25 roots)$^{-1}$ and that for the 72 hour control was 2050 µg (25 roots)$^{-1}$. The difference between these two control values was not significant (P=0.22). The level following waterlogging increased significantly to 2840 µg (25 roots)$^{-1}$ (P<0.001), however, and following the recovery period it was reduced again to 2390 µg (25 roots)$^{-1}$. Waterlogging thus apparently caused a marked increase (46%) in the total acyl lipids which decreased again during 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 have any significant effect on the level compared with the 48 hour control (P=0.38), 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 Table 8.2 and Figure 8.2. 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% linoleic 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 linolenic 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

Table 8.2 Effect of waterlogging on the percentage fatty acid compositions in the cultivar S-12

Lipid fraction	Germination time (hours)	Fatty acids					
		Unknown	C16:0	C18:0	C18:1	C18:2	C18:3
Neutral lipids	48h control	2.9±0.6	24.4±1.6	8.8±0.9	15.8±1.4	37.7±0.8	10.3±2.6
	72h control	2.2±0.3	23.9±1.4	8.9±1.3	16.9±0.6	34.9±1.7	12.2±2.8
	After flooding	25.0±7.1	21.2±2.0	5.1±0.3	11.4±2.0	30.4±3.0	6.9±0.4
	After recovery	9.2±1.3	23.9±1.3	5.9±0.9	16.9±1.5	33.6±1.2	10.6±3.6
Glycolipids	48h control	1.3±0.2	24.9±1.2	7.9±1.3	15.1±1.2	23.1±2.6	27.7±3.9
	72h control	0.5±(tr)	25.4±1.3	4.5±1.5	11.1±1.0	21.9±4.4	37.0±6.7
	After flooding	63.6±7.6	12.9±1.0	2.4±0.2	4.6±0.4	8.3±1.0	6.4±0.3
	After recovery	1.2±0.2	32.0±0.5	3.9±0.2	9.3±1.1	18.5±1.3	35.1±1.7
Phospholipids	48h control	0.6±(tr)	32.7±1.4	2.8±0.3	4.9±0.2	39.2±1.3	19.9±0.7
	72h control	0.6±(tr)	31.8±0.5	3.1±0.5	5.0±0.2	39.5±1.0	20.2±0.7
	After flooding	7.3±1.1	31.2±1.2	1.8±0.4	4.0±0.2	38.2±1.4	17.6±0.2
	After recovery	0.6±(tr)	32.6±0.7	1.9±0.3	4.5±0.1	41.7±0.9	18.8±0.6
Free fatty acids	48h control	2.8±0.4	36.7±3.2	13.1±2.5	11.5±2.2	25.2±2.9	10.7±1.7
	72h control	1.4±0.3	31.2±1.4	6.7±0.2	9.9±0.9	33.0±3.2	17.8±4.0
	After flooding	2.8±0.8	33.3±1.3	8.5±2.5	11.8±3.7	29.2±4.4	14.1±2.8
	After recovery	2.3±0.3	28.9±3.4	8.6±1.8	16.0±2.9	33.4±4.6	10.7±3.9

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

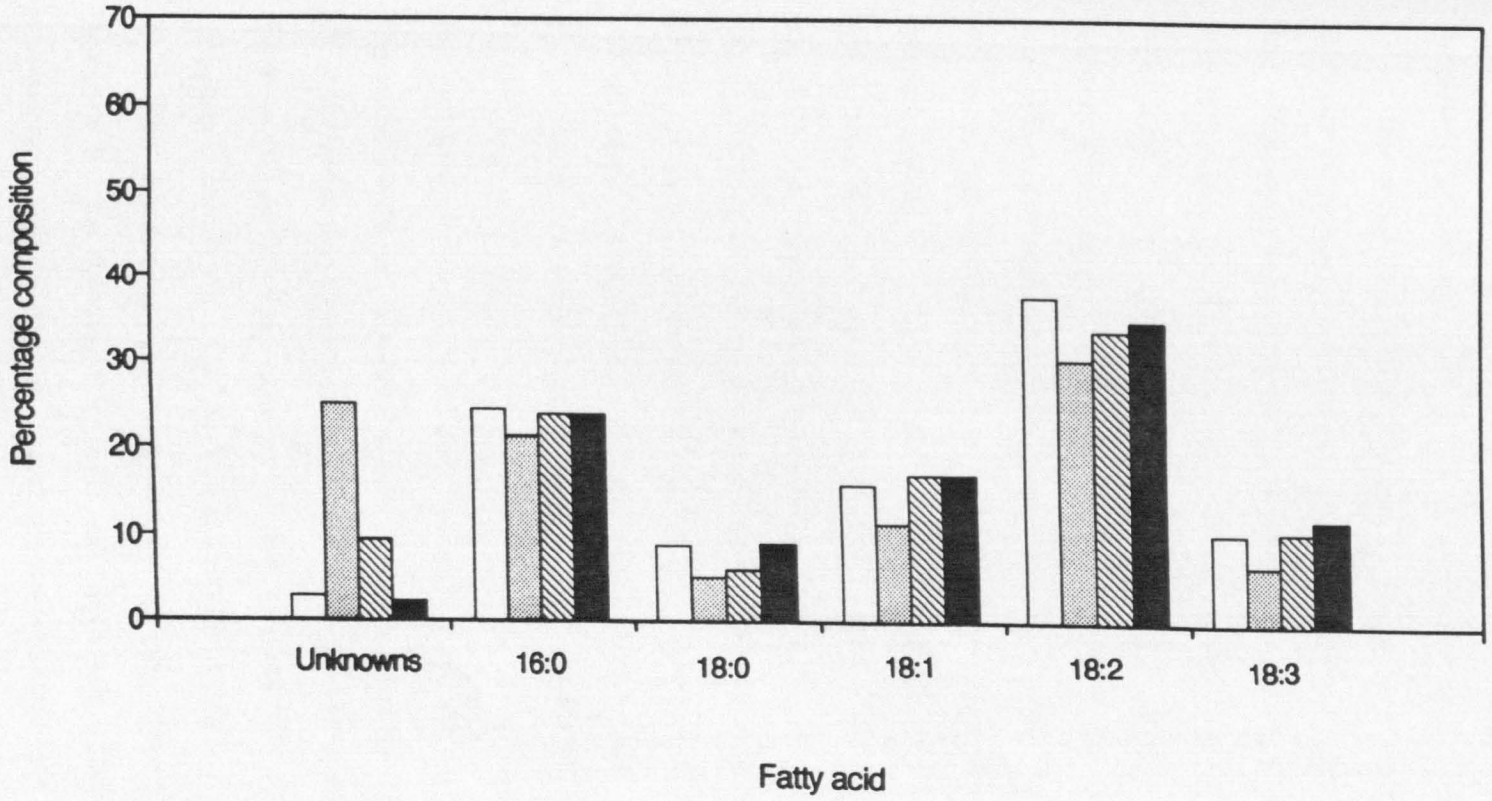


Figure 8.2 Effect of waterlogging on fatty acid composition in the neutral lipid fraction in cultivar S-12. ▭ ,48h control; ■ , 72h control; ▨ , after waterlogging; ▧ , after recovery.

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 levels of the 72 hour controls.

The results for the glycolipid fraction are also shown in Table 8.2 and in Figure 8.3. 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 proportions were observed. The largest increase was observed in the 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 latter value. The relative amounts of palmitic and linoleic acids remained constant. Seedlings waterlogged for 6 hours showed a very large increase in the unknowns from 1.3 to 63.6% and marked decreases in all of the other fatty acids ($P<0.001$). The greatest decrease was in the proportion of linolenic acid from 27.7 to 6.4%. At this stage a small amount (2.0%) of palmitoleic acid (C16:1) was also found (tentatively identified and data not shown). At the end of the 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 latter declined from 63.6 to 1.2%.

The proportions of fatty acids in the phospholipid fraction (Table 8.2 and Figure 8.4) 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

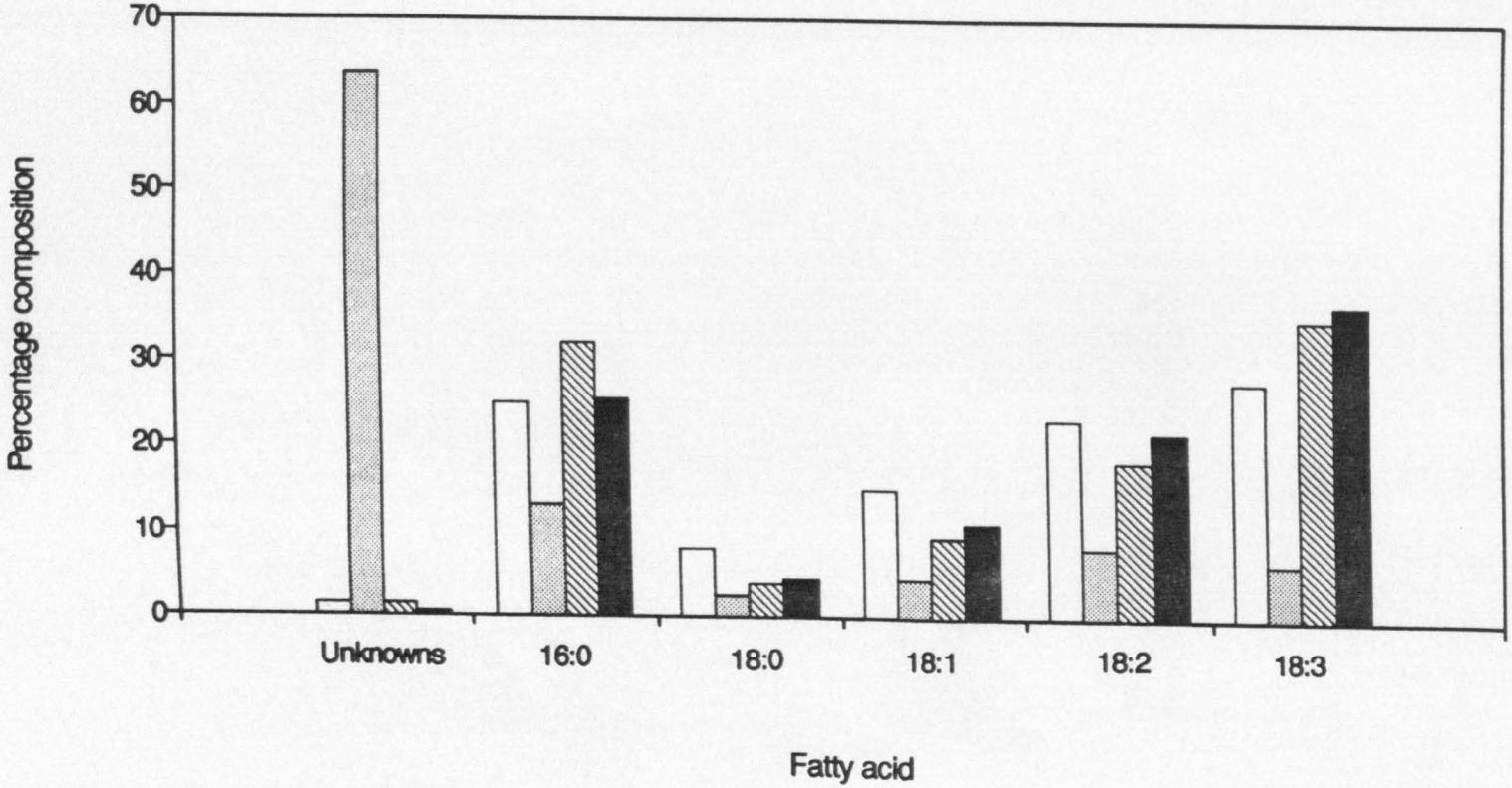


Figure 8.3 Effect of waterlogging on fatty acid composition in the glycolipid fraction in cultivar S-12. ▭, 48h control; ■, 72h control; ▩, after waterlogging; ▨, after recovery.

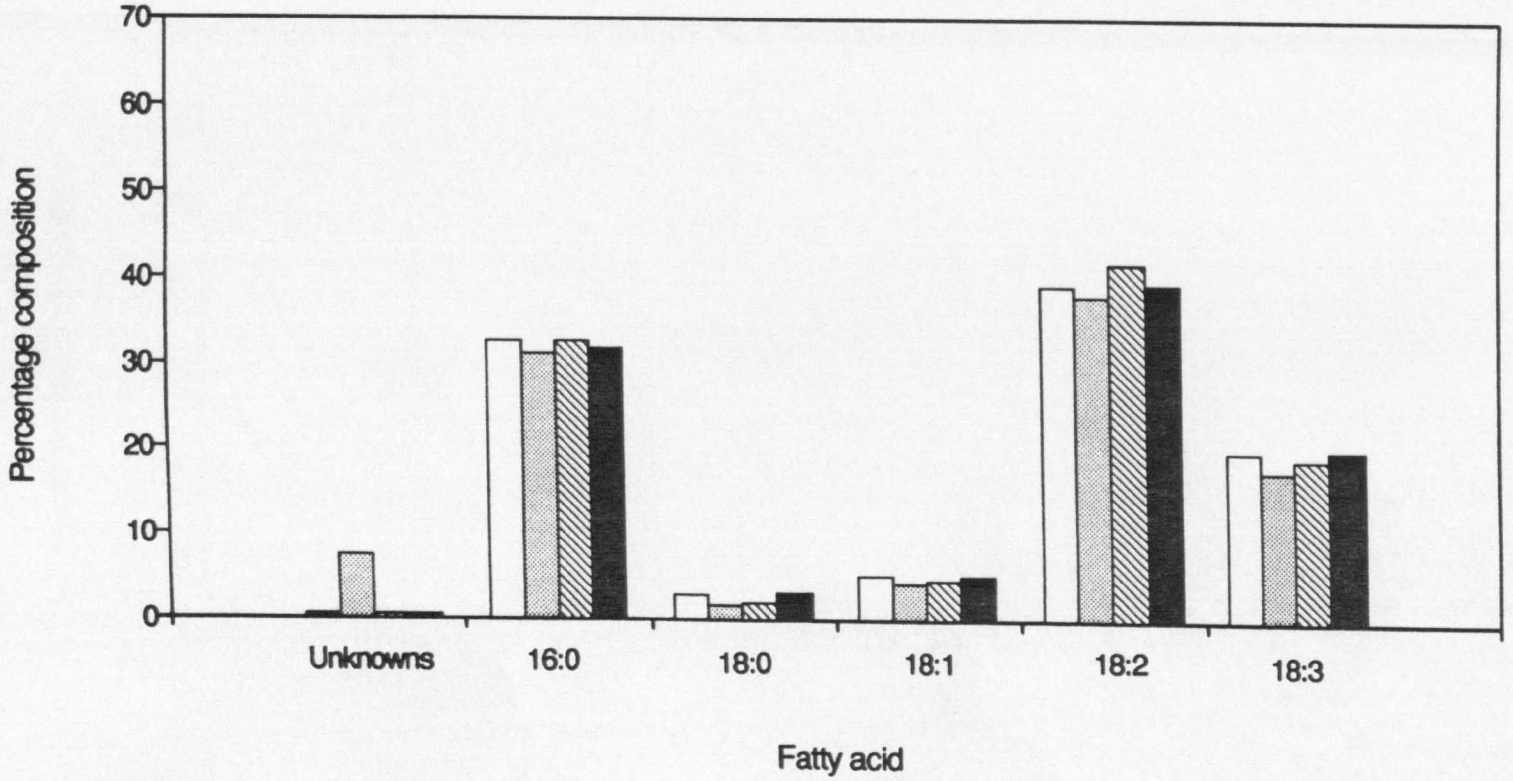


Figure 8.4 Effect of waterlogging on fatty acid composition in the phospholipid fraction in cultivar S-12. □, 48h control; ■, 72h control; ▨, after waterlogging; ▧, after recovery.

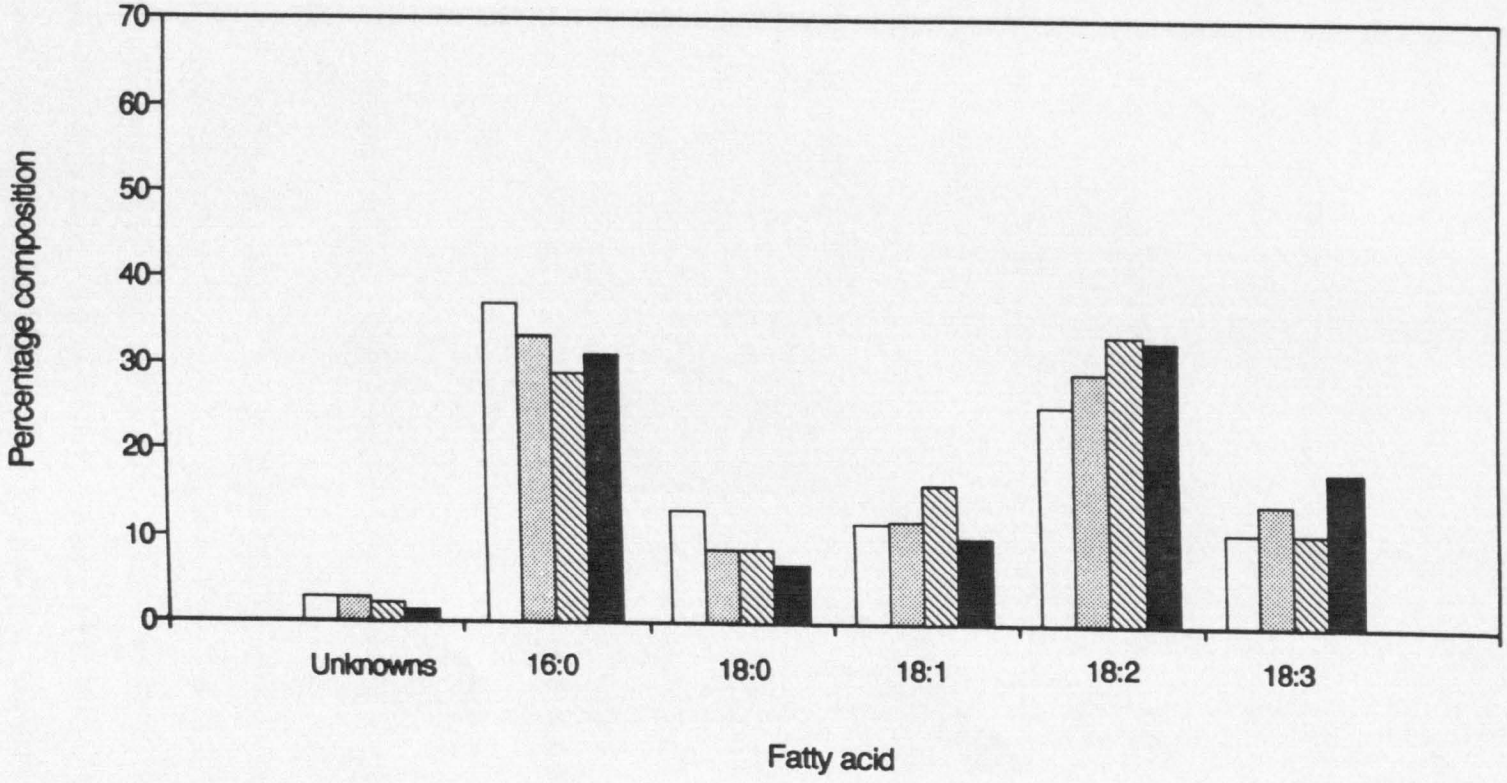


Figure 8.5 Effect of waterlogging on fatty acid composition in the free fatty acids in cultivar S-12. ▭, 48h control; ■, 72h control; ▩, after waterlogging; ▨, after recovery.

at the expense of all the known fatty acids. This increase was 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 latter felt back to about 0.6%.

In the roots of seeds germinated for 48 hours, the proportions of the free fatty acids were 36.7% palmitic acid, 13.1% stearic acid, 11.5% oleic acid, 25.2% linoleic acid, 10.7% linolenic acid and 2.8% unknowns. During the next 24 hours germination, the most significant increases were in the proportions of linoleic acid, which rose from 25.2 to 33.0%, and linolenic acid, which rose 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 increases in the percentages of the unsaturated fatty acids and decreases in palmitic and stearic acids. All of these changes were insignificant however ($P>0.12$). At the end of the recovery period, an insignificant increase was observed in the percentage of oleic acid ($P=0.17$) and there was a further increase in linoleic acid ($P=0.30$) at the expenses of palmitic and linolenic acids ($P>0.08$).

8.3.2 Effect of waterlogging on fatty acid composition in the cultivar MNH-93

The total levels of fatty acids all in the various fractions are presented in Table 8.3 and in Figure 8.6. The amount at 48 hour germination was 2270 µg (25 roots)$^{-1}$ which increased significantly in the 72 hour control to 3330 µg (25 roots)$^{-1}$ ($P<0.001$). In the seedlings waterlogged for 6 hours there was a small but insignificant increase (12%) in the value to 2690 µg (25 roots)$^{-1}$ ($P=0.10$), but after recovery

Table 8.3 Effects of waterlogging on the esterified and free fatty acids in the cultivar MNH-93

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.

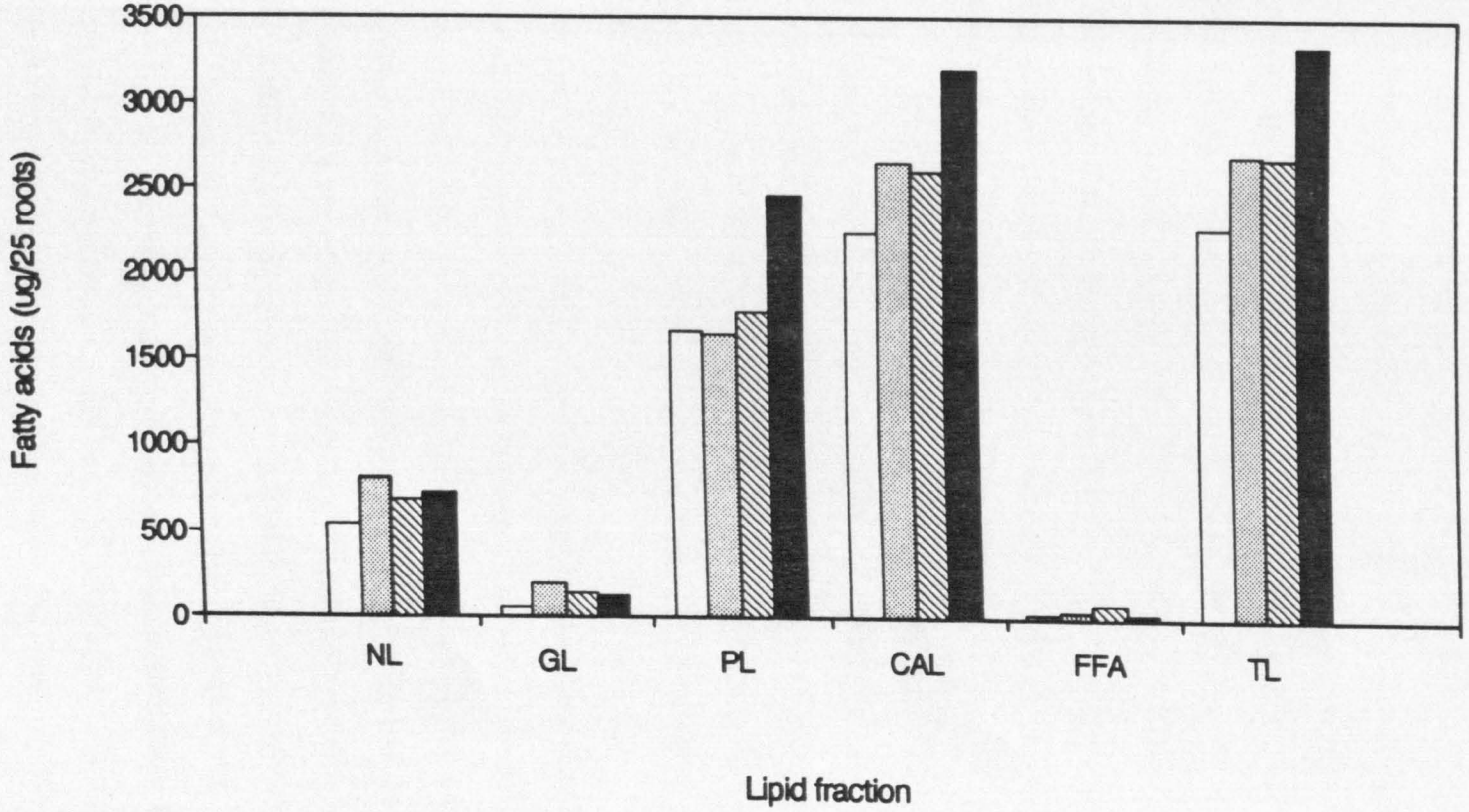


Figure 8.6 Effects of waterlogging on total fatty acids in various lipid fractions in cultivar MNH-93. ▭, 48h control; ■, 72h control; ▭, after waterlogging; ▨, after recovery. NL, neutral lipid; GL, glycolipid; PL, phospholipid; CAL, combined acyl lipids ; FFA, free fatty acids; TL, total lipids.

the level was not changed further at 2680 µg (25 roots)$^{-1}$. The latter value was significantly reduced when compared with 72 hour control value however (P=0.004).

The increase in the total fatty acids during germination from 48 to 72 hours was reflected in all of the acyl lipid fractions, but not in the free fatty acid fraction. Thus, an apparent increase in neutral lipid fatty acids was found. This increase was from 537 to 718 µg (25 roots)$^{-1}$. Seedlings waterlogged for 6 hours also showed a marked increase to 809 µg (25 roots)$^{-1}$. At the end of the recovery period following waterlogging, this level had declined again to 677 µg (25 roots)$^{-1}$. Because of the large standard deviation values, however, none of these changes were significant (P>0.14).

The glycolipid fatty acids increased significantly from 52 to 123 µg (25 roots)$^{-1}$ during germination between 48 and 72 hours (P=0.01). Seedlings waterlogged for 6 hours also showed a significant increase from 52 to 195 µg (25 roots)$^{-1}$ (P=0.04). At the end of the recovery period, this level had declined again from 195 to 144 µg (25 roots)$^{-1}$.

A relatively large increase in the amount of phospholipid fatty acids from 1655 to 2453 µg (25 roots)$^{-1}$ was found during germination from 48 to 72 hours (P<0.001). Seedlings waterlogged for 6 hours did not show any change at 1648 µg (25 roots)$^{-1}$. At the end of the recovery period, the value had increased to 1776 µg (25 roots)$^{-1}$, which was 28% and significantly short of the 72 hours control value (P<0.001).

Table 8.3 and Figure 8.6 also show the data for the combined acyl lipids (neutral lipid + glycolipid + phospholipid). Because these combined fractions made up 99% of the total lipid fatty acids, they naturally underwent changes which were the same as those described above for the total lipid.

In the free fatty acid fraction, the level also increased slightly (but insignificantly) during germination from 25 µg (25 roots)$^{-1}$ at 48 hours to 32 µg (25 roots)$^{-1}$ at 72 hours (P=0.39). Seedlings waterlogged for 6 hours also showed a small but insignificant increase to 35 µg (25 roots)$^{-1}$ (P=0.20). At the end of the recovery, the level showed a further and significant increase to 78 µg (25 roots)$^{-1}$ (P=0.001).

The ratios of the individual fatty acids in the neutral lipid fraction are shown in Table 8.4 and Figure 8.7. In roots germinated for 48 hours, the fatty acid composition of this fraction was 27.5% palmitic acid, 6.2% stearic acid, 20.3% oleic acid, 40.4% linoleic acid, 3.9% linolenic acid and 2.3% unknowns. During the next 24 hours of germination, there were only limited changes in these proportions. In particular, the significant proportion of linolenic acid increased to 9.1% at the expense of palmitic acid (P<0.02). In seedlings waterlogged for 6 hours, the largest change was in the set of unknown low molecular weight compounds which increased from 2.3 to 15.9% (P<0.001). This was at the expense of decreases of oleic and linoleic acids (P<0.01). A small proportion of palmitoleic acid was also observed (data no reported). At the end of the recovery period following waterlogging, increases were observed in the proportions of all the unsaturated fatty acids at the expense of the unknown low molecular weight compounds. These changes were also significant statistically (P<0.02). The level of stearic acid remained unchanged.

Results for the glycolipid fraction showed larger changes than those seen in the neutral lipids. In the roots of seeds germinated for 48 hours, the proportions of the fatty acids were 36.8% palmitic acid, 3.9% stearic acid, 18.2% oleic acid, 25.3% linoleic acid and 15.7% linolenic acid. During the next 24 hours germination, marked increases

Table 8.4 Effect of waterlogging on the percentage fatty acid compositions in the cultivar MNH-93

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±(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 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

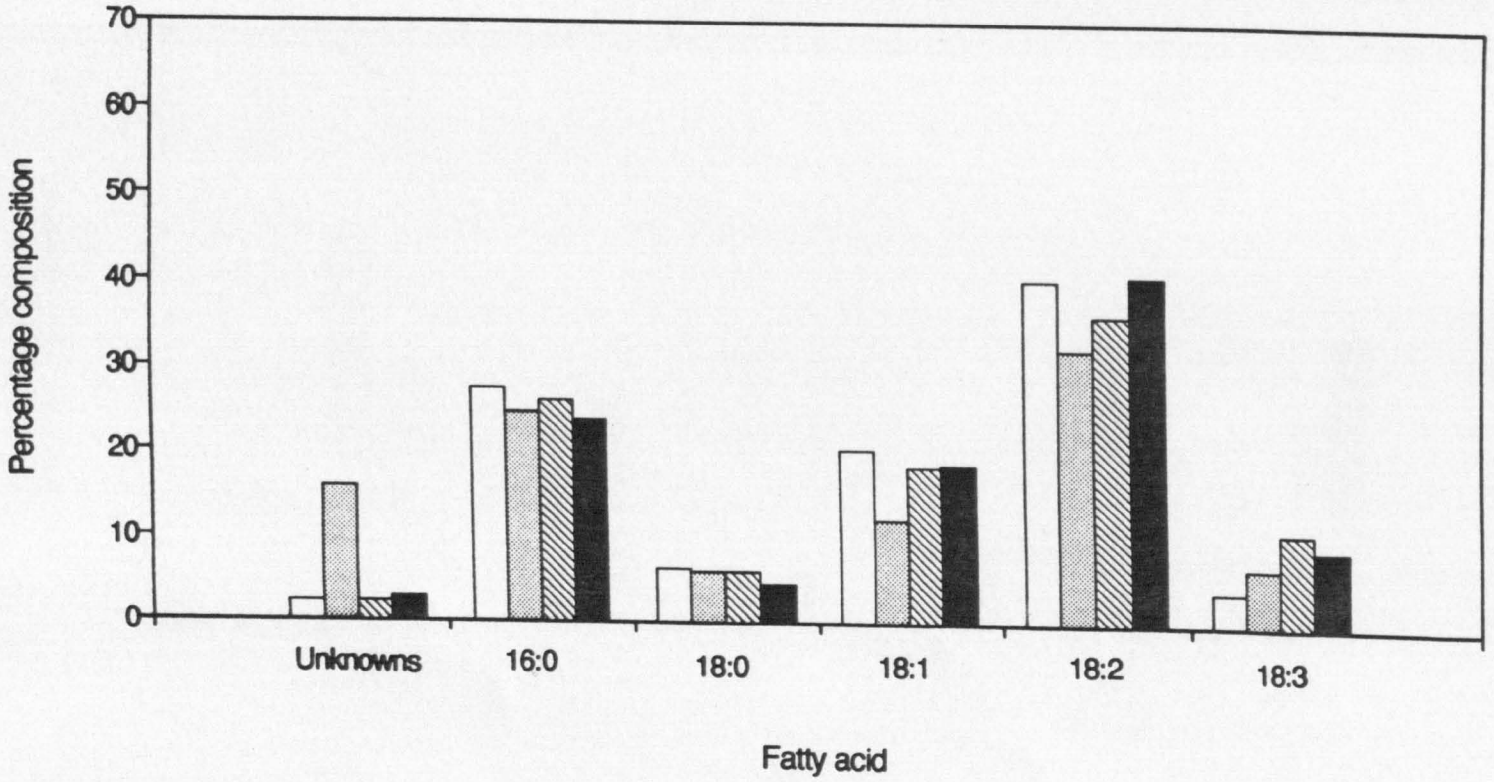


Figure 8.7 Effect of waterlogging on fatty acid composition in the neutral lipid fraction in cultivar MNH-93. ▭, 48h control; ■, 72hcontrol; ▨, after waterlogging; ▨, after recovery.

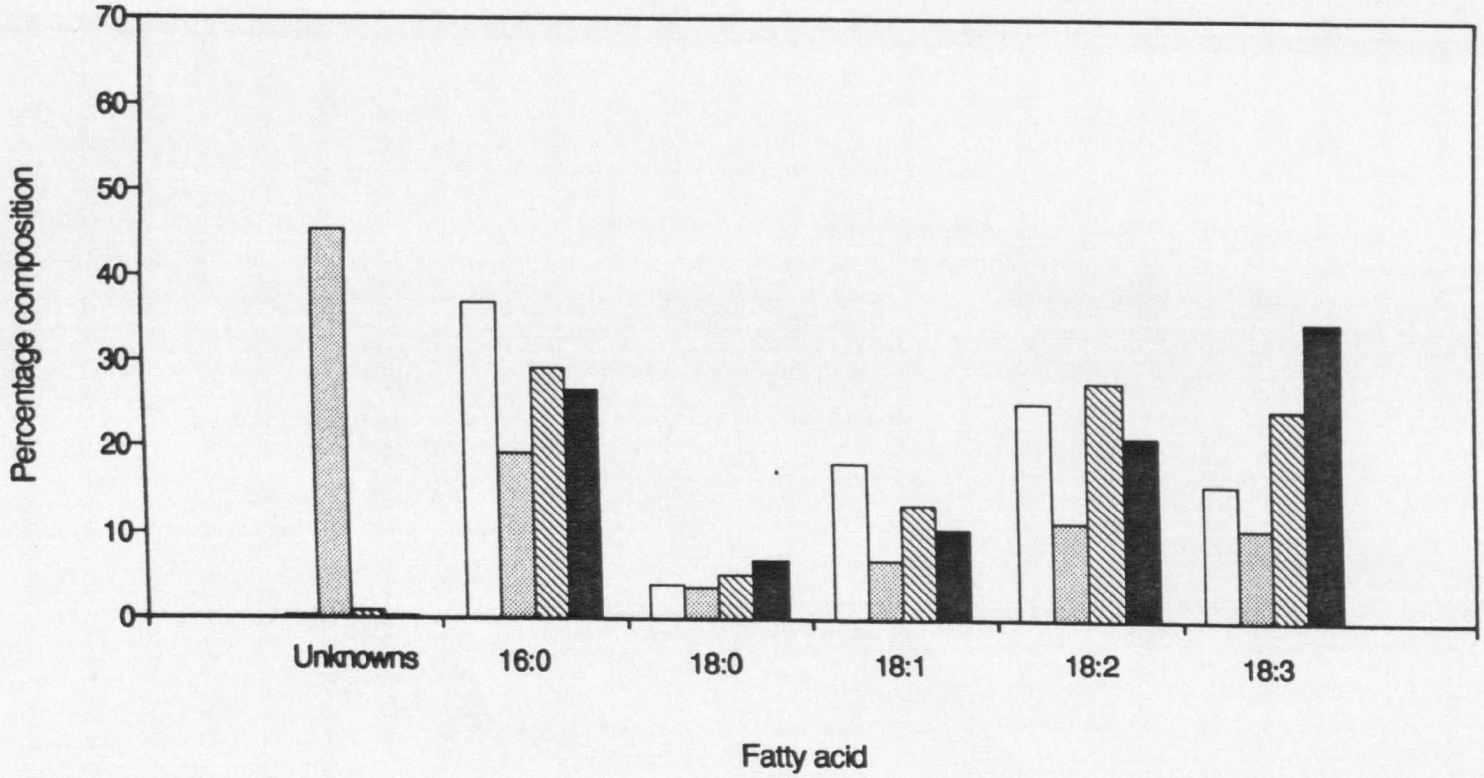


Figure 8.8 Effect of waterlogging on fatty acid composition in the glycolipid fraction in cultivar MNH-93. ☐, 48h control; ■, 72h control; ▨, after waterlogging; ▨, after recovery.

took place in the proportions of stearic acid from 3.9 to 6.7% and linolenic acid from 15.7 to 35.0% at the expense of decreases in palmitic and oleic acids. All of these changes were statistically significant ($P<0.05$), except that of stearic acid which had large standard deviation values. The proportion of linoleic acid also decreased. Seedlings waterlogged for 6 hours showed a very large increase in the set of unknowns from zero before waterlogging to 45.5%. There were corresponding decreases in the proportions of all the known fatty acids except stearic. The greatest decrease was in the proportion of palmitic acid from 36.8 to 19.2% ($P<0.001$). A small proportion of palmitoleic acid was again observed. At the end of the recovery period, recoveries in the proportions of all the characterised fatty acids were correlated with a decrease in the set of unknowns. The proportion of linoleic acid by this time was actually above the 72 hours control value, while the level of linolenic acid was below its 72 hours control. These values were effectively the small as their 72 hour control values ($P>0.12$).

The phospholipid fraction was less affected by the various experimental treatments (Table 8.4 and in Figure 8.9). In the roots germinated for 48 hours, the proportions of the fatty acids were 34.1% palmitic acid, 3.0% stearic acid, 5.8% oleic acid, 40.1% linoleic acid, 16.6% linolenic acid and 0.5% unknowns. The values underwent only small changes between 48 and 72 hours. The largest changes were in the proportion of linolenic acid, which increased from 16.6 to 20.8% at the expense mainly of palmitic acid. Both of these changes were significant ($P<0.04$). The seedlings waterlogged for 6 hours did not show any significant changes except in oleic acid, which decreased from 5.8 to 4.3% ($P=0.003$). Any changes during recovery were also very small.

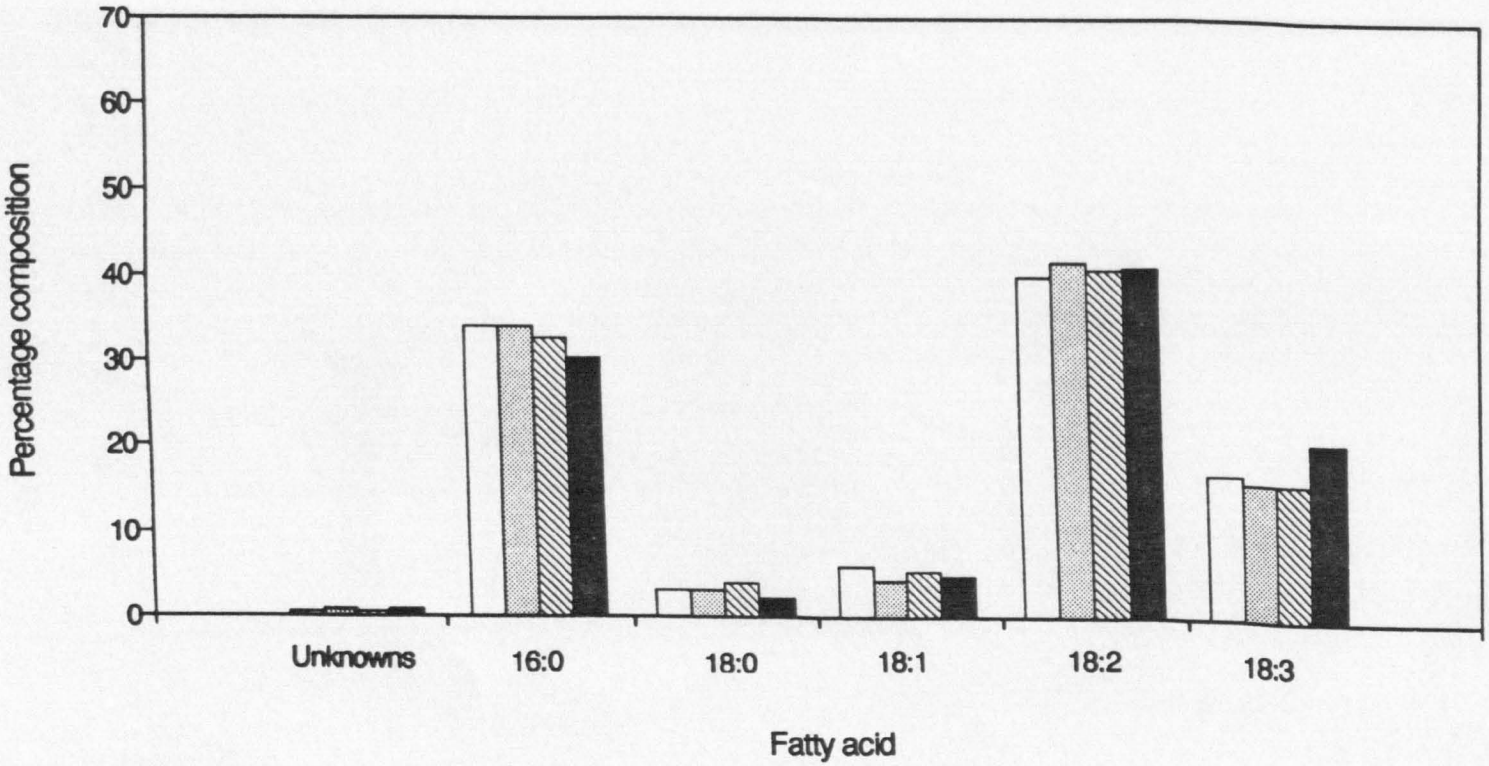


Figure 8.9 Effect of waterlogging on fatty acid composition in the phospholipid fraction in cultivar MNH-93. ☐, 48h control; ■, 72h control; ▨, after waterlogging; ▨, after recovery.

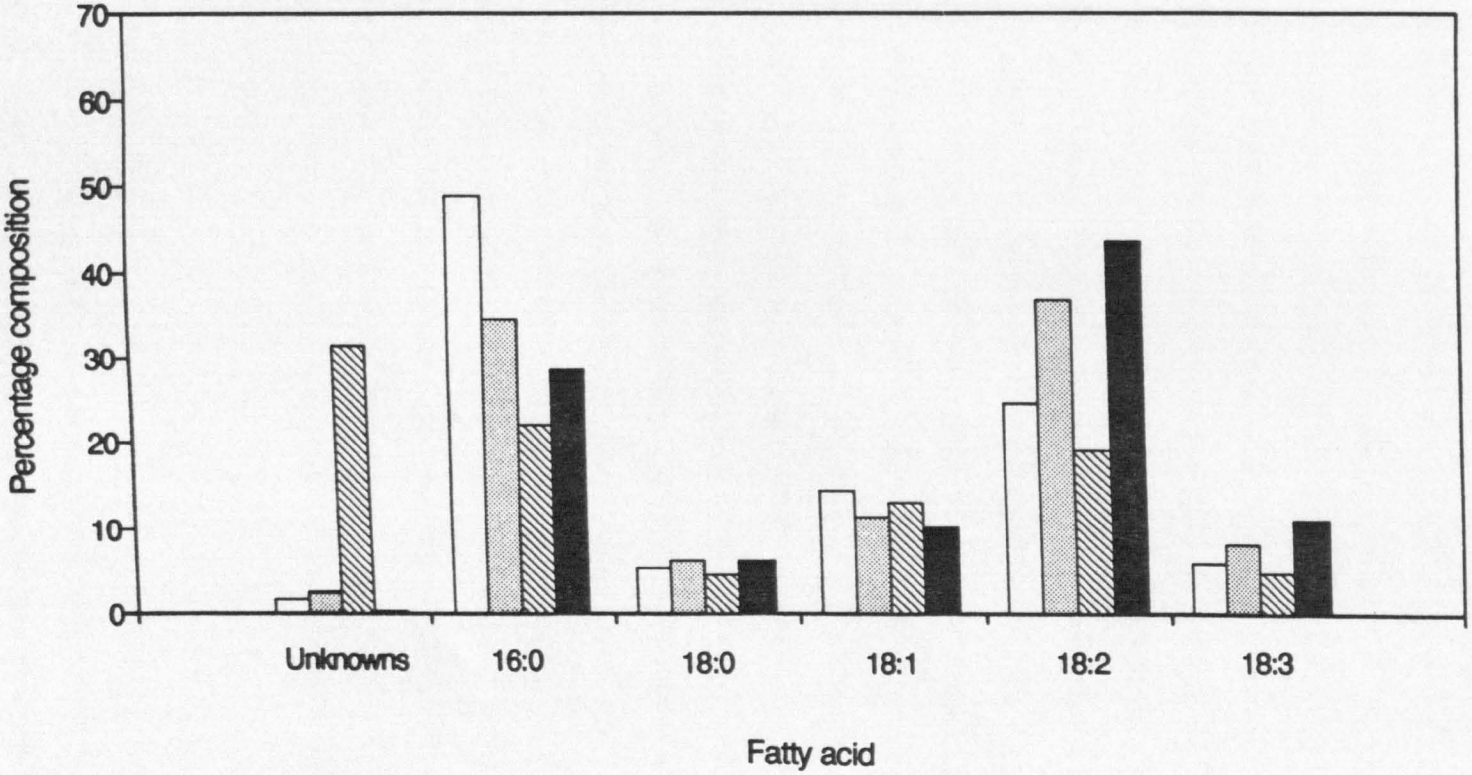


Figure 8.10 Effect of waterlogging on fatty acid composition in the free fatty acids in cultivar MNH-93. ☐, 48h control; ■, 72h control; ▨, after waterlogging; ▨, after recovery.

The results for the free fatty acid fraction showed larger changes. In the roots of seeds germinated for 48 hours, the proportion of the fatty acids in this fraction were 48.7% palmitic acid, 5.3% stearic acid, 14.5% oleic acid, 24.8% linoleic acid, 5.9% linolenic acid and 1.8% unknowns. During the next 24 hours of germination, the proportions of the unsaturated fatty acids (linoleic and linolenic acids) increased ($P<0.001$). The largest increase was noted in the proportion of the linoleic acid, which went from 24.8 to 43.8%. There was a similar but still significant decrease in the proportion of palmitic acid ($P=0.03$). A small but insignificant decrease in the proportion of oleic acid was also observed ($P=0.28$). At this stage, the low levels of the unknown low molecular weight compounds present at 48 hours had disappeared. Seedlings waterlogged for 6 hours showed significant increases in linoleic and linolenic acids ($P<0.01$), especially in linoleic acid which changed from 24.8 to 36.8%. Corresponding decreases occurred in the proportions of palmitic and oleic acids. The proportion of palmitic acid decreased significantly ($P=0.05$). The amounts of the set of unknown compounds remained low at this time, but at the end of the recovery period they increased dramatically from 2.4 to 31.4%. Corresponding significant decreases occurred in the percentages of palmitic, linoleic and linolenic acids ($P<0.01$). The greatest decrease was noted in the proportion of linoleic acid. A small proportion of palmitoleic acid was found at this time.

8.4 Discussion

As we discussed in Chapter 5, some of the changes following heat shock were found to be statistically insignificant. Despite the statistics, however, several of the changes were considered to be real

because they were so large. The statistics problem arose due to large standard deviations obtained with some of the analyses. In the present chapter, this problem was not so serious. Most of the observed changes were found to be statistically significant in the student T-test.

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 plus free fatty acids increased with increasing germination time. This increase was much greater in MNH-93 than in S-12. The increases were 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 in both cultivars, with the exception of the phospholipids in the cultivar S-12. The greater increase in fatty acids in the phospholipid fraction in MNH-93 was presumably due to the formation of more new membrane in this cultivar. The fresh weight values for the roots used in the experiment confirms this; the fresh weight of the MNH-93 roots increased from 1.8g at 48 hours to 3.3g at 72 hours, while in cultivar S-12 the fresh weight increased only from 2.0g to 2.5g.

Seedlings waterlogged for 6 hours showed a marked increase in the amount of total esterified fatty acids in both cultivars. This increase was greater in the cultivar S-12 than in MNH-93. 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. The fresh weight of the roots used in the present experiment showed no further increase in growth during waterlogging however. This indicates

that the increase in total esterified lipids due to waterlogging must be due to the 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 increases in the levels of neutral lipids and glycolipids in both cultivars. These increases were again greater in the cultivar S-12 than in MNH-93. The 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. Vartapetian *et al.* (1978a) similarly found higher amounts in the rice coleoptiles growing under anaerobic conditions. The amounts of phospholipids remained unchanged during this period in both cultivars.

During recovery from waterlogging, the levels of fatty acids in the neutral lipid and glycolipid fractions decreased, while the levels of phospholipid increased slightly. These increases in phospholipids were very small, however, and they were not significant statistically.

A significant increase in free fatty acids was observed in MNH-93 but not in S-12. Increased levels of free fatty acids might cause the disruption of membrane structures and decrease the stability of membranes. Weiss (1980) has reported that the formation of free fatty acids by endogenous lipases decreases the stability of membrane structures within plant cells. In the context of the present results, this would suggest that S-12 should be more tolerant to waterlogging than MNH-93.

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. In the phospholipid fraction, greater changes were found in MNH-93 than in S-12. El-Nockrashy *et al.* (1974) and John and Christiansen (1976) have 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. This is similar to the present observations. 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 characterised fatty acids and greatly enhanced the synthesis of a set of low molecular weight compounds in both cultivars. 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 acids in the cultivar MNH-93 and no formation of the low-molecular-weight compounds. In S-12, however, waterlogging stimulated the formation of these low molecular weight compounds in the phospholipid fraction and reduced the proportions of almost all the characterised 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. Young and Anderson (1974) reported 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, our earlier results (Chapter 6) 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. The present experiments are unable to choose between these two possibilities.

It is obvious from the present results that, at the end of the recovery period following waterlogging, the amount of combined acyl lipids in the cultivar MNH-93 remained significantly below the 72

hour control value, while the level in cultivar S-12 was significantly higher than its 72 hour control. The patterns for neutral lipids and phospholipids were similar, but unfortunately the statistical significance of these patterns could not be demonstrated. Nevertheless, from these results, we might anticipate that S-12 is a relatively waterlogging-tolerant cultivar compared with MNH-93, because it can maintain higher membrane lipid levels under stress conditions. Similar results were obtained from the root growth data (see Section 6.3) where S-12 was a relatively better cultivar under short-term waterlogging conditions.

CHAPTER NINE

Chapter Nine

Summary and General Discussion

The experiments described in this thesis were undertaken to study changes in the physiological and biochemical properties of cotton seedling roots with respect to high temperature and waterlogging treatments. In the case of seedling roots subjected to high temperature stress, these properties were root growth, fresh weight, dry weight, total phospholipid levels, (Me-^{14}C) choline uptake and incorporation into phospholipid and fatty acid composition. In the case of seedlings subjected to waterlogging, root growth, respiratory activity and fatty acid composition were studied. It is clear from these studies that both high temperature and waterlogging significantly damage cotton seedling roots and affect their physiological and biochemical activities.

It is quite obvious that both of the cultivars studied (MNH-93 and Qalandari) behaved similarly with respect to their root growth at normal germinating conditions (25°C). Following a 2 hour heat shock, given after 48 hours germination at 25°C, both cultivars showed an increase in root growth for stresses up to 30°C but they responded differently when stressed above this temperature. Cultivar MNH-93 performed well up to about 38°C, whilst Qalandari showed an inhibition at temperatures of 35°C and above. Both cultivars failed to grow following post-germination stress at 40°C and above. A stress temperature of 45°C was lethal to both cultivars. Thus, MNH-93 appeared to be more heat tolerant than Qalandari. Careful statistical analysis of the data indicated, however, that the difference was small and very difficult to prove. Fresh weight and dry weight values were only determined for MNH-93, but they confirmed the findings of the

root growth measurements for that cultivar.

Investigations reported in Chapter 4 in which total phospholipid levels were determined in the cultivar MNH-93 showed that, as germination proceeds, there is a continuous increase in total phospholipid levels. A 2 hour heat-shock to seedlings, after germination for 48 hours at 25°C, caused a reduction in total phospholipids levels. Stress at up to 40°C resulted in an insignificant reduction, but stress temperatures above 40°C caused a loss of phospholipids possibly due to its severe effects on membrane integrity. Although these results suggested that longer roots are less affected than the shorter roots, the differences were very small and statistically insignificant. Similar effects of temperature were found with respect to fresh and dry weights. In fact, a high degree of correlation was observed between changes in root length, fresh weight, dry weight and total phospholipid levels. Between any pair of variables, the coefficient of correlation (r) was greater than 0.95. In other words, all were affected in the same way by temperature. It seems obvious that all the parameters are different aspects of a general effect of temperature on seedling development.

Each of four cultivars showed different rates of (Me-^{14}C) choline incorporation into PC under normal germinating conditions at 25°C. The cultivars MNH-93 and Qalandari had higher rates than the other two cultivars Rehmani and S-12. In MNH-93 at least, longer roots incorporated more (Me-^{14}C) choline than shorter roots. At stress temperatures up to 35°C, there were still differences between cultivars. Above this temperature, the rate of synthesis of PC (choline incorporation) decreased with increasing temperature. At 50°C, more than 80% of the activity was lost in all the four cultivars studied. In general, despite the above differences between the

cultivars, no significant differences were found between the four cultivars with respect to the temperature effects above 35°C.

Esterified fatty acid levels in both MNH-93 and S-12 increased with increasing germination time at 25°C reflecting increases in the levels of neutral and polar lipids. Increasing germination also resulted in increases in the proportion of unsaturated fatty acids and decreases in saturated fatty acids. Heat shock for 2 hours caused increases in neutral lipid and glycolipid levels and a small decrease in phospholipid content compared with untreated tissue. The situation for both cultivars were similar. High temperature also enhanced the synthesis of saturated fatty acids and suppressed the synthesis of unsaturated fatty acids. A great increase of free fatty acids was found in MNH-93 but not in S-12 at the end of the recovery following heat shock. In this respect, cultivar S-12 appeared to be relatively better cultivar than MNH-93, in light of the belief that free fatty acids are damaging to cellular structures especially membranes.

It is clear from all the data obtained that, there were some differences between cultivars following post-germination high temperature stress. In some experiments (for example root growth measurements), MNH-93 appeared to be a better cultivar, while in another experiment (fatty acid analysis) S-12 appeared to be the relatively better cultivar. All of these differences were very small, however, and near to the limits of significance. We must conclude that there were no real differences between the cultivars studied.

Waterlogging had a profound effect on root growth in the all the four cultivars. A 6-hour waterlogging treatment caused variable effects, while waterlogging for 12 hours or more greatly damaged the root system. Waterlogging for 24 hours stopped further root growth

completely, followed by a breakdown of root tissues. This treatment caused great damage to almost all the seedlings. In terms of both root growth and visible damage, S-12 was clearly a more tolerant cultivar than MNH-93, Rehmani or Qalandari. Aerated waterlogging also caused reductions in root growth in two cultivars studied (Qalandari and S-12). These reductions were much lower than those seen in the waterlogging without aeration. In this treatment, no visible damage to the roots was seen.

Waterlogging caused large reductions in respiration rates. The reduction was much greater in Qalandari than in S-12. During recovery following waterlogging treatments, Qalandari recovered its respiratory activity much faster than the cultivar S-12, however. In this respect, Qalandari appeared to be a slightly better cultivar than S-12. The pattern was very similar for long and short roots in both cultivars.

Fatty acid analysis following waterlogging showed changes in both their levels and their relative composition. Thus, seedlings waterlogged for 6 hours showed a marked increase in the amounts of esterified fatty acids; the increase was greater in the cultivar S-12 than in MNH-93. The results also showed that waterlogging enhanced the synthesis of a set of low molecular weight compounds and suppressed the synthesis of all the "normal" fatty acids. Cultivar S-12 synthesized a higher proportion of these low molecular weight compounds than MNH-93 did. During recovery following waterlogging, a reduction in the level of phospholipid fatty acids was observed in the cultivar MNH-93, whilst a small increase was found in S-12. A significant increase in free fatty acids was observed in MNH-93 but not in S-12. These results suggest that S-12 is a relatively better cultivar than the MNH-93, because it can synthesise membrane

phospholipids better after imposition of stress conditions and because it does not accumulate free fatty acids.

It is clear from the data obtained following post-germination waterlogging stress that there were some differences between cultivars. In some experiments (for example root growth measurements and in fatty acid analysis), S-12 appeared to be a relatively better cultivar, while in another (respiratory activities) Qalandari showed better tolerance to waterlogging. Since root growth, fresh weight gain and dry weight gain are measuring the overall response of the roots to stress, they must carry the greatest meaning. They suggest that S-12 is more tolerant than the other three cultivars to waterlogging.

The large variability in root length and root growth rate within single seed lots of all the cultivars studied has been noted. This was quite clear from the standard deviation values for the roots in a seedling lot, which lay between 30 to 50% of the mean root length values. Obviously, there was a large heterogeneity in seedling vigour in the seed lots studied. Such variability is a common property of cotton. Genetic causes are less likely to be involved. Several other factors may be responsible for causing this, including biotic and abiotic effects. This variability should be of great concern to the cotton industry. The establishment of a population of vigorously growing seedling is one of the first pre-requisite of crop production (Christiansen and Rowland, 1981). A farmer may fail to achieve his prime goal of harvesting a good crop, due to non-uniform seedling emergence and even the inability of some seedlings to emerge under unfavourable environmental conditions. Heydecker (1972) stated that those seeds which produce seedlings with faster growth rates are better ones, because they can sustain their growth in unfavourable

growing conditions and ultimately produce higher yields. These general relationships between vigour, tolerance to stress and ultimate crop yield have been demonstrated for several species. It is therefore surprising that the present study revealed little or no differences between cotton seedling quartiles with respect to their heat tolerance or their tolerance to waterlogging. This suggests that, in the case of cotton, germination vigour on the one hand and heat tolerance or waterlogging tolerance on the other hand are not directly related to each other.

The amount of storage materials present in a seed and the efficiency with which they are mobilised are all important aspects of seedling growth and development. Seedling establishment is significantly influenced by the quality of the seeds that are planted (Kreig and Bartee, 1975; Leffler, 1981). Several factors, including preharvest deterioration in the field, premature harvesting of seeds and post-harvest deterioration due to poor storage conditions, may adversely affect the physiology and biochemistry of the seed and hence its quality. Gipson (1970) reported that cottonseed produced under low night temperatures (10-15°C) are small, light in weight and of poor quality. Several other workers reported that storage under poor conditions such as high temperature and high relative humidity severely reduces the physiological quality of cotton seed and reduce viability. Rajana (1972) reported that heat injury to cotton seed can occur during any of the several stages and operations from harvest through to final packaging of the processed treated seeds. It is most likely to occur, however, between harvest and ginning when the cotton seeds are stored in trailers or field houses and during the bulk storage of cotton seed after ginning.

In cotton, the cotyledons supply important nutrients to the

growing seedlings. Plants contain about 13 mineral elements, which play essential roles in metabolism, growth and development and ultimately successful reproduction (Epstein, 1972; Rains, 1976; Marschner, 1986). These elements form either an integral part of the cellular structure or they are essential for providing conditions required for metabolism. For example, nitrogen is a essential constituent of proteins and potassium is essential for ionic and osmotic regulation in the cell (Leigh and Storey, 1991). In addition to supplies from the cotyledons, the rapidly growing radicle must also obtain adequate amounts of calcium from the surrounding environment in order to prevent leakage of other solutes and maintain nutrient transport generally. The presence of calcium is essential, for example, for the uptake of potassium by cells (Lauchi and Epstein, 1970). The observations of Yoshiyoki *et al.* (1990) indicated that calcium prevents the leakage of intercellular potassium and thereby supports the elongation of roots. Mineral ion exchange thus greatly affects the further development of seedlings via several metabolic processes.

At the cellular level, vigour and viability depend on the integrity of cellular processes such as DNA replication and transcription, protein synthesis and the functions of subcellular organelles and membranes (Bradbeer, 1988). It is likely that seeds stored for long periods or under adverse conditions are more likely to suffer peroxidative damage (Simon, 1974) leading to disturbance of the basic biochemical processes. Seeds of soybean and pea stored at high temperature and high relative humidity have reduced total phospholipid contents, in particular reduced phosphatidyl choline and phosphatidyl ethanolamine (Priestly and Leopold, 1979; Powell and Metthews, 1981). In pea seeds (Herman and Mattick, 1976) soybean (Steward and Bewley,

1980) and peanut (Pearce and Abdel Samad, 1980) the unsaturated fatty acids decrease during ageing. As seeds age, they germinate more slowly, they respire more slowly and they are more susceptible to diseases. These authors suggest that the above changes indicate membrane destruction, which reduces the viability and eventually leads to the death of the seed. Extending these observations to cotton seedlings, there is the expectation that the more vigorous roots (first quartile) might contain more phospholipid than the less vigorous ones (second and third quartiles). In fact, there was such a difference on a per root basis. When phospholipid levels were calculated on a per gram fresh weight basis, however, there were no significant differences between the quartiles. Therefore, tissue phospholipid levels in the cotton roots do not reflect seedling vigour.

Since seed quality plays such an important role in seedling development and subsequent plant growth, the world-wide demand for high quality cotton seed is increasing. Techniques to differentiate between seeds with poor and good vigour should therefore be developed. A study should be undertaken to select seeds according to their size, harvesting conditions, storage conditions *etc.*, and to correlate these functions with germination vigour. The present study provides an excellent basis for the development of suitable seed testing procedures.

As mentioned in the earlier part of this chapter, there is a high correlation between root length, fresh weight, dry weight and total phospholipid levels. In future, many more cultivars could be screened to select the best ones on the basis of their seed quality by measuring especially root length, fresh weight and dry weights. The measurement of electrolyte efflux would also provide valuable

information about seed quality. Sethar (1993), working on cotton roots, reported that the electrolyte efflux from low vigour seeds was considerably greater than that from high vigour seeds. The determination of total phospholipid levels and (Me-^{14}C) choline incorporation could provide excellent information about seed quality, but these techniques are quite expensive and relatively time consuming.

Although temperature change in sub-tropic regions, such as Pakistan can be rapid, plants in the field are not normally exposed to a temperature shock as such, but to a relatively gradual increase in temperature during the diurnal cycle (Howarth, 1991). During the present study, sudden temperature stress were given to the seedling roots. In the future, a study involving gradual increase and decreases in temperature should be performed to determine changes resulting from "real" diurnal rhythms.

Although the present study of the cotton plant has been mainly carried out on seedling roots, an extension of the study is desirable in order to evaluate heat shock tolerance (and also waterlogging tolerance) at the later stages of development. This is particularly important since pollen development and seed set are believed to be particularly sensitive to heat stress.

The present finding that cotton seedlings grow well up to 30°C but are adversely affected by higher temperatures agree with reports from other researchers. Pearson *et al.* (1970) reported an optimum daily temperature range from 25 to 30°C for maximum seedling growth in cotton. Observations reported by several workers (Tharp, 1960; Gerard, 1971; Wanjura and Buxton, 1972; Sethar, 1993) also confirm our findings that cotton radicle elongation is inhibited above 38°C. The mortality of the majority of the roots following temperature stress at

45°C in our studies was without doubt associated with the death of root cells due to extensive physiological damage including cell membrane damage leading to tissue dehydration. Murtazi and Portnova (1971); Kharlamov and Moesienko (1992), working on wheat seedlings, reported that mitotic cell division is inhibited at temperature between 36 and 48°C. Peacocke and Walker (1962) reported that nucleic acids like proteins can also be denatured by heat. Levitt (1980) explained that, with few exceptions, the guanine and cytosine contents of rRNA tend to increase and the adenine and uracil contents decrease with increasing temperature. This suggests that disturbance of nucleic acid metabolism occurs.

Prolonged growth at non-optimal temperatures alters the plastochrone and often changes in the morphology of the plant (Thomas, 1983). Changes in temperature to either lower or higher temperatures can have profound effects by slowing or hastening the cell cycle (Francis and Barlow, 1988). This in turn affects the duration of the cell cycle and ultimately the growth of the plant. The work of Francis and Barlow further suggests that the rate of cell division alters with changes of temperature in a tissue and species-specific manner. The temperature at which the mitotic cycle has a minimum duration (the optimum temperature) is, for example, 28°C in *Vicia faba* and 32°C in *Allium cepa* (Grif, 1981). The cycle is prolonged at above or below these optima. Thus, at extreme temperatures the high coupling which exists between nuclear division and cell growth may be dissociated (Francis and Barlow, 1988). This may have been a major reason causing reduced elongation and finally a general mortality of cotton roots at high temperatures. Similar studies can also be conducted in future to see the effect of temperature on the cell cycle in cotton roots.

Differences in the total phospholipid levels between longer and

shorter roots was apparent at every stage of germination. The levels rose in all the roots during germination reflecting the requirement for extensive synthesis of phospholipids associated with cell elongation and the production of new cells by cell division. Phospholipid forms the bulk lipid in membrane systems, such as endoplasmic reticulum, mitochondria and plasma membranes (Hitchcock and Nichols, 1971). The lower lipid levels in the shorter roots may be due to a slower rate or delayed onset of cell division. Roots increase in length mainly by cell enlargements rather than by cell division, however (McDonnell, 1981). The rate of root elongation is greater in longer roots than in shorter roots (Zainon, 1993). Longer roots have a smaller number of cells but they are larger in size than those in the shorter roots (Chachar, personal communication). This suggests that the higher phospholipid content of long roots arises because larger cells have more membranes than the smaller cells.

In the present study, two hour heat shock caused a loss of phospholipid possibly due to effects on membrane integrity. Evidence from many sources suggest that loss of membrane integrity is a primary response to high temperature stress in plants. This can be expected to result in a loss of electrolytes and water from the cell, leading to a loss of homeostasis and eventually death of the cell (Salisbury and Ross, 1991). In this context, heat injury may also involve changes in membrane proteins as well as in lipids. The reduction of lipid content due to high temperature may cause extra carbon to be channelled into protein accumulation. At low temperatures, Quinn (1988) reported that growth usually results in an increase in the proportion of lipid to proteins. Perhaps the opposite occurs at high temperatures. There is also evidence that temperature-induced phase changes occur in the lipid of membranes. The main consequences of such a phase transition

are to increase membrane contraction and even cause cracking at low temperatures, as suggested by Lyons (1973), or membrane expansion at high temperatures. Tischner *et al.* (1978) found that swelling and partial damage to the Golgi apparatus, endoplasmic reticulum and especially mitochondria occur after heat-shock treatment. Niki *et al.* (1978) described the importance of the endoplasmic reticulum and Golgi apparatus in supplying lipid precursors for membrane assembly and they state that an alteration in the normal function of the endoplasmic reticulum, either in a reversible or irreversible manner, may result in a serious change in the membrane metabolism of the cell as a whole. They further added that an abrupt cessation of a continuous supply of membrane building materials and/or the energy which is necessary to maintain the integrity of the membranes may cause decay of membranes such as the tonoplast and plasma membrane.

Several pieces of evidence suggest that high temperatures causes denaturation of membrane-bound enzymes. Hydrogen bonding between molecules of the membrane is important in maintaining membrane integrity (Hale and Orcutt, 1987). High temperature is assumed to weaken this hydrogen bonding (Hochachka and Somero, 1973) and it is considered to be responsible for alterations in protein stability at supraoptimal temperatures (Langridge, 1963). The subsequent loss of enzyme regulation and build-up of metabolites to toxic levels may have a damaging effect on cells exposed to high temperatures. Benzioni and Itai (1973) reported that such damage to membranes will affect the activities of membrane-bound enzymes as well as compartmentation of metabolites in the tissue. The latter effect might also cause a build-up of metabolites to toxic levels.

High temperature results in the release of degradative enzymes. Increased phospholipid breakdown due to high temperature may lead to

still further lipid breakdown and one possible mechanism for this was proposed by Galliard (1971) who reported that free fatty acids released during lipid breakdown of homogenised cucumber fruits stimulated further deacylation of PC and PE by lipolytic acyl hydrolases. The free fatty acids and peroxides, which are the products of lipid breakdown, have extremely deleterious effects particularly on membranes when coupled with a loss of membrane components such as proteins. The concentration of lipolytic enzymes is thought to be high in the vacuoles and therefore any damage to the tonoplast will cause further lipid breakdown in other membranes. In future work on cotton, a study should be conducted to study the turnover of phospholipids following temperature stress in order to understand breakdown rates.

The degree of fluidity of the cell membrane greatly affects the ability of cells to survive and function. In the present study, it has been shown that high temperature affects the fatty acid composition and causes an increase in the proportion of saturated fatty acids and decreased proportions of unsaturated fatty acids in polar lipids. The greater fatty acid saturation may lead to decreased membrane fluidity, as suggested by Thompson (1979), and affect the activity of the fatty acyl desaturase enzyme (Brenner, 1984). Harris and James (1969 a and b) suggested that temperature could also influence the rate of fatty acid desaturation through an effect on oxygen solubility. Thus, the changes in fatty acid composition in cotton following heat shock may be due to changes in relative activities of enzymes including the fatty acyl desaturase.

Waterlogging creates an anaerobic environment for roots. This results in the disruption of root metabolism which may lead to induced water and nutrient stresses. Many other effects such as reduction in hormonal supply may result (Reid and Crozier, 1971; Leyshon and

Sheard, 1974; Drew, 1983). Marschner (1991) reported that cessation of mineral nutrient uptake is an immediate response to waterlogging. Other changes in mineral ion movement may be directly damaging. In waterlogged soil, Clarkson and Sanderson (1969) have shown that primary cell walls in the root tip have a very low resistance to Al^{+3} movement, apparently owing to the low exchange capacity, so that aluminum ions rapidly penetrate to the meristematic cells and inhibit DNA synthesis. Ions can also play positive role in enzyme functions, as a structural component of the enzyme molecule (eg. Fe in cytochrome) or simply as an activator (eg. K in pyruvate kinase). Ionic imbalances, therefore, are peculiarly capable of disrupting cellular activities.

Energy metabolism is, of course, central to all cellular functions. A continuous supply of energy is required for all synthetic and maintenance processes. Anaerobic respiration produces insufficient ATP for growth and maintenance (Fitter and Hay, 1987). For example, it produces insufficient energy for the initiation of S-phase (DNA-synthesis phase) of mitosis in the root meristem (Amoore, 1962a and b). Root extension rates depend on rates of cell division and elongation. Lopez-Saez *et al.* (1969), working on roots of *Allium cepa*, reported that the root extension rate is reduced from 21.3 to 8.8 mm per day over of range of oxygen concentration from 0.2 to 0.02 atmospheres. They further reported that the retarded growth at the lower oxygen concentrations is due to a slower production of new cells. Elimination of oxygen may be the primary reason for reduction in respiration rate during waterlogging. The cytochrome chain in the root cells ceases to function in the absence of molecular oxygen, and this in turn leads to the accumulation of NADH and the suppression of Kreb's cycle activity. The resulting build-up of acetaldehyde induces

the synthesis of alcohol dehydrogenase activity which catalyses the transformation of the acetaldehyde to ethanol. Jackson *et al.* (1982) showed, however, that a low concentration of ethanol displayed little toxicity to pea roots or even to cell protoplasts.

Changes in membrane lipid composition due to waterlogging can be expected to modify membrane lipid fluidity and alter permeability and membrane transport processes. These effects are presumably similar to those caused by heat shock. In mitochondria extracted from yeast cells, degeneration under anoxia is closely associated with interference in the synthesis of unsaturated fatty acids. This in turn increases permeability of the inner mitochondrial membrane and causes uncoupling of ADP phosphorylation from respiration (Quinn and Chapman, 1980). These may be reasons causing the reduced elongation, respiration and consequently a general mortality of cotton roots under waterlogging conditions.

The present study highlights the need to produce new cotton varieties suited to the extreme growing conditions experienced in Pakistan. During the cotton growing season in Pakistan, the temperature often reaches 40-50°C. Under these conditions, crops such as cotton will suffer and will have great problems in maintaining physiological and biochemical integrity. Therefore, there is a great need to identify or to breed plants with high temperature tolerance. Unfortunately, the results obtained during the present study show that there is little difference between the Pakistan cotton cultivars in this respect. In summers in Pakistan, soil waterlogging after planting is not uncommon as a result of over-flowing of river-banks, heavy rainfall and sometimes over-irrigation. Under these conditions, summer crops including cotton suffer from anaerobiosis and will have difficulty in maintaining growth. Maintenance of growth under these

conditions requires that plants should possess roots with properties which can allow tolerance to anaerobic conditions for a limited time. Several forms of roots generation occur in flooded plants. The most common type of regeneration is the development of roots on the stem above the soil usually within the flood zone. They are called adventitious water roots. This type of root has been observed on many species such as tomato, sunflower and maize which tolerate flooding or recover from flooding damage quickly and completely. Sena-Gomes and Kozlowski (1980b) found that *Fraxinus pennsylvanica* seedlings with adventitious water roots have higher water-absorbing efficiency than those seedling without adventitious water roots. They further reported that there was a correlation between adventitious water roots and stomatal reopening. In some species, flooding induces the formation of aerenchyma in developing roots. A rapid rate of oxygen diffusion is possible by the presence of large, continuous air spaces in the root cortex, which in some cases are permanent features of the roots (eg. rice, John, 1977). In addition, the development of aerenchyma may improve root aeration by reducing the volume of respiring tissue (i.e. root demand for oxygen). Most of these properties of root aerenchyma development and regeneration after flooding unfortunately are not well developed in cotton (Reicosky *et al.*, 1985), which is a semi-arid zone plant. It would, however, be of great advantage if modern genetic techniques could be used to incorporate some of these properties.

It seems that genetic variation for stress tolerance within a single species is often limited particularly in the cultivated *G. hirsutum* cotton. Greater variation may exist in diploid species (old-world cotton) and their wild relatives, however. Tissue culture and molecular biology are two important and interesting components of plant biotechnology, which may be used to transfer genes between

species. The successful development of these new techniques have made it possible to generate improved plants resistant to environmental stresses (Mirza and Shaikh, 1983). The development of recombinant DNA technology and its application to plants has provided opportunities for the investigation and understanding of the molecular basis of plant development and for the identification and cloning of the desirable plant genes. The successful routine use of Ti plasmid of *Agrobacterium tumefaciens* to introduce foreign genes stably into plant cells has created unparalleled opportunities for the molecular genetic manipulation of dicotyledonous plants. In the case of cotton (*G. hirsutum*), several workers, for example, Firoozababy *et al.* (1987), have reported that cotyledon tissues can be efficiently transformed and plants regenerated that express the newly incorporated genes. With the help of these techniques, improved properties (stress resistant) can be transferred from wild species and from diploid species of cotton to the cultivated species (*G. hirsutum* L.).

LITERATURE CITED

LITERATURE CITED

Abdul Baki, A.A. (1969), Crop Sci. 9: 732-737. Relationship of glucose metabolism in germinability and vigour in barley and wheat roots.

Abdel Magid and A.M. Osman (1977), Ann. Botany 41: 697-706. On the germination and changes in the chemical composition during seedling emergence and development of cotton seeds.

Adelusi, S.A. and A.O. Lawanson (1978), J. Agri. Sci. 91: 349-358. Heat-induced changes in dry weight, leaf size and number of chloroplasts per cell in maize and cowpea shoots.

Al-Ani, A.; F. Bruzau; P. Raymond; V. Saint-Ges; J.M. Leblanc and A. Pradet (1985), Plant Physiol. 79: 885-890. Germination, respiration and adenylate energy charge of seeds at various oxygen partial pressures.

Amoore, J.E. (1962a), J. Cell Biol. 13: 365-371. Oxygen tension and the rates of mitosis and interphase in roots.

Amoore, J.E. (1962b), J. Cell Biol. 13: 373-381. Participation of a non-respiratory ferrous complex during mitosis in roots.

Anaya, M. (1972), Effects of soil aeration, water and nitrogen fertilization on nutrition, yield and quality of wheat. Unpublished dissertation, University of California, Riverside. (Cited B.D. Meek and L.H. Stolzy, 1978).

Anon (1982), J. Am. Oil Chem. Soc. 59: 752A-766A. World forecast: record oil seed production.

Ansel, G.B. and S. Spanner (1982), Phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl choline. In, New Comprehensive Biochemistry, (ed. A. Neuberger and L.L.M. van Deenen), Vol. 4, pp. 1-50, Elsevier, New York.

Aref, A.; Abdul Baki and J.D. Anderson (1973), Crop Sci. 13: 630-633. Vigour determination in soybean seed by multiple criteria.

Arndt, C.A. (1945), Plant Physiol. 20: 200-220. Temperature growth relations of the roots and hypocotyls of cotton seedlings.

Arnon, I. (1972), In, Crop Production in Dry Regions. II: 261-344. (ed. N. Polunin). Leonard Hill London.

Babaev, M.U.; Kh. S. Mukhamedova and S.T. Akramov (1974), Khim. Prir. Soedin., No. 3:28. Phospholipids of the Tashkent-1 cotton cultivar (Cited Rakhimov, 1986).

Beadle, N.C.W. (1962), Ecology 43: 281-288. Soil phosphate and the delineation of plant communities in eastern Australia.

Becker, W.M; C.J. Leaver; C.M. Weir and H. Reizman (1978), Plant Physiol. 62: 542-549. Regulation of glyoxysomal enzymes during germination of cucumber. I. Developmental changes in cotyledonary proteins, RNA and enzyme activities during germination.

Belehdradck, J. (1935), Protoplasma 8: 277. Temperature and living matter.

Benedict, C.R. (1984), Physiology. In, Cotton, (ed. R.J. Kohel and C.F. Lewis), pp. 151-192. Amer. Soc. Agron. Pub.

Benson, A.A. and B. Maruo (1958), Biochim. Biophys. Acta 227: 189-195. Plant phospholipids. I. Identification of phosphatidyl glycerols.

Benson, A.A. and E.H. Strickland (1960), Biochim. Biophys. Acta 41: 328-333. Plant Phospholipids. III. Identification of Phosphatidyl glycerol.

Benzioni, A. and C. Itai (1973), Physiol. Plant. 28: 493-497. Short and long term effects of high temperature (47-49°C) on tobacco leaves. III. Efflux and phosphorus-32 incorporation into phospholipids.

Berckley, D.M. and E.E. Berckley (1933), Ann. Missouri Bot. Gard. 20: 583-604. Super-optimal and thermal death temperatures of the cotton plant as affected by variations in relative humidity.

Berger, J. (1969), In, The World's major fibre crops, their cultivation and manuring. Pub. Centre D'etude L'azote Zurich.

Berry, J. and O. Bjorkman (1980), Ann. Rev. Plant Physiol. 31: 491-543. Photosynthetic response and adaptation to temperature in higher plants.

Berry, J.A.; D.C. Fork and S. Garrison (1975), Carnegie Inst. Year Book 74: 751-759. Mechanistic studies of thermal damage to leaves.

Bettaieb, L.; M. Gharselli and A. Cherif (1980), In, Biogenesis and Function of Plant Lipids. (ed. P. Mazliak; P. Benveniste; C. Costes and R. Douce), pp. 243-247. Elsevier Amsterdam.

Bewley, J.D. and M. Black (1978), Physiology and Biochemistry of Seeds. Vol. 1, Springer-Verlag, Berlin.

Bhatti, A.S. (1975), Plant and Soil 41: 681-683. Treatment of cotton seeds for germination.

Bielorai, H; A. Mental and S. Moreshet (1983), Water relations of cotton. In, Water Deficits and Plant Growth, pp. 49-87, (ed. T.T. Kozlowski), Vol. VII, Academic Press.

Bland, W.L. (1993), Agron. J. 85: 906-911. Cotton and soybean root systems growth in three soil temperature regimes.

Botha, F.C. and J.G.C. Small (1985), Plant Physiol. 77: 79-82. Effect of water stress on the carbohydrate metabolism of *Citrullus lanatus* seeds during germination.

Bowen, G.D. (1991), Soil temperature, root growth and plant functions. pp. 309-330. In, (ed. Y. Waisel *et al.*,), Marcel Dekker, New York. (Cited Bland, 1993).

Bowman, R.D. and R.O. Mumma (1967), Biochim. Biophys. Acta 144: 501-510. The lipids of *Pythium ultimum*.

Boynton, D.; J.I. Davilliers and W. Reuther (1938), Science 88: 569-570. Are there different cortical oxygen levels for the different phases of root activity?

Bradbeer, J.W. (1988), Seed dormancy and germination. Chapman and Hall, New York.

Bradford, K.T. and T.C. Hsiao (1982), Physiological responses to moderate water stress. In, Encyclopedia of Plant Physiology, (ed. O.L. Lange; P.S. Nobel; C.B. Osmand and H. Ziegler), Vol. 12B, pp. 264-324. Spriger, Berlin Heidelberg, New York.

Brady, N.C. (1984), The nature and properties of soils (9th ed.) Macmillan Publishing Co. New York.

Brenner, R.R. (1984), Prog. Lipid Res. 23: 69-96. Effect of unsaturated acids on membrane structure and enzymatic kinetics.

Briggs, P. (1973), Rampage: the story of disastrous floods, broken dams, and human fallibility. In, Flooding and Plant Growth, (ed. T.T. Kozlowski, 1984), McKay, New York.

Broadfoot, M.W. and H.L. Williston (1973), J. For. 71: 584-587. Flooding effects on southern forests.

Brohult, S and P. Sandegren (1954), In, The Proteins. 2A: 487.Acad. Press New York.

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

Burke, J.J.; J.L. Hatfield; R.R. Klein and J.E. Mullet (1985), Plant Physiol. 78: 394-398. Accumulation of heat shock proteins in field grown cotton.

Burrows, W.J. and D.T. Carr (1969), Physiol. Plant. 22: 1105-1112. Effect of flooding the root system of sunflower plants on the cytokinin content in the xylem sap.

Buttrose, M.S. and J.S. Swift (1975), Aust. J. Plant Physiol. 2: 225-234. Effect of killing by heat or desiccation on membrane structure in pea roots.

Caers, M.; P. Rudelsheim; H.V. Onckelen and S. Horemans (1985), Plant Cell Physiol. 26: 47-52. Effect of heat stress on photosynthetic activity and chloroplast ultrastructure in correlation with endogenous cytokinin concentration in maize seedlings.

Cal, J.P. and R.L. Obendroff (1972), Crop. Sci. 12: 369-373. Inhibitional chilling injury in *Zea mays* L. altered by initial kernal moisture and maternal parent.

Cannon, W.A. (1925), Carnegie Ins. Washington Publ. No. 368: 1-168. Physiological features of roots with special reference to the relation of roots to aeration of the soil.

Chapman, S.R. and L.P. Carter (1976), Principles and practices. In, Crop Production. Freeman, W.H and Co. San Francisco, USA.

Chernov, I.A.; T.M. Khlyustova; D.Z. Shakirova and L.I. Grishina (1971), Fotosin. Ispol'z. Energ. 205-209. (Russ.) Effect of temperature on photosynthesis of isolated chloroplasts. (Cited Chem. Abst. 76, 151187).

Cherry, J.P. (1983), J. Am. Oil. Chem. Soc. 60: 360-367. In, Cotton, (ed. R.J. Kohel and C.F. Lewis). Amer. Soc. Agron.

Cherry, J.P.; L.A. Jones and W.H. Powell (1981), Cotton seed quality: factors affecting feed and food uses. In, Proc. Beltwide Cotton Prod. Res. Conf. New Orleans, La.

Chirkova, T.V.; K.L. Khoang and Yu. A. Blyudzin (1981), Fiziol. Rast. 28: 358. Effect of anaerobic conditions on fatty acid composition of phospholipids in wheat and rice roots.

Chittenden, C.; D.L. Laidman; N. Ahmad and R.G. Wyn Jones (1978), Phytochem. 17: 1209-1220. Amino acids and quaternary nitrogen compounds in germinating wheat grains.

Christiansen, M.N. (1986), Influence of atmospheric parameters on growth and development. In, Cotton Physiology, (ed. J.R. Mauny and J.M. Stewart), pp. 39-46. Natl. Cotton Council Momphis TN. USA.

Christiansen, M.N. and R. Rowland (1981), In, Cotton Physiology 3: 315-318. Seed and germination. Proc. Beltwide Cotton Prod. Res. Conf. 1981, New Orleans, La.

Christiansen, M.N. and R.P. Moore (1959), Agron. J. 51: 282-284. Seed coat structural differences that influence water uptake and seed coat quality in hard seed cotton.

Christiansen, M.N. and R.P. Moore (1961), Crop Sci. 1: 385-386. Temperature influence on the *in vivo* hydrolysis of cotton seed oils.

Clarkson, D.T. and J. Sanderson (1969), Planta 89: 136-154. The uptake of polyvalent cation and its distribution in the root apices of *Allium cepa*.

Cobon, G.S. and J.M. Haslam (1973), Biochim. Biophys. Res. Comm. 52: 320-326. Effect of altered membrane sterol composition on the temperature dependence of yeast mitochondrial ATPase.

Colborne, A.J. (1974), Developmental processes in the aleurone cells of the germinating wheat grains. Ph.D. Thesis, Univ. of Wales.

Colborne, A.J. and D.L. Laidman (1975), Phytochem. 14: 2639-2645. The extraction and analysis of wheat phospholipids.

Copeland, L.O. (1976), In, Principles of Seed Science and Technology. Burgess Publishing Company USA.

Crawford, R.M.M. (1969), Ber. dtsch. bot. ges. 82: 111-114. The physiological basis of flooding tolerance. (Cited Vartapetian *et al.*, 1971).

Crawford, R.M.M. (1978), Metabolic adaptation to anoxia. In, Plant Life in Anaerobic Environments. Ann. Arbor, Sci. Publ. Ann. Arbor., Michigan.

Czarnecka, E.; L. Edelman; F. Schoffl and J.L. Key (1984), Plant Mol. Biol. 3: 45-58. Accumulation of heat shock proteins in field grown cotton.

Dalians, C.D. (1982), Seed Sci. Technol. 10: 35-45. Rate of radicle emergence as a measure of seedling emergence and vigour in cotton.

Dawson, R.M.C; N. Clarke and R.H. Quarles (1969), Biochem. J. 114: 265-270. N-Acyl phosphatidyl ethanolamine, a phospholipid that is rapidly metabolized during the early germination of pea seeds.

Del-Rosario, D.A. and F.F. Fajardo (1991), Philippine Agriculturist 74: 51-58. Waterlogging resistance in crops.

Demel, R.A. and B. de Kruyff (1976), Biochim. Biophys. Acta 457: 109-132. The function of sterols in membranes.

Dexter, S.T.; W.E. Tottingham and L.H. Graber (1932), Plant Physiol. 7: 63-78. Investigations of hardiness of plants by measurement of electrical conductivity.

Dieckert, J.W. and M.C. Dieckert (1972), The deposition of vacuolar proteins in oil seeds. In, Seed Proteins, (ed. G.E. Inglett), pp. 52-58. AVI Publishing Co. Westport Conn.

Dieckert, J.W. and M.C. Dieckert (1976), J. Food Sci. 41: 475-482. The chemistry and cell biology of the vacuolar proteins of seeds.

Douce, R. and Joyard, J. (1980), In, The Biochemistry of Plants, Vol. 4, pp. 321-333, (ed. P.K. Stumpf). Acad. Press New York.

Douce, R.; R.B. Halz and A.A. Benson (1973), J. Biol. Chem. 248: 7215-7222. Isolation and properties of the envelope of spinach chloroplasts.

Draper, S.R. (1969), Phytochem. 8: 1641-1647. Lipid changes in senescing cucumber cotyledons.

Drew, M.C. (1983), Plant and Soil 75: 179-199. Plant injury and adaptation to oxygen deficiency in the root environment.

Drew, M.C. (1992), Soil Sci. 259-268. Soil aeration and plant root metabolism.

Drew, M.C. and E.J. Sisworo (1979), New Phytolog. 82: 301-314. The development of waterlogging damage in young barley plants in relation to plant nutrient status and change in soil properties.

Ehling, C.F. (1986), Effects of high temperature and controlled fruiting on cotton yield. In, Cotton Physiology, (ed. J.R. Mauney and J.M. Stewart), pp.57-62.

El-Nockrashy, A.S.; H.M. Mostafa; Y. El-Shattory and M.H. Abbassy (1974), Nahrung 18: 285-293. Biochemical changes in cotton seed during germination. (Cited Chem. Abst. 81, 88051).

Epstein, E. (1972), Mineral nutrition of plants: Priciples and practices. John Wiley and Sons, New York.

Erdei, L.; C.E.E. Stuiver and P.J.C. Kuiper (1980), Physiol. Plant. 49: 315-319. The effect of salinity on lipid composition and activity of Ca^{++} and Mg^{++} stimulated ATPase in salt-sensitive and salt-tolerant species.

Erdei, L. and E. Taleisnik (1992), Physiol. Plant. 85(part 2): A66. Water relation parameters under osmotic and salt stress in maize and sorghum.

FAO (1978), Production Year Book, Vol. 31, 1977. Food and Agri. Rog. Rome.

Feo, F.; R.A. Canuto; R. Garcea and L. Gabriel (1975), Biochim. Biophys. Acta 413: 116-134. Effect of cholesterol content on some physical and functional properties of mitochondria isolated from adult rat liver, fetal liver, cholesterol-enriched liver and hepatomas AH-130, 3924A and 5123.

Finean, J.B.; R. Coleman and R.H. Micheal (1978), Membranes and their cellular functions. Blackwell Scientific Publications, Oxford.

Firoozababy, I.; D.L. Debor; D.M. Merlo; E.L. Halk; L.N. Amerson; K.E. Rashka and E.M. Murray (1987), Plant Mol. Biology 10: 105-116. Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration of transgenic plants.

Fitter, A.H. and R.K.H. Hay (1987), Environmental Physiology of Plants. Acad. Press London.

Fork, D.C. and N. Murata (1977), Special issue of Plant Cell Physiol. pp. 427-436. The relationship between changes of the physical phase of membrane lipids and photosynthesis in the thermophilic alga *Cyanidium caldarium*.

Francis, D. and P.W. Barlow (1988), Temperature and cell cycle. In, Plants and Temperature, (ed. S.P. Long and F.I. Woodward). Sym. Soc. Exp. Biol. 42: 181-201. Cambridge Co. of Biologists.

Fransolet, S.; R.B. Rojer-Doltour and C. Van De Walle (1979), Planta 146: 7-18. Changes in ultrastructure and transcription induced by elevated temperature in *Zea mays* embryonic root cells.

Fryxell, P.A.; L.A. Craven and J.McD. Stewart (1992), Systematic Botany 17: 91-114. A revision of *Gossypium Sect. Grandicalyx* (*Malvaceae*), including the description of six new species.

Galliard, T. (1971) Eur. J. Biochem. 21: 90-98. Enzymic deacylation of lipids in plants. The effects of free fatty acids on the hydrolysis of phospholipid by the lipolytic acyl hydrolase of potato tubers.

Garcia-Huidobro, J.; J.L. Monteith and G.R. Squire (1985), J. Exp. Botany 36: 338-343. Time, temperature and germination of pearl millet (*Pennisetum typhoides*): 3. Inhibition of germination by short exposure to high temperature.

Gardner, H.W. (1979), J. Agri. Food Chem. 27: 220-229. Lipid hydroperoxide reactivity with proteins and amino acids: a review.

Gerard, C.J. (1971), Agron. J. 63: 555-558. Influence of osmotic potential, temperature and calcium on growth of plant roots.

Getz, G.S. (1970), Adv. Lipid Res. 8: 175-223. Lipids in membrane development.

Gipson, J.R. (1970), Cotton Grow. Rev. 47: 264-271. Temperature-variety interrelationship in cotton. II. Seed development and composition.

Gipson, J.R. and H.E. Joham (1968), Agron. J. 61: 292-295. Influence of night temperature on growth and development of cotton (*G. hirsutum*). I. Fruiting and boll development.

Glady, C.G. (1938), Plant Physiol. 13: 835-844. Temperature effects upon the growth of excised root tips.

Gounaris, K.; A.P.R. Brain; P.J. Quinn and W.P. William (1983a), FEBS. Letts. 153: 47-52. Structural and functional changes associated with heat-induced phase-separations of non-bilayer lipids in chloroplast thylakoid membranes.

Gounaris, K.; W.P. William and P.J. Quinn (1983b), Biochem. Soc. Trans. 11: 388-389. Heat stress induces non-bilayer lipid structures in chloroplast membranes.

Grace, J. (1988), Temperature as a determinant of plant productivity. In, Plants and Temperature, (ed. S.P. Long and F.I. Woodward). Sym. Soc. Exp. Biol. 42: 91-107. Cambridge Co. of Biologists.

Gregory, P.J. (1978), Biochemistry of Photosynthesis. J. Wiley and Sons Ltd. London.

Grif, V.G. (1981), Tsitologiya 23: 166-173. The use of temperature coefficients for studying the mitotic cycle in plants.

Grisham, C.M. and R.E. Barnett (1973), Biochem. 12: 2635-2637. The role of lipid phase transitions in the regulation of the (sodium + potassium) adenosine triphosphate.

Grunwald, C. (1975), Ann. Rev. Plant Physiol. 26: 209-236. Plant sterols.

Grunwald, C. (1978), Phil. Tran. Res. Soc. Lond. Ser. B. 284: 541-548. Function of sterols.

Gulliver, R.L. and W. Heydecker (1973), Establishment of seedlings in a changeable environments. In, Seed Ecology (ed. W. Heydecker), pp. 433-462. Butterworth London.

Gupta, U.S. (1977a), Physiological aspects of crop nutrition and resistance. Atma Ram and Sons, Delhi. pp. 383.

Gupta, P.K. (1977b), Can. J. Botany 55: 1186-1194. A study of photosynthesis and leakage of solutes in relation to desiccation effects in bryophytes.

Guyot, C. and J.C. Prioul (1985), Agronomie 5: 743-750. Alleviation of waterlogging effects in winter wheat by mineral fertilizer application. I. Experiments on soil.

Hale, M.G. and D.M. Orcutt (1987), The physiology of plants under stress. John Wiley and Sons Inc. New York.

Hanson, J.B.; A.E. Vatter; M.E. Fisher; R.F. Bils (1959), Agron. J. 51: 295-301. The development of mitochondria in the sculettum of germinating corn.

Harman, G.E. and L.R. Mattick (1976), Nature 260: 323-324. Association of lipid oxidation with seed ageing and death.

Harris, P. and A.T. James (1969a), Biochim. Biophys. Acta 187: 13-18. Effects of low temperature on fatty acid biosynthesis in seeds.

Harris, P. and A.T. James (1969b), Biochem. J. 112: 325-330. The effect of low temperatures on fatty acid biosynthesis in plants.

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.

Harwood, J.L. (1983), Biochem. Soc. Transl. 11: 343-346. Adaptive changes in the lipids of higher plant membranes.

Harwood, J.L. (1984), Effects of environment on the acyl lipids of algae and higher plants. In, Developments in Plant Biology, Structure, function and metabolism of plant lipids, Vol. 9, (ed. P.A. Siegenthaler and W. Eichenberger), pp. 543-550. Elsevier, Amsterdam.

Haugen, L.M. and S.E. Smith (1992), Plant and Soil 145: 71-80. The effect of high temperature and fallow period on infection of mungbean and cashew roots by the vesicular-arbuscular mycorrhizal fungus *Glomas intradices*.

Hetherington, A.M.; M.I.S. Hunter and R.M.M. Crawford (1982), Phytochem. 21: 1275-1278. Contrasting effects of anoxia on rhizome lipids in IRIS species.

Hetherington, P.R.; H.L. Broughton and B.D. McKeersie (1988), Plant Physiol. 86: 740-743. Ice-Encasement injury to microsomal membranes isolated from winter wheat crowns.

Heydecker, W. (1972), Vigour. In, Viability of Seeds. pp. 209-252. (ed. E.H. Roberts). Chapman and Hall, London.

Hiatt, A.J. and R.H. Lowe (1967), Plant Physiol. 42: 1731-1736. Loss of organic acids, amino acids, K and Cl from barley roots treated anaerobically and with metabolic inhibitors.

Hiron, R.W.P. and S.T.C. Wright (1973), J. Exp. Botany 24: 769-781. The role of endogenous abscisic acid in the response of plants to stress.

Hitchcock, C. and B.W. Nichols (1971), Plant Lipid Biochemistry. Acad. Press London.

Hochachka, P.W. and G.N. Somero (1973), In, Strategies of Biological Adaptation, pp. 179-270. W. Saunders, Philadelphia.

Hocking, P.J.; D.C. Reicosky and W.S. Meyer (1985), Plant and Soil 87: 375-391. Nitrogen status of cotton subjected to two short term periods of waterlogging of varying severity using a sloping plot water-table facility.

Hocking, P.J.; D.C. Reicosky and W.S. Meyer (1987), Plant and Soil 101: 211-222. Effects of intermittent waterlogging on the mineral nutrition of cotton.

Hodgson, A.S. (1982), Aust. J. Agri. Sci. 33: 1019-1028. The effects of duration, timing and chemical amelioration of short-term waterlogging during furrow irrigation of cotton in a cracking grey clay.

Hodgson, A.S. and K.Y. Chan (1982), Aust. J. Agri Sci. 33: 109-116. The effect of short-term waterlogging during furrow irrigation of cotton in a cracking grey clay.

Holloway, J.T. (1962), N.Z. For. Serv. Info. Ser. No. 36. Forests and water- the New Zealand problem.

Hook, D.D. and R.M.M. Crawford (1978), Plant Life in Anaerobic Environments. Ann. Arbor Sci. Publi. Arbor Michigan.

Hopkins, R.M. and W.H. Patrick, Jr. (1969), Soil Sci. 108: 408-413. Combined effect of oxygen content and soil compaction on root penetration.

Houslay, M.D. and K.K. Stanely (1982), Dynamics of Biological Membranes. pp. 25-90. Wiley ltd. U.S.A.

Howarth, C.J. (1991), Plant Cell and Environ. 14: 831-841. Molecular responses of plants to an increased incidence of heat shock.

Huck, M.G. (1970), Agron. J. 62: 815-818. Variation in tap root elongation rate as influenced by composition of the soil air.

Iordanov, I.T. and V.S. Vasil'eva (1976), Fiziol. Rast. (Mosc.) 23: 812-817. Effect of elevated temperature on the rate of photosynthesis and the activity of ribulose diphosphate and phosphoenolpyruvate carboxylases. (Cited Chem. Abst. 63, 71537).

Irvine, R.F.; A.J. Letcher and R.M.C. Dawson (1980), Biochem. J. 192: 279-283. Phosphatidyl inositol phospho diesterase in higher plants.

Itai, C. and Y. Vaadia (1965), Physiol. Plant. 18: 941-944. Kinetin-like activity in root exudates of water-stressed sunflower plants.

Jackson, M.B.; B. Herman and A. Goodenough (1982), Plant Cell Environ. 5: 163-172. An examination of the importance of ethanol in causing injury to flooded plants.

Jackson, M.B. and M.C. Drew (1984), Effect of flooding on growth and metabolism of herbaceous plants. In, Flooding and Plant Growth, (ed. T.T. Kozlowski), pp. 47-128. Academic press, Orlando, Fla.

Janiszowska, W.; E. Sobocinska and Z. Kasprzyk (1979), Phytochem. 18: 427-430. Distribution of different forms of sterols in three cellular subfractions of *Calendular officinalis* leaves.

John, C.D. (1977), Plant and Soil 47: 269-274. The structure of rice roots grown in aerobic and anaerobic environments.

Jones, R.L. and I.D.J. Phillips (1966), Plant Physiol. 41: 1381-1386. Organs of gibberellin synthesis in light grown sunflower plants.

Jordan, W.R. (1982), Water relations of cotton. In, Crop Water Relations, (ed. I.D. Teare and M.M. Peet). Pub. Willey Chichester.

Kagawa, T.; J.M. Lord and H. Beevers (1973), Plant Physiol. 51: 61-65. The origin and turnover of organelle membranes in castor bean endosperm.

Kailasanathan, K.; G.G.S.N. Rao and S.K. Sinha (1976), Ind. J. Plant Physiol. 19: 171-177. Effects of temperature on the partitioning of seed reserves in cowpea and sorghum.

Karim, Z.; M.S. Khan; M. Rahman and D. Nessa (1986), Bangladesh J. Agriculture 11: 39-45. Effect of permeability on the shoot and root growth of cotton (*Gossypium hirsutum* L.).

Kates, M. (1955), Can. J. Biochem. Biophys. 33: 575-589. (Cited Vakharia, 1986).

Kates, M. (1960), Biochim. Biophys. Acta 41: 315-328. Chromatographic and radioisotopic investigations of the lipid components of runner bean leaves.

Kates, M. (1970), Plant phospholipids and glycolipids. In, Advances in Lipid Research, Vol. VIII, (ed. R. Paoletti and D. Kritchevsky), pp. 225-265. Acad. Press New York.

Katsuhara, M. and M. Tazawa (1986), Protoplasma 135: 155-161. Salt tolerance in *Nitellopsis obtusa*.

Kemp, R.J. and E.I. Mercer (1968), Biochem. J. 110: 111-118. The sterol esters of maize seedlings.

Kennedy, E.P. and S.B. Weiss (1956), J. Biol. Chem. 222: 193-214. The function of cytidine coenzymes in the biosynthesis of phospholipids.

Key, J.L.; C.Y. Lin and Y.M. Chen (1981), Proc. Natl. Acad. Sci. U.S.A. 78: 3526-3530. Heat-shock proteins of higher plants.

Key, J.L.; J.A. Kimple; E. Vierling; C.Y. Lin; R.T. Nagao; E. Czarnecka and F. Schoffl (1985), In, Changes in Eukaryotic Gene Expression in Response to Environmental Stress, (ed. B.G. Atkinson and B.D. Walden), pp. 327-348. Acad. Press.

Kharlamov, A.V. and N.N. Mousienko (1992), Physiol. Plant. 85: A67. The effect of high temperature on the meristem cell proliferations.

Knowles, L.O. and R.A. Kennedy (1984), Phytochem. 23: 529-532. Lipid biochemistry of *Echinochloa crus-galli* during anaerobic germination.

Kozlowski, T.T. and S.G. Pallardy (1984), Effect of flooding on water, carbohydrate and mineral relations. In, Flooding and Plant Growth, (ed. T.T. Kozlowski), pp. 165-193. Acad. Press.

Kreig, D.R. and J.D. Carrol (1978), Agron. J. 70: 21-25. Cotton seedling metabolism as influenced by germination temperature, cultivar, and seed physical properties.

Kreig, D.R. and S.N. Bartee (1975), Agron. J. 67: 343-347. Cotton density: Associated germination and seedling emergence properties.

Krishnamoorthy, H.N.; C.L. Goswami and Jai-Dayal (1987), Ind. J. Plant Physiol. 30: 387-389. Effect of waterlogging and growth retardants on gram (*Cicar arietimum* var. H.335).

Kuhn, H.; J. Belkner; R. Wiesner and A.R. Brash (1990), J. Biol. Chem. 265: 18351-18361. Oxygenation of biological membranes by the pure reticulocyte lipoxygenase.

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.

Kunce, C.M.; R.N. Trelease and D.C. Doman (1984), Planta 161: 156-164. Ontogency of glyoxysomes in maturing and germinated cotton seeds. A morphometric analysis.

Lal, R. and G.S. Taylor (1970), Soil Sci. Soc. Amm. Proc. 34: 245-248. Drainage and nutrient effects in a field lysimeter study. II. Mineral uptake by corn.

Langridge, J. (1963), Ann. Rev. Plant Physiol. 24: 445-466. Biochemical aspects of temperature response.

Lauchi, A. and E. Epstein (1970), Plant Physiol. 45: 639-641. Transport of potassium and rubidium in plant roots. The significance of calcium.

Leffler, H.M. (1981), Developmental aspect of cotton seed planting quality. pp. 283-286. Proc. Beltwide Cotton Prod. Res. Conf. New Orleans, La.

Leffler, H.R. and R.D. Williams (1983), Crop Sci. 23: 161-165. Seed density classification influences germination and seedling growth of cotton.

Lee, D.K. and J.C. Lee (1991), J. Korean Soc. For Hort. Sci. 32: 97-101. Studies of flooding tolerance and its physiological aspects in fruit trees. II. Physiological changes associated with flooding.

Leigh, R.A. and R. Storey (1991), Nutrient compartmentation in cells and its relevance to the nutrition of the whole plant. In, Plant Growth: Interactions with nutrition and environment, (ed. J.R. Porter and D.W. Lawlor), pp. 33-54. Society for Experimental Biology, Cambridge Univ. Press.

Lem, N.W.; M. Khan; G.R. Watson and J.P. Williams (1980), J. Exp. Botany 31: 289-298. The effect of light intensity, day length and temperature on fatty acid synthesis and desaturation of *Vicia faba* L.

Letey, J.; O.R. Lunt ; L.H. Stolzy and T.E. Szuszkiewicz (1961), Soil Sci. Soc. Am. Soc. 25: 183-186. Plant growth, water use and nutritional response to rhizosphere differentials of oxygen concentration.

Letey, J.; L.H. Stolzy and C.B. Blank (1962), Agron. J. 54: 34-36. Effect of duration and timing of low oxygen content on shoot and root growth.

Levitt, J. (1980), Chilling, freezing, and high temperature stresses. In, Responses of Plants to Environmental Stresses, 2nd. ed., Vol. I. Academic Press New York.

Leyshon, A.J. and R.W. Sheard (1974), Can. J. Soil Sci. 54: 463-473. Influence of short term flooding on the growth and plant nutrient composition of barley.

Liljenberg, C. and M. Kates (1982), An effect of water stress on lipid composition of oat seedling root cell membranes. In, Develpments 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 Biomedical Press B.V.

Lin, C.Y.; J.K. Roberts and J.L. Key (1984), Plant Physiol. 74: 152-160. Acquisition of thermotolerance in soybean seedlings synthesis and accumulation of heat-shock proteins and their cellular localizations.

Lin, S.S. (1990), Revista-Brasilevia-de-Fisiologia Vegetal. 2: 1-6. Changes in electrolyte leaching, germination and vigour of bean seeds under high relative humidity and high temperature. (Cited CD-ROM 1990-91).

Lindquest, S. (1986), Ann. Rev. Biochem. 55: 1151-1191. The heat shock response.

Lopez-Saez, J.F.; F. Gonzalez; A. Gonzalez-Fernadez and G. Garcia-Ferrero (1969), Protoplasma 67: 213-221. Effect of temperature and oxygen tension on root growth, cell cycle and cell elongation.

Lord, J.M.; T. Kagawa; T.S. Moore and H. Beevers (1973), J. Cell Biol. 57: 659-667. Endoplasmic reticulum as the site of lecithin formation in castor bean endosperm.

Lyons, J.M. (1973), Ann. Rev. Plant Physiol. 24: 445-466. Chilling injury in plants.

Lyons, J.M.; T.A. Wheaton and K. Pratt (1964), Plant Physiol. 39: 262-268. Relationship between the physical nature of the mitochondrial membranes and chilling sensitivity in plants.

MacCarthy, J.J. and P.K. stumpf (1980), Planta 147: 389-395. The effect of different temperatures on fatty acid synthesis and polyunsaturation in cell suspension cultures.

MacMurray, T.A. and W.R. Morrison (1970), J. Sci. Food Agri. 21: 520-528. Composition of wheat flour lipids.

Marschner, H. (1986), Mineral nutrition in higher plants. Acad Press London.

Marschner, H. (1991), Plant-soil relationships: acquisition of mineral nutrients by roots from soils. In, Plant Growth: Interactions with nutrition and environment, (ed. J.R. Porter and D.W. Lawlor), pp. 125-156. Society for Experimental Biology, Cambridge Univ. Press.

Marshall, M.O. and M. Kates (1973), FEBS Letts. 31: 199-203. Biosynthesis of phosphatidyl ethanolamine and phosphatidyl choline in spinach leaves.

Marshall, M.O. and M. Kates (1974), Can. J. Biochem. 52: 469-482. Biosynthesis of nitrogen phospholipids in spinach leaves.

Matsuzaki, T.; S. Iwai and A. Koiwai (1988), Agri. Biol. Chem. 52: 1283-1285. Effects of temperature on seed fatty acid composition in ovary culture of tobacco.

Mazliak, P. (1977), Glyco-and phospholipids of biomembranes in higher plants. In, Lipids and Lipid Polymers in Higher Plants, (ed. M. Tevini and H.K. Lichtenthaler). Springer-Verlag, Berlin.

Mazliak, P. (1980), Prog. in Phytochem. 6: 49-102. Synthesis and turnover of plant membrane phospholipids.

Mazliak, P.; A. Jolliot and C. Bonnerot (1982), Biosynthesis and metabolism of phospholipids. In, Develpments in Plant Biology, Biochemistry and metabolism of plant lipids, (ed. J.F.G.M. Wintermans and P.J.C. Kuiper), Vol. 8, pp. 89-98. Elsevier Biomedical Press Amsterdam.

McDonnell, E.M. (1980), A study of the glycolipids in germinating wheat. Ph.D. Thesis, Univ. of Wales.

Mead, J.F. (1976), In, Free Radicals in Biology, (ed. Pryor) Vol. 1, p. 51. Acad. Press New York.

Meek, B.D.; E.C. Owen-Bartlett; L.H. Stolzy and C.K. Labunuskas (1980), Soil Sci. Soc. Am. J. 44: 301-05. Cotton yields and nutrient uptake in relation to water table depth.

Meek, B.D. and L.H. Stolzy (1978), Short-term flooding. In, Plant Life in Anaerobic Environments, (ed. D.D. Hook and R.M.M. Crawford). Ann. Arbor, Sci. Publ. Ann. Arbor, Michigan.

Mengal, D.B. and S.A. Barber (1974), Agron. J. 66: 341-344. Development and distribution of the corn root system under field conditions.

Metzler, D.E. (1977), Biochemistry, Acad. Press New York.

Meyer, W.S.; D.C. Reicosky; H.D. Barrs and R.C.G. Smith (1987), Plant and Soil 102: 161-170. Physiological responses of cotton to a single waterlogging at high and low N-levels.

Milborrow, B.V. (1974), Ann. Rev. Plant Physiol. 25: 259-307. The chemistry and physiology of ABA.

Mirbahar, R.B. (1981), Studies on the metabolism of membrane lipids in germinating wheat. Ph.D. Thesis, Univ. of Wales.

Mirza, M.A. and A.L. Shaikh (1983), The Pak. Cottons 28: 117-125. In-ovulo embryo culture of some incompatible species hybrids of *Gossypium*.

Mishra, P.K. and G.S. Singhal (1992), Plant Physiol. 98: 1-6. Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids.

Misra, S.; A. Choudhury; Pal, P.K. and G. Amitabha (1986), Phytochem. 25: 1083-1087. *Effect on the leaf lipids of three species of* mangrove of periodic submergence in tidal water.

Mohanty, B.; P.M. Wilson and T.A. Rees (1993), Phytochem. 34: 75-82. Effects of anoxia on growth and carbohydrate metabolism in suspension cultures of soybean and rice.

Moldau, H. (1973), Photosynthetica 7: 1-7. Effects of various water regimes on stomatal and mesophyll conductance of bean leaves.

Moore, T.S. (1976), Plant Physiol. 57: 383-386. Phosphatidyl choline synthesis in castor bean endosperm.

Moore, T.S.; M.J. Price-Jones and J.L. Harwood (1983), Phytochem. 22: 2421-2425. The effect of indoleacetic acid on phospholipid metabolism in pea stems.

Morisset, C. (1973), C.R. Hebd. Seances Acad. Ser. D276: 311-314. Compartment des raeines isolees de *Lycopersicon esculentum* (Solanacees) cultivees *in vitro* et soumises al anoxie. In, Flooding and Plant Growth, (ed. T.T. Kozlowski), pp. 64. Acad. Press.

Morisset, C. (1978), Structural and cytoenzymological aspects of mitochondria in excised roots of oxygen-deprived *Lycopersicon* cultivated in vitro. In, Plant Life in Anaerobic Environments, (ed. D.D. Hook and R.M.M. Crawford), pp. 497-537. Ann. Arbor, Michigan.

Morris, D.A. (1979), Planta 146: 603-606. Effect of temperature on the velocity of exogenous auxin transport in intact chilling-sensitive and chilling resistant plants.

Morrison, W.R. and L.M. Smith (1964), J. Lipid Res. 4: 600-608. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with BF_3-MeOH.

Mudd, J.B. (1980), Phospholipid biosynthesis. In, The Biochemistry of Plants, (ed. P.K. Stumpf), Vol. 4, pp. 249-282. Acad. Press New York.

Mukherjee, I; D. Drakeford and D.M. Reid (1986), Physiol. Plant. 67: 55-60. ATPase activity of sunflower root membranes as affected by flooding.

Muller, M. and K.A. Santarius (1978), Plant Physiol. 62: 326-329. Changes in chloroplast membrane lipids during adaptation of barley to extreme salinity.

Murata, N.; J.H. Troughton and D.C. Fork (1975), Ibid 56: 508-517. Relationship between the transition of the physical phase of membrane lipids and photosynthetic parameters in *Anacystis nidulans* and lettuce and spinach chloroplasts.

Murtadha, H.M.; J.M. Maranville; R.B. Clark and M.D. Clegg (1989), J. Plant Nutrition 12: 535-545. Effects of temperature and relative humidity on growth and calcium uptake, translocation and accumulation in sorghum.

Murtazi, F.F. and N.A. Partnova (1971), Fiziol. Vodoobmena Ustoich. Rast, No. 2: 152-188. Fractional changes of the total level of nucleic acid as a index of the functional state of wheat seed germs during the action of high temperature. (Cited Chem. Abst. 77, 31636).

Natr, L. and J. Purs (1971), Sci. Agri. Biochemoslov 3: 259-271. Effect of deficits in mineral nutrients at different root temperature. (Cited Chem. Abst. 79, 65099).

Nightingale, G.T. (1935), Botanical Gazette 96: 581-639. Effects of temperature on growth, anatomy and metabolism of apple and peach roots.

Niki, T; S. Yoshida and A. Sakai (1978), Plant Cell Physiol. 19: 139-148. Studies on chilling injury in plant cells. I. Ultrastructural changes associated with chilling injury in callus tissues of *Cornus stolonifera*.

Nikulina, G.N. (1985), Fiziol. Biokhim. Kul't Rast. 17: 131-134. The effect of high temperature on respiration and ATP content in pea roots. (Cited Biol. Abst. 80, 99249).

Nishiyama, I. (1975), Plant Cell Physiol. 16: 533-536. A break on the Arrhenius plot of germination activity in rice seeds.

Obendrof, R.L. and P.R. Hobbs (1970), Crop Sci. 10: 536-566. Effect of seed germination on temperature sensitivity during imbibition of soybean.

Oliveira, L. (1977), Protoplasma 91: 267-280. Changes in ultrastructure of mitochondria roots of *Triticale* subjected to anaerobiosis.

Ong, C.K. and C.K. Baker (1985), Temperature and leaf growth. In, Control of Leaf Growth, (ed. N.R. Baker; W.J. Davies and C.K. Ong), pp. 175-200, Seminar Series, Society for Experimental Biology No. 27 Cambridge. Cambridge Univ. Press.

Opik, H. (1965), J. Exp. Botany 49: 667-682. Respiration rate, mitochondrial activity and mitochondrial structure in the cotyledons of *Phaseolus vulgaris* L. during germination.

Orchard, P.W. and H.B. So (1985), Plant and Soil 88: 407-419. The response of sorghum and sunflower to short-term flooding.

Orchard, P.W.; H.B. So and R.S. Jessop (1985), Plant and Soil 88: 421-430. The response of sorghum and sunflower to short-term waterlogging. III. Root growth effects.

Ordin, L.; C. Itai; A. Benzioni; C. Musolan and J.A. Kindinger (1974), Plant Physiol. 53: 118-121. Effects of heat-shock on plant growth and on lipid and ß-glucan synthesis.

Pace, B. and L.L. Campbell (1967), Proc. Natl. Acad. Sci. USA. 57: 1110-1116. Correlation of maximum growth temperatures and ribosome heat stability.

Pagano, S. N. and K. Arens (1977), Phyton. Rev. Int. Bot. Exp. 35: 123-126. Effect of temperature on potassium leaching in leaflets of *Phaseolus vulgaris* L.

Parsons, D.S. (1975), Biological Membranes. Oxford Univ. Press.

Patterson, D.T.; M.T. Highsmith and E.P. Flint (1988), Weed Sci. 36: 751-757. Effects of temperature and CO_2 concentration on the growth of cotton (*G. hirsutum*), spurred anoda, a valvet leaf.

Peacock, H.A. and B.S. Hawkins (1970), Crop Sci. 10: 667-670. Effect of seed source on seedling vigor, yield and lint characteristics of upland cotton *Gossypium hirsutum* L.

Peacocke, A.R. and I.O. Walker (1962), J. Mol. Biol. 5: 560-563. The thermal denaturation of sodium deoxyribonuclease.

Pearce, R.C. and J.H.M. Williams (1985), Planta 163: 304-316. A freeze-etch study of the effects of extracellular freezing on cellular membranes of wheat.

Pearce, R.S. and J.M. Abdel Samad (1980), J. Exp. Botany 31: 1283-1290. Changes in fatty acid content of polar lipids during ageing of seeds of peanut (*Arachis hypogea* L.).

Pearson, R.W.; L.F. Ratliff and H.M. Taylor (1970), Agron. J. 62: 243-246. Effect of soil temperature, strength and pH on cotton seedling root elongation.

Pham-Thi, A.T. and J. Viera-da-Silva (1975), C.R. Academic Sci. Paris 280: 2857-2860. (Cited Pham-Thi *et al.* 1982).

Pham-Thi, A.T. and J. Viera-da-Silva (1980), Z. Pflanzenphysiol. 100: 351-358. (Cited Pham-Thi *et al.*, 1982).

Pham-Thi, A.T.; C. Flood and J.V.D. Silva (1982), Effects of water stress on lipid and fatty acid composition of cotton leaves. In, Developments in Plant Biology, Biochemistry and metabolism of plant lipids, (ed. J.F.G.M. Wintermans and P.J.C. Kuiper), Vol. 8, pp. 451-454. Elssevier Biomedical Press, B.V.

Pham-Thi, A.T.; C. Borrel-Flood; J. Vierira-da-Silva; A.M. Justin and P. Mazliak (1987), Physiol. Plant. 69: 147-150. Effects of drought on (1-^{14}C) oleic acid and (I-^{14}C) linoleic acid desaturation in cotton leaves.

Pollock, C.J. (1990), J. Agri. Sci. Cambridge 115: 1-5. The response of plants to temperature change.

Ponnamperuma, F.N. (1972), Adv. Agron. 24: 29-96. The chemistry of submerged soils.

Powell, A.A. and S. Matthews (1981), Ann. Botany 47: 709-712. Associated phospholipid changes with early stages of seed ageing.

Priestly, P.A. and A.C. Leopold (1979), Plant Physiol. 63: 726-729. Absence of lipid oxidation during accelerated ageing of soybean seeds.

Quarles, R.H. and R.M.C. Dawson (1969), Biochem. J. 112: 787-794. The distribution of phospholipase D in developing and mature plants.

Quinn, P.J. (1988), Effects of temperature on cell membrane. In, Plants and Temperature, (ed. S.P. Long and F.I. Woodward). Sym. Soc. Exp. Biol. 42: 237-258. Cambridge Co. of Biologists.

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

Quinn, P.J. and W.P. Williams (1985), In, Photosynthetic Mechanisms and the Environments, (ed. J. Barber and N.R. Baker), pp. 1-47. Elsevier Sci. Publ. B.V. Amsterdam.

Railton, I.D. and D.M. Reid (1973), Planta 111: 261-266. Effects of benzyladenine on the growth of waterlogged tomato plants.

Rains, D.W. (1976), Mineral nutrition. In, Plant Biochemistry, (ed. J. Bonner and J.E. Varner), pp. 561-597. Acad. Press New York.

Raison, J.K.; J.A. Berrg; P.A. Armond and T.K. Pike (1980), Membrane properties in relation to the adaptation of plants to high and low temperature stress. In, Adaptation of Plants to Water and High Temperature Stress, (ed. N.C. Turner and P.J. Kramer), pp. 261-273. Willey Interscience New York.

Raison, J.K.; J.K.M. Roberts and J.A. Berry (1983), Biochim. Biophys. Acta 688: 218-228. Acclimation of higher plant *Nerium oleander* to growth temperature: Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids.

Rajana, B. (1972), Heat damage to cotton seed and its effects on plant growth and development. Ph.D. Thesis, Univ. of Missipissi, U.S.A.

Rakhimov, M.M.; M.M. Abdullaeva; M.N. Balikhanov and M.U. Babaev (1986), Translated from Fiziologiya Rastenni 33: 1121-1129. Role of phytase and phospholipase in changes of phospholipids during germination of cotton seeds.

Raymond, P.; A. Al-Ani and A. Pradet (1985), Plant Physiol. 79: 879-884. ATP production by respiration and fermentation and energy change during anaerobiosis in twelve fatty and starchy germination seeds.

Rebeille, F.; R. Bligny and R. Douce (1980), Biochim. Biophys. Acta 620: 1-9. Role of L'oxygene et de la temperature sur la composition en acides gras des cellules isolees D'erable (*Acer Pseudoplatanus* L.).

Reicosky, D.C.; W.S. Meyer; N.L. Schaefer and R.D. Sides (1985), Agri. Water. Manag. 10: 127-143. Cotton response to short term waterlogging, imposed with a water-table gradient facility.

Reid, D.M. and A. Crozier (1971), J. Exp. Botany 22: 39-48. Effects of waterlogging on the gibberellin content and growth of tomato plants.

Rich, S.(1964), A. Rev. Phytopathology 2: 253. Ozone damage to plants.

Rivera, C.M. and D. Penner (1978), Phytochem. 17: 1269-1272. Rapid changes in soybean root membrane lipids with altered temperatures.

Roberts, E.H. (1988), Temperature and seed germination. In, Plants and Temperature, (ed. S.P. Long and F.I. Woodward), Sym. Soc. Exp. Biol. 42: 109-133. Cambridge Co. of Biologists.

Roberts, T.K.M.; J. Callis; O. Jardetsky; V. Walbot and M. Freeling (1984a), Proc. Natl. Acad. Sci. U.S.A. 81: 3379-3383. Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia.

Roberts, T.K.M.; J. Callis; O. Jardetsky; V. Walbot and M. Freeling (1984b), Proc. Natl. Acad. Sci. U.S.A. 81: 6029-6033. Cytoplasmic acidosis as a determinant of flooding intolerance in plants.

Robinson, G.W. (1949), Soils: Their origin, constitution and classification. (3rd. edition). Thomas Murby and Co. London.

Rodionov, V.S. and L.S. Zakharova (1978), Sov. Plant Physiol. 25: 141-144. Rate of endogenous composition of phospholipids in potato leaves as a result of frost influence.

Rumpho, M.E. and R.A. Kennedy (1981), Plant Physiol. 68: 165-168. Anaerobic metabolism in germinating seeds of *Echinochloa crus-galli* (barnyard grass). Metabolite and enzyme studies.

Russel, E.W. (1973), Soil Conditions and Plant Growth, 10th ed. Longman London.

Roughan, P.G. and Batt, R.D. (1969), Phytochem. 8: 363-369. The glycerolipid composition of leaves.

Rouser, G.; A.N. Siakotos and S. Fleischer (1966), Lipids 1: 85-86. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots.

Rouser, G.; G. Kritchevsky and A.Yamamoto (1967), In, Lipid Chromatographic Analysis, (ed. G.V. Marinetti), Vol. III, pp. 713. M. Dekker Inc. New York.

Ryan, C.A. (1973), Ann. Rev. Plant Physiol. 24: 173-196. Proteolytic enzymes and their inhibitors.

Sachs, M.M. and T.H.D. Ho (1986), Ann. Rev. Plant Physiol. 37: 363-376. Alteration of gene expression during environmental stress on plants.

Sachs, M.M.; M. Freeling and R. Oktimoto (1980), Cell 20: 761-767. The anaerobic proteins of maize.

Safaralieva, R.A.; S.I. Alekperova and R.M. Mekhtizade (1980), Deposited Doc. VINITI 5439-5480. Changes in growth regulator activity during the process of wheat seed germination under constant and variable temperature conditions. (Cited Chem. Abst. 96, 17325).

Saglio, P.H.; P. Raymond and A. Pradet (1980), Plant Physiol. 66: 1053-1057. Metabolic activity and energy charge of excised maize root tips under anoxia.

Salam, A. and H.D. Scott (1987), Soil Sci. 144: 61-66. Effects of prolonged flooding on soybeans during early vegetative growth.

Salim, M. and R.C. Saxena (1991), Crop Sci. 31: 1620-1625. Temperature stress and varietal resistance in rice. Effects on white backed planthopper.

Salisbury, F.B. and C.W. Ross (1991), Plant Physiology. Acad. Press London.

Santarius, K.A. (1980), Physiol. Plant. 49: 1-6. Membrane lipids in heat injury of spinach chloroplasts.

Sastry, P.S. and M. Kates (1965), Can. J. Biochem. 43: 1445-1453. Biosynthesis of lipids in plants.

Sattelmacher, B.; H. Marschner and R. Kuhne. (1990), J. Agri. and Crop Sci. 165: 131-137. Effects of root zone temperature on root activity of two potato (*Solanum tuberosum* L.) clones with different adaptation to high temperature.

Schlesinger, M.J.; M. Ashburner and A. Tissieres (1980), Heat shock from bacteria to man. Cold Spring Harbor New York.

Semikhatova, O.A. and G.N. Nikulina (1987), Fizio. Biokhim. Kul't. Rast. 19: 467-472. The effect and after effect of high temperature on oxygen uptake by leaves. (Cited Chem. Abst. 108, 19282).

Sena-Gomes, A.R. and T.T. Kozlowski (1980a), Can. J. For. Res. 10: 308-311. Responses of *Pinus halepenis* seedlings to flooding.

Sena-Gomes, A.R. and T.T. Kozlowski (1980b), Plant Physiol. 66: 267-271. Growth responses and adaptation of *Fraxinus pennsylvanica* seedlings to flooding.

Sethar, M.A. (1993), A physiological study of heat stress in cotton seedlings. Ph.D. Thesis, Univ. of Wales.

Sij, J.W. and C.A. Swanson (1973), Plant Physiol. 51: 368-371. Effect of petiole anoxia on phloem transport in squash.

Simolenka, G. and P.J.C. Kuiper (1977), Physiol. Plant. 41: 29-35. Effect of low temperature upon lipid and fatty acid composition of roots and leaves of winter rape plants.

Simon, E.M. (1974), New Phytol. 73: 377-420. Phospholipids and plant membrane permeability.

Simon, E.M.; A. Minchin; M.M. Mcmenamin and J.M. Smith (1976), New Phytol. 77: 301-311. The low temperature limit for seed germination.

Sisson, W.B. and M.M. Caldwell (1977), J. Exp. Botany 2: 692-705. Atmospheric ozone depletion: Reduction of photosynthesis and growth of a sensitive higher plant exposed to enhanced UV-B radiation.

Sivakumar, M.V.K.; H.M. Taylor and R.H. Shaw (1977), Agron. J. 69: 670-673. Top and root relations of field grown soybeans.

Skogqvist, I. (1974), Exp. Cell Res. 86: 285-294. Induction of heat sensitivity of wheat roots and its effects on mitochondria, adenosine triphosphate, triglyceride and total lipid content.

Slack, C.R. and R.G. Roughan (1978), Biochem. J. 170: 437-440. Rapid temperature changes in the fatty acid composition of certain lipids in developing linseed and soybean cotyledons.

Slack, P.T.; M. Black and J.M. Chapman (1977), J. Exp. Botany 28: 569-577. The control of lipid mobilization in *Cucumis* cotyledons.

Smillie, R. M. (1979), Aust. J. Plant Physiol. 6: 121-134. Coloured components of chloroplast membranes as intrinsic membrane probes for monitoring the development of heat injury in intact tissues.

Sojka, R.E. and L.H. Stolzy (1980), Soil Sci. 130: 350-358. Soil oxygen effects on stomatal response.

Soomro, A.W. and S.A. Waring (1987), Aust. J. Agri. Sci. 38: 91-99. Effect of temporary flooding on cotton growth and nitrogen nutrition in soils with different organic matter levels.

Sparace, S.A. and T.S. Moore (1979), Plant Physiol. 63: 963-972. Phospholipid metabolism in plant mitochondria.

Speer, M.; J.E. Schmidt and Kaiser, W.M. (1988), Effects of water stress on photosynthesis and related processes in plant membranes, structure, assembly and function (ed. J.L. Harwood and T.T. Walton) pp. 209-221. The Biochemical Society London.

St. Angelo, A.J. and A.M. Altschul (1964), Plant Physiol. 39: 880-883. Lipolysis and free fatty acid pool in seedlings.

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

Stassart, J.M. (1992), Physiol. Plant. 85 (part 2): A78. Changes in metabolite partitioning in barley leaves due to salt stress conditions.

Steward, R.R. and J.D. Bewley (1980), Plant Physiol. 65: 245-248. Lipid peroxidation associated with accelerated ageing of soybean axes.

Stolzy, L.H.; J. Letey; T.E. Szuszkiewicz and O.R. Lunt (1961), Soil Sci. Soc. Am. Proc. 25: 463-467. Root growth and diffusion rates as functions of oxygen concentrations.

Stumpf, P.K. (1975), In, Plant Biochemistry, (ed. J. Bonner and J.E. Varner), pp. 427-461. Acad. Press London.

Stumpf, P.K. (1980), Biosynthesis of saturated and unsaturated fatty acids. In, The Biochemistry of Plants, (ed. P.K. Stumpf), Vol. 4, pp. 177-204. Acad. Press London.

Tackett, J.L. and R.W. Pearson (1964), Soil Sci. Soc. Am. Proc. 28: 600-605. Oxygen requirement of cotton seedlings roots for penetration of compacted soil cores.

Tang, Z.C. and T.T. Kozlowski (1982a), Plant and Soil 66: 243-255. Physiological, morphological and growth responses *Platanus occidentalis* seedlings to flooding.

Tang, Z.C. and T.T. Kozlowski (1982b), Can. J. For. Res. 12: 196-202. Some physiological and morphological responses of *Quercus macrocarpa* seedlings to flooding.

Tappel, A.L. (1973), Fed. Proc. Fed. Am. Soc. Exp. Biol. 32: 1870-1874. Lipid peroxidation damage to cell components.

Tevini, M. and H.K. Lichtenthaler (1977), Lipids and lipid polymers in higher plants. Springer- Verlag, Berlin.

Tharp, W.H. (1960), U.S. Agri. Dept. hand book, 178: 17.

Thebud, R. and S.A. Santarius (1982), Plant Physiol. 70: 200-205. Effects of high temperature stress on various biomembranes of leaf cells *In situ* and *In vitro*.

Thomas, H. (1983), Physiol. Plant. 57: 509-513. Analysis of the response of leaf extension to chilling temperatures in *Lolium temulentum* seedlings.

Thompson, G.A. Jr. (1979), In, Low Temperature Stress in Crop Plants, The role of membrane, (ed. J.M. Lyons; D. Graham and J.K. Raison), pp. 343-363. Acad. Press New york.

Thompson, L.W. and S. Zalik (1973), Plant Physiol. 52: 268-273. Lipids in rye seedlings in relation to vernalization.

Thomson, W.W.; W.M. Dugger and R.L. Palmer (1966), Can. J. Botany 44: 1677-1682. Effects of ozone on the fine structure of the palisade parenchyma cells of bean leaves.

Tikhonov, A.N. and E.K. Ruuge (1978), Mol. Biol. (Mosc.) 12: 1028-1036. ESR Study of electron transport in photosynthetic systems. VII. Effects of temperature on the process of electron transport between 2 photosystems and on the structural state of chloroplast membrane.

Tischner, R.; K.P. Heise; R. Nelle and H. Lorenzen (1978), Planta 139: 29-33. Changes in pigment content, lipid pattern and ultrastructure of synchronous Chlorella after heat and cold shocks.

Trelease, R.N. (1984), Ann. Rev. Plant Physiol. 35: 321-347. Biogenesis of glyoxysomes.

Tremolieres, H.; A. Tremolieres and P. Mazliak (1978), Phytochem. 17: 685-687. Effects of light and temperature on fatty acid desaturation during the maturation of rapeseed.

Trought, M.C.T. and M.C. Drew (1980a), Plant and Soil 54: 77-94. The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.) Shoot and root growth in relation to changes in the concentrations of dissolved gases and solutes in the soil solution.

Trought, M.C.T and M.C. Drew (1980b), Plant and Soil 56: 187-199. The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.). II Accumulation and redistribution of nutrients by the shoot.

Trought, M.C.T. and M.C. Drew (1980c), J. Exp. Botany 31: 1573-1588. The development of waterlogging damage in young wheat plants in anaerobic solution culture.

Tsuji, H. (1972), Bot. Mag. 85: 207. Respiratory activity in rice seedling germinated under strictly anaerobic conditions.

Tsuji, H. (1973), Environ. Control Biol. 11: 79. Growth and metabolism in plants under anaerobic conditions.

Turner, F.T.; J.W. Sij; G.N. mcCauley and C.C. chen (1983), Crop Sci. 23: 40-44. Soybean seedling response to anaerobiosis.

Ueda, K. and H. Tsuji (1971), Protoplasma 2: 203-215. Ultrastructural changes of organelles in coleoptile cells during anaerobic germination of rice seeds.

Vakharia, D.N. (1986), Biochemical aspects of membrane formation in wheat aleurone tissue. Ph.D. Thesis, Univ. of Wales.

Van-Hasselt, P.R. (1972), Acta Bot. Neerl. 21: 539-548. Photooxidation of leaf pigments in *Cucumis* leaf discs during chilling.

Van Waalwijkn van Doorn, J.J.L. (1982), J. Am. Oil Chem. Soc. 59: 588A-597A. U.S. sun oil exports expected to rise.

Vantoi, T.T.; N.R. Fausey and M.B. McDonald Jr. (1987), Plant and Soil 102: 33-39. Anaerobic metabolism enzymes as markers of flooding stress in maize seeds.

Vartapetian, B.B.; I.N. Andreeva and I.P. Maslova (1971), Dokl. Akad. Nawk. SSSR, Ser. Biol. 196: 1231-1233. In, The Biochemistry of Plants, Vol. 2, (ed. P.K. Stumpf and E.E. Conn). Acad. Press.

Vartapetian, B.B.; I.P. Maslova; I.N. Andreeva and G.I. Kozlova (1973), Fiziol. Rast. 20: 1279. Cytochromes of mitochondria of rice coleoptiles grown under conditions of strict anoxia. Cited Plant Life in Anaerobic Environments, (ed. D. Hook and R.M.M. Crawford). Ann. Arbor, Sci., Michigan.

Vartapetian, B.B.; A.I. Maslova and I.N. Andreeva (1975), Plant Sci. Lett. 4: 1-8. Cytochromes and respiratory activity of mitochondria in anaerobically grown coleoptoles.

Vartapetian, B.B.; I.N. Andreeva; G.I. Kozlova and L.P. Agapova (1977), Protoplasma 91: 243-256. Mitochondrial ultrastructure in roots of mesophytes and hydrophytes at anoxia and after glucose feeding.

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

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

Vartapetian, B.B.; I.N. Andreeva and N.N. Nutridinov (1978c), Plant cells under oxygen stress. In, Plant Life in Anaerobic Environments, (ed. D. Hook and R.M.M. Crawford), pp. 13-88. Ann. Arbor, Sci., Michigan.

Varty, K. (1975), Aspects of nucleic acid and phospholipid metabolism in germinating wheat. Ph.D. Thesis, Univ. of Wales.

Wanjura, D.F. and D.R. Buxton (1972), Agron. J. 64: 431-434. Hypocotyl and radicle elongation of cotton as affected by soil environment.

Weiss (1980), Cited Membrane lipids in heat injury of spinach chloroplasts, Santarius, K.A., 1980.

Wignarajah, K and H. Greenway (1976), New Phytol. 77: 575-584. Effect of anaerobiosis on activities of alcohol dehydrogenase and pyruvate decarboxylase in roots of *zea mays*.

Willemot, C. (1975), Plant Physiol. 55: 356-359. Stimulation of phospholipid biosynthesis during frost hardening of winter wheat.

Willemot, C. and W.G. Boll (1967), Can. J. Botany 45: 1863-1876. Incorporation of serine-^{14}C and ethanolamine-^{14}C into nitrogen containing phosphatides and effects of medium containing ethanolamine on phosphatide biosynthesis in excised tomato roots.

Williams, J.P.; M.U. Khan; K. Mitchell and G. Johnson (1988), Plant Physiol. 87: 904-910. The effect of temperature on the level and biosynthesis of unsaturated fatty acids in diacylglycerols of *Brassica napus* leaves.

Williamson, R.E. (1968), Agron. J. 62: 80-83. Effect of soil gas composition and flooding on growth of *Nicotiana tobacum*.

Wilson, J.M. and R.M.M. Crawford (1974), J. Exp. Botany 25: 121-131. Leaf fatty-acid content in relation to hardening and chilling injury.

Wintermans, J.F.G.M. (1960), Biochim. Biophys. Acta 44: 49-54. Concentrations of phosphatides and glycolipids in leaves and chloroplasts.

Wintermans, J.F.G.M. (1963), Coll. Int. Centre Nat. Res. Sci. 119: 381-385. Phosphatides and glycolipids of the photosynthetic apparatus.

Wolf, R.B.; J.F. Cavins; R. Kleiman and L.T. Black (1982), J. Am. Oil. Chem. Soc. 59: 230-232. Effects of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids and sugars.

Woodstock, L.M. and R.B. Taylorson (1981), Physiol. Plant. 53: 263-268. Soaking injury and its reversal with polyethylene glycol in relation to respiratory metabolism in high and low vigour soybean seeds.

Woodstock, L.M.; K. Furman and H.R. Leffler (1985), Crop Sci. 25: 459-466. Relationship between weathering deterioration and germination, respiratory metabolism and mineral leaching from cotton seeds.

Young. O.A. and J.W. Anderson (1974), Biochem. J. 137: 423-433. Properties and substrate specificity of some reactions catalysed by a short-chain fatty-acyl synthetase from seeds of *Pinus radiata*.

Yarden, R. and A.F. Mayer (1981), Phytochem. 20: 2669-2674. Phospholipid metabolism in cotyledons of wild and cultivated peas.

Yatsu, L.Y. (1965), J. Cell Biol. 25: 193-198. The ultrastructure of cotyledonary tissue from *G.hirsutum* L. seeds.

Yoshiyuki, N.; K. Tanaka; E. Ohto and M. Sakata (1990), Plant Cell Physiol. 31: 815-821. Protective effect of external Ca^{+2} on elongation and intercellular concentration of K^{+} in intact mung bean roots under high NaCl stress.

Yuldashev, P.Kh. and M.M. Rakhimov (1970), Khim. Prir. Soedin., No. 6, 782. Lypolytic enzymes in germinating cotton seeds. (Cited Rakhimov, 1986).

Zaerr, J.B. (1983), Fore. Sci. 29: 71-78. Short-term flooding and net photosynthesis in seedlings of three conifers.

Zainon, A.R. (1993), A study of cell elongation and peroxidase activity in cotton seedling roots. M.Sc. Thesis, Univ. of Wales.

Zarrouk, M. and A. Cherif (1981), Z. Pflanzenphysiol. 105: 85-92. Cited Zarrouk and Cherif, 1984.

Zarrouk, M. and A. Cherif (1984), Effect of sodium chloride on chloroplast lipid composition of olive tree leaves (*Olea europea* L.). In, Developments in Plant Biology, Structure, function and metabolism of plant lipids (ed. P.A. Siegenthaler and W. Eichenberger), Vol. 9, pp. 595-599. Elsevier, Amsterdam.

Zvareva, E.G. and B.I. Bartkov (1976), Fiziol. Biokhim. Kult. Rast. 8: 204-208. Distribution of assimilates in soybeans during fruit growth in flooded soil. (Cited Flooding and plant growth, ed. T.T. Kozlowski, 1984).

Zwiazek, J.J.; C. Petrovic and E. Sailerova (1992), Physiol. Plant. 85 (part 2): A73. Effects of triadimefon and osmotic stress on water relation, plasma membrane composition and ATPase activity in white spruce (*Picea Glauca*) needles.