GENOTYPIC DIFFERENCES AND WATER STRESS-INDUCED CHANGES IN LIPIDS OF MAIZE HYBRIDS

by

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(ABSTRACT)

Eighteen-day-old seedlings of five maize hybrids (A619xH60, B73xMO17, B73xPA91, B73xVA17 and A632xH96) were grown hydroponically and compared for inherent differences in lipid concentration and composition. These seedlings were also evaluated for their ability to tolerate mild osmotic stresses (-0.4 and -0.6 MPa, polyethylene glycol-induced), osmotically induced changes in lipid composition, and differences in membrane stability as measured by electrolyte leakage.

Inherent differences among the hybrids included reduced dry matter accumulation, and lower total lipid and free fatty acid concentration in the leaves and roots of B73xVA17 and A632xH96 compared to A619xH60, B73xMO17, and B73xPA91. No differences were apparent in the free, glycosidic or esterified steryl fractions among hybrids. Distributional patterns of fatty acid and sterol composition differed among tissues but were similar in all hybrids.

Osmotic treatments of -0.4 and -0.6 MPa resulted in significant reductions in the dry weight of B73xVA17 and

A632xH96. Total lipid concentration increased significantly in the roots of all hybrids while there was a general trend for moderate increases in stems and leaves. Few, mostly insignificant differences, were observed in the free fatty acid, free sterol, steryl glycoside and steryl ester fractions. However, the stigmasterol to sitosterol ratio increased in all three steryl fractions in the roots of B73xVA17 and A632xH96 as a result of the osmotic treatments. B73xVA17 and A632xH96 also exhibited the greatest electrolyte leakage when leaf discs were subjected to osmotic stress.

B73xVA17 and A632xH96 appear to be less tolerant to osmotic stress than the other hybrids. This may be due to their comparatively earlier developmental growth stage at the onset of osmotic stress. Modification of lipids in these sensitive hybrids may reflect an initial stabilization of cellular membranes which, in turn, may have some adaptive value in terms of drought tolerance.

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Chapter I

INTRODUCTION

Environmental stresses, such as water stress, reduce growth and harvestable yields of agricultural crop species. Water is often the major limiting factor in crop productivity. Severe drought may result in major economic losses and is exemplified by the 1983 United States drought in which crop losses amounted to $10 billion dollars (Le Rudulier et al. 1984). Transcient periods of drought also contribute to low agricultural productivity. In the State of Virginia, recent rainfall patterns are more critical, than the stored moisture in shallow soils, in determining maize growth and yield (McClane 1986). Thus, periods of prolonged and brief water-deficit stress may limit the growth potential of crop species such as maize.

Drought tolerance mechanisms include plant characteristics that impart resistance to water stress. These plant characteristics include a large root system or increased root to shoot ratio, small cell size, thickened leaf cuticle, changes in leaf angle and movements, stomatal frequency and behavior, and osmotic adjustment (Parsons 1979). Some of these adaptations enhance the drought tolerance of plants which allows survival under water-limiting conditions. Attention has focused on the

role of osmotic adjustment, which is the active accumulation of solutes in the cell cytoplasm, that allows the maintenance of turgor and growth. However, the possible adaptive role of osmotic adjustment is uncertain because differences in drought resistance were not correlated with osmotic adjustment of barley (Hanson et al. 1977), sorghum (Jones and Turner 1978) and other crop species. Recent interest in drought tolerance mechanisms has shifted to the possible role of lipid metabolism under water-deficit stress.

Lipid constituents of cellular membranes can be modified in plants exposed to water-deficit stress. Changes in membrane lipids may influence the permeability and fluidity of the membrane which, in turn, affects the activity of membrane-bound enzymes. These changes in the bulk lipid environment or microenvironment of specific enzymes may alter important physiological processes such as photosynthesis and respiration. Furthermore, the extent of these dynamic changes in lipid concentration and composition may depend on the intensity and duration of water stress. Thus, complex responses of plant lipid metabolism to water stress may involve a number of integrated changes in cellular membranes. Genotypic differences in lipid content, and stress-induced changes may be an important facet of drought tolerance in some species.

The purpose of the present study was to develop a better understanding of the impact of mild water stress on the lipids of maize hybrids grown in nutrient solution. The first objective was to compare the lipid concentration and composition among five maize hybrids to determine if genotypic differences exist in specific lipid classes (free fatty acids, free sterols, steryl glycosides, and steryl esters). The second objective was to consider the impact of PEG-induced osmotic stresses (-0.4 and -0.6 MPa) on the same lipid classes of these five maize hybrids. The investigation of water stress-induced changes in lipid metabolism may contribute to an overall understanding of plant responses to water stress.

Chapter II

LITERATURE REVIEW

Biomembranes in Higher Plants

Membranes of plant cells are highly specialized structures that function in numerous essential cellular processes. Plant membranes such as those of the nucleus, chloroplast, mitochondria, endoplasmic reticulum, Golgi apparatus, and the tonoplast and plasma membranes provide an environment for such diverse metabolic functions as charge separation, light-trapping and synthesis of proteins and lipids (Robertson 1983). The membrane also serves to compartmentalize cellular metabolic events by functioning as a selective barrier to the diffusion of metabolites (Sitte 1977). Therefore, plant cell membranes have many important functions relative to the interaction between the cell and its surroundings.

Lipid constituents of membranes

Lipids are constituents of biomembranes and contribute to the molecular organization of the membrane. The lipids of plant cell membranes comprise four major types; phospholipids, glycolipids, sterols, and neutral lipids (free fatty acids, triglycerides, and hydrocarbons; Sitte

1977). The polar lipids, phospholipids and glycolipids, predominate in membranes and are structurally derived from a glycerol derivative as are triglycerides (Mazliak 1977). The fatty acid substituents of these acyl lipids and the free fatty acids are denoted by the number of carbon atoms and double bonds. Saturated fatty acids in higher plant cells include lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0), while unsaturated fatty acids (those containing double bonds) include oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3).

Phospholipids are the major polar lipids in non-chloroplastic membranes and are structurally derived from a glycerol derivative (Harwood 1980). The head groups of phospholipids are highly polar moieties that are ester linked to phosphoric acid at the terminal hydroxyl group of the glycerol backbone. Fatty acids are ester linked to the two remaining hydroxyl groups of glycerol and one fatty acid chain is usually saturated while the other is unsaturated. Phospholipid species, differentiated by the polar head groups of choline, ethanolamine, inositol or serine, may dominate in specific organelles of plant cells. Chloroplasts contain phosphatidlycholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE) with PE localized in the envelope and PC found in both the envelope and thylakoid membrane systems (Goodwin and

Mercer 1983). The phospholipid species present in the mitochondria are mainly PC and PE with smaller amounts of PI. Diphosphatidylglycerol (PG) is also present but only in the mitochondrial inner membrane.

Glycolipids are the major polar lipids in the chloroplasts of leaves but are also present in trace amounts in a wide variety of plant tissues (Douce and Joyard 1980). Monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG) and sulfoquinovosyldigalactosyldiglyceride or sulfolipids (SGDG), each contain two fatty acid substituents. The ratio of MGDG to DGDG in the chloroplast envelope is about 1:1, whereas, in the thylakoid membranes this ratio is 2:1 (Goodwin and Mercer 1983). The fatty acids of MGDG and DGDG are highly unsaturated and usually contain high concentrations of linolenic acid (18:3).

The structure of mono-, di- and triglycerides is similar to phospholipids and glycolipids in that fatty acids are esterified to a glycerol backbone. Both the number of carbons and degree of saturation of these fatty acid substituents determines whether triglycerides occur as fats or oils (Goodwin and Mercer 1983). Apparently there is a highly specific positional placement of saturated and unsaturated fatty acids on the glycerol backbone in seeds and tissues of higher plants (Weber 1983). Thus, the degree of saturation of triglycerides, as well as other

acyl lipids, is a non-random process in higher plant cells. Triglycerides mainly occur in the cytoplasm of cells as lipid droplets which may contain small amounts of sterols, phospholipids and hydrocarbons (Trelease 1969). These lipid droplets serve as storage forms of lipid and often disappear during periods of high metabolic activity and reappear during lowered metabolic activity or environmentally-induced stress (Douglas and Paleg 1981).

Sterols are important constituents of plant cell membranes and occur not only as free sterols but also as steryl glycosides, acylated steryl glycosides and steryl esters. As a general rule, all three sterol forms are found in the microsomal fraction which is composed of membranes from the endoplasmic reticulum, Golgi vesicles, and the plasmalemma (Goodwin and Mercer 1983). Free sterols are planar, heterocyclic structures that are characterized by a side chain attached at C-17 and a hydroxyl group attached at the 3-B ring position of ring A of the molecule (Grunwald 1974). The major free sterols in higher plants are cholesterol, campesterol, stigmasterol and sitosterol. They differ in the presence or absence of a methyl or ethyl group attached to C-23 of the side chain. Cholesterol has no substituents at this position while campesterol has a methyl group and stigmasterol and sitosterol each have an ethyl group at C-23. Stigmasterol also contains a double bond at C-22 which distinguishes it

from other free sterols. The free sterols are localized primarily in the microsomal fraction and mitochondria (Goodwin and Mercer 1983).

Sterols occur in the free form and modified as glycosides, acylated glycosides and esters. Steryl glycosides are sterols modified by addition of a glycosidic residue to the hydroxyl position of the ring structure. Sitosterol is the preferred sterol for glycosylation although stigmasterol, campesterol and cholesterol are also found glycosylated with various sugars. The physiological role of this sterol form is controversial, although recent evidence indicates the steryl glycosides may be present in membranes in addition to their role as transport forms of sterols. Acylated steryl glycosides are sterols modified by the addition of an acylated glycoside to the hydroxyl position. Steryl esters are sterols modified by the addition of a fatty acid in an ester linkage at the reactive hydroxyl site. Steryl esters are present in both nuclear and mitochondrial membranes.

Molecular organization of the membrane

The molecular organization of the membrane is partly defined by the amphipathic (polar and nonpolar) character of the phospholipids. The hydrophilic head group of the molecule is oriented to the exterior and the hydrophobic fatty acid chains to the interior of the membrane

(Robertson 1983). The degree of unsaturation of the fatty acid chains, which restricts motion of the acyl chains, also determines membrane structure. Highly unsaturated acyl chains occupy a greater cross-sectional area which increases the fluidity of the membrane.

Associated with the phospholipids are sterols which intercalate with the fatty acid chains of the phospholipid molecules. The molecular organization of free sterols in the membrane is determined by the C_3-hydroxyl group, which interacts by ion dipole and H-bonding, with the nitrogenous base moiety of the phospholipid (McKersie and Thompson 1979). Other structural requirements for sterol insertion in the membrane are that the sterol have a flat configuration, with at least one double bond in the B-ring, and a C-17 side chain without bulky substituents (Grossman et al. 1985).

The relative ability of free sterols to physically interact with fatty acid chains of phospholipids depends upon the nature of side chain saturation and substitution. Side chains substituted with a methyl or ethyl group alter the planar configuration of the sterol molecule and may sterically hinder sterol incorporation into the membrane (Grunwald 1968, 1974). Cholesterol and campesterol are the least highly substituted and have a more planar ring structure than stigmasterol and sitosterol which are more highly substituted and have a less planar ring

configuration. Exogenously applied cholesterol and campesterol were more effective in reducing the leakage of electrolytes from barley roots (Grunwald 1971) and in protecting membranes from ethanol-induced leakage in red beet tissue (Grunwald 1968) and barley roots (Grunwald 1974) than more highly substituted sterols such as stigmasterol and sitosterol.

Free sterols rather than steryl glycosides or steryl esters were more effective in regulating the ionic permeability of plant cell membranes (Grunwald 1971). Therefore, sterols differ in their ability to penetrate the phospholipids and interact with them by means of ion-dipole or hydrogen bonding (Grossman et al. 1985).

Fluidity and permeability considerations

The fluidity of the membrane is affected by the degree of unsaturation and chain length of fatty acids of acyl lipids (Chapman 1975), concentration and composition of sterols in the phospholipid bilayer (Ford and Barber 1983), and interaction between the phospholipid head groups (Robertson 1983). This, in turn, affects the permeability of the lipid bilayer. The general rule is that the more fluid a membrane is the more permeable it is.

Membrane fluidity is of significance not only in terms of permeability to water and other small molecules (Simon 1974), but also with respect to the structural and

enzymatic proteins that are interspersed in the lipid matrix. Proteins may be membrane-bound to varying degrees as extrinsic proteins that are weakly bound to the membrane surface or as intrinsic proteins that partially or totally span the lipid bilayer. Some intrinsic proteins, such as plastoquinone and ubiquinone, are highly diffusible in the lipophilic region of the bilayer and may transverse the bilayer depending on the oxidation state of prosthetic groups. Lipid concentration and composition may affect the membrane fluidity, which in turn, affects the conformational state as well as activity of enzymes such as ATPases (Cocucci et al. 1981, Douglas and Walker 1984, Parks 1984).

The sidedness of many membrane activities may be accounted for by the structural and functional asymmetry of the lipid to protein ratio. Membrane asymmetry is further evidenced in the heterogenous distribution of lipid molecules which coexist with highly specialized domains of lipids in the vicinity of proteins (Parks 1984). Membrane fluidity and permeability of the bulk phase of the lipid matrix may differ from these lipid microenvironments. Restoration of the lipid microenvironment of proteins associated with the membrane may be a crucial facet of adaptation to environmentally-induced stress.

Dynamic state of membrane lipids

Membrane lipids may diffuse laterally in the bilayer or exhibit what has been termed flip-flop movement in which the lipid relocates 180 degrees, from its original position, to the opposite side of the membrane (Parks 1984). Lipids containing hydrophilic head groups may totally leave the bilayer by moving from the membrane into the cytosol (Cossins 1980).

Sterols may also be differentially partitioned among intracellular membranes based on the relative affinity of membranes to specific sterols (Wattenberg and Silbert 1983). The mechanisms for this type of transfer has been studied most extensively for cholesterol of mammalian cells in which cholesterol is rapidly transferred between membranes (Bell 1975). This transfer probably involves the partitioning of cholesterol, to the greatest extent, in membranes that possess the highest affinity for the sterol (Wattenberg and Silbert 1983). Presumably, the distribution of sterols of higher plant cells is also directed at the cellular level by the sterols and phospholipids characteristic of specific membranes. Thus, regulation of sterol incorportion into membranes, may allow for optimization of membrane fluidity and functionality (Ford and Barber 1983).

Lipid metabolism may vary depending on the environment of the cell and plant (Kuiper 1985). The lipid

constituents of membranes may be modified, in both concentration and composition, by changes in the environment. In this respect, membranes are dynamic structures, in which components are synthesized, degraded and partitioned into various intracellular membranes (Robertson 1983, Wattenberg and Silbert 1983). This biosynthetic adaptability may represent an elegant system by which the plant cell is able to buffer the impact of external changes.

Factors affecting lipid metabolism

The lipid metabolism of higher plants is affected by numerous factors which emphasizes the point that a dynamic state is characteristic of cellular membranes. Chemical probes that have served as useful tools in the study of lipid metabolism include anesthetics which alter the molecular configuration of membranes (Jackson and St. John 1984), plant growth hormones (Wood and Paleg 1972), plant growth retardants (Henry 1985), and herbicides (Lehoczki et al. 1985). The role of plant host sterols in the resistance or susceptiblity to pathogenic agents, such as *Phythiaceous* fungi, has also been investigated (Moore and Orcutt 1982). The chemical and biotic factors mentioned above, while clearly important factors affecting lipid metabolism, will not be considered further in this review. Of primary interest are changes in lipid metabolism

associated with abiotic, environmentally-induced stresses.

Lipid metabolism is affected by many environmental factors including light, low temperature, salinity and water-deficit stress (Levitt 1980). The diversity of lipid molecules found in higher plant cells suggest the evolution of complex responses to environmental signals. These responses may have various outcomes for higher plants. Environmental changes in lipid metabolism may be interpreted as variations that confer some adaptive advantage that allows survival in a particular environmental niche. Other changes in lipid metabolism may represent consequential or degradative events without a significant role in adaptation to the environment (Kuiper 1985). In this review, abiotic environmental stresses are considered separately to identify the relative contribution of each stress factor to the integrated response of plants to the environment.

Light stress

Light induces ultrastructural changes in plant cellular membranes at both high and low light intensities. Therefore, light sensitivity of higher plants may involve two types of metabolic response. A reduction in light intensity has been correlated with an increase in the degree of thylakoid stacking of the chloroplast (Tevini 1977) which increases the relative amount of granal to

stromal lamellae. This ultrastructural change involves breakdown and reassembly of membrane systems and their lipid components.

The greening of etiolated tissue has been associated with changes in sterol composition in some plant systems. Etiolated barley shoots contained 38% more total sterols than light-grown shoots primarily due to an increase in the free sterols (Bush et al. 1971). Etiolated tissue was characterized by higher levels of sitosterol in all steryl fractions except the steryl esters. During greening of barley shoots there was a light-stimulated shift from sitosterol to stigmasterol. Another study showed that etiolated corn seedlings contained 60% higher concentrations of total sterols than seedlings exposed to light (Hirayama and Nushida 1968). In contrast, no significant changes occurred in unsaturated free sterols during greening of etiolated pea seedlings (Gaunt and Stowe 1967).

A similar shift in sterol composition was observed when wild potato plants were transferred form long-day to short-day conditions (Bae and Mercer 1970). However, in this case the decline in sitosterol and cycloartenol was associated with an increase in cholesterol concentration. Sterol concentrations returned to initial levels after 2-3 weeks which may suggest the need for a specific light regime during tuber formation. Changing the photoperiod

from long-day to short-day conditions (8 hrs) also increased the concentration of stigmasterol relative to sitosterol in tobacco, possibly due to an decrease in the synthesis of sitosterol (Grunwald 1978).

Low light resulted in an increase in free sterols in the diatom, *Nitzschia closterium*, while total sterols remained the same (Orcutt and Patterson 1974). However, shading nearly 90% of the incident light of field grown tobacco did not significantly alter the free sterol concentration (Grunwald 1978).

Low temperature stress

Cold hardening involves the gradual transition from a freeze-sensitive to freeze-tolerant state in plants exposed to acclimating conditions (Li 1985). Acclimation of plants to lowered temperature requires changes in membrane lipids to allow optimal fluidity of functionally important membranes (Chapman et al. 1983). Membrane fluidity is maintained by increasing both the unsaturation of acyl lipids (Smolenska and Kuiper 1977), as well as the relative amounts of the less-planar sterols such as stigmasterol and sitosterol (Quinn and Williams 1978). These adjustments in lipid composition are particularly important since the temperature at which phase transition occurs is correlated

with the activity of many membrane-bound enzymes (Lyons and Raison 1970).

Low temperature often induces changes in the total free sterols and in the relative proportions of individual sterol components. The total free sterol levels decreased in leaves of winter rape plants that were fully hardened (Sikorska and Farkas 1982). The decrease in sitosterol was associated with an increase in the level of cholesterol in these leaves. There was also a reduction in total free sterol levels in the shoots of winter wheat seedlings grown at 1 C but not at 10 C. (Davis and Finkner 1972). In contrast, the free sterol concentration of the roots of wheat seedlings increased at both temperatures due to greater proportions of campesterol and sitosterol. An increase in sitosterol is characteristic of low temperature adapted roots and may contribute to greater membrane fluidity and therefore, enhanced uptake of water and ions at low temperature (Kuiper 1980).

Specific changes in the fatty acid composition of the phospholipids and glycolipids has been correlated with chilling resistance. An increase in the degree of unsaturation of phospholipids, as a result of increased linolenic acid synthesis, has been observed in the leaves and roots of rape plants (Smolenska and Kuiper 1977). Linolenic acid concentrations were also elevated in young apple leaves but were reduced in older apple leaves exposed

to low temperatures (Ketchie and Kuiper 1979). The most pronounced modification in the PG of chloroplast thylakoids of chilling resistant plants is an increase in unsaturation due to an increase in the relative amount of 18:3, 18:2, and 18:1. This increase in unsaturation was evident in PG of thylakoid membranes from leaves of winter and summer grown pea plants (Chapman et al. 1983). The linolenic acid content was greater in the winter than summer grown leaves. This is significant with respect to chilling resistance because PG may be preferentially associated with the light harvesting complex (LHC)-proteins which are thermally unstable (Armond et al. 1980). Thus, relatively small adjustments in thylakoid lipids could have an impact on the functional activity of these complexes.

Ultrastructural changes occur during cold acclimation that allow cells of cold tolerant plants to survive extracellular freezing at lower temperatues than cold-sensitive plants. Intramembrane particles (IMP) aggregate in the plasmalemma of freeze-tolerant but not freeze-sensitive potato cells for about 15 days after exposure to cold acclimating conditions (Li 1985). After 15 days these aggregates are redistributed. IMP aggregation is thought to represent partial lipid crystallization during the reversible induction of gel to liquid crystalline phase transition of the membrane. IMP probably consist of transcient clusters of small, densely-

packed, relatively ordered lipid that is dispersed in the fluid matrix (Cossins 1980). Aggregation of IMP occurs when the membrane is cooled towards the phase-tranistion temperature and may induce self-regulatory mechanisms that restore membrane fluidity in higher plants (Li 1985).

One of the major causes of injury in plants exposed to freezing temperature is the formation of intracellular ice which may rupture the cell (Levitt 1980). Cold tolerance is related to the removal of water from cells, which reduces cell size and causes dehydration, and results in extra- rather than intracellular ice formation. The permeability of cellular membranes to water may be expected to partially determine the freezing tolerance of plants. Modification of the sterol:phospholipid ratio affects water efflux (Demel and De Kruyff 1972) which may be a factor in the avoidance of intracellular freezing and tolerance to low temperature stress, as well as stabilization of the membrane upon rehydration after freezing (Morris and McGrath 1981).

Sudden exposure of acclimated plants to freezing temperatures often induces changes that may differ from those observed during cold hardening. For example, the gradual acclimation of winter rape plants to cold temperatures results in a decrease in total free sterols of shoot, an increase of sitosterol in the roots, and an increase in both the level and unsaturation of leaf

phospholipids (Sikorska and Farkas 1982). However, when frost hardened seedlings were abruptly exposed to freezing temperatures there was a dramatic increase in both free sterol and the sterol to phospholipid ratio. This increase in sterol was due to an increase in the proportion of campesterol relative to sitosterol. A reduction in phospholipid content of leaves was previously observed in winter rape (Sikorska and Kacperska-Palacz 1979) and winter wheat cultivars (De La Roche 1973). Therefore, the different modifications of membrane lipids that occur during cold hardening and exposure to freezing temperature may indicate that several types of adjustments in membrane lipids may be essential for survival of these two temperature stresses.

Salinity stress

Salinity stress includes exposure of plant cells to high concentrations of ions, such as Na^+ and Cl^-, in addition to a lowered osmotic potential of the growth medium. Higher plants that are resistant (halophytic) compared to those sensitive (glycophytic) to salt stress may exhibit several adaptive mechanisms including Cl^- exclusion by restricted uptake by roots and/or tranport to shoots, salt

accumulation in vacuoles of leaves, or salt excretion which prevents accumulation in the leaves (Levitt 1980). In some cases, these adaptive mechanisms have been correlated with salt-induced changes in lipid components of cellular membranes. In other cases, inherent differences in lipid composition of plants may play a greater role in salt tolerance.

Genotypic differences in lipid composition may contribute to salt resistance in several species and varieties. In grape varieties that differ in salt tolerance, the free sterol concentration was highest in the most tolerant rootstock (Kuiper 1968). Cl^- accumulation in the leaves was correlated directly with the level of MGDG and inversely with the content of lignoceric acid (24:0) in the two phospholipids, PC and PE. Comparative determinations among plant species that differ in salt sensitivity indicate that the free sterols plus steryl esters and sulfolipid contents were higher in sugar beet (halophyte) than barley (less halophytic) and bean (glycophyte; Stuiver et al. 1978). Sugar beet roots also contained higher concentrations of long chain fatty acids (> 20:0) and greater amounts of linoleic acid than linolenic acid in their total fatty acids compared to salt-sensitive plants. These inherent differences in lipid content may be involved in the relative salt resistance of these varieties and species.

Salt-induced changes in lipid composition may also contribute to salt resistance in higher plants by affecting the passive movement of ions through root cell membranes. The concentration of free sterols and steryl esters in plant tissue may be affected by exposure to salinity stress. The level of free sterols was maintained in the roots of the halophytic *Plantago maritima* and *P. coronopus* but decreased in the salt-sensitive *P. media* (Erdei et al. 1980) during salinity stress. The effect of salinity on two sugar beet inbred lines was for a general increase in the free sterols plus steryl esters in the roots of both the salt-tolerant (FIA) and salt-sensitive (ADA) lines (Stuiver et al. 1981). Differences in free sterol content were also demonstrated in citrus rootstocks differing in their salt-exclusion capacity (Douglas and Walker 1983). The level of esterified sterols but not free sterols, decreased in the salt-sensitive rootstocks, Kharna katta (*Citrus kharna* Raf.) and Etrog citron (*Citrus medica* L.) This same pattern was not observed in the salt-tolerant rootstock, Rangpur lime (*Citrus reticula'a* Blanco var. *austera*). Instead there was an increase in the total free and esterified sterol fraction which may restrict the passive movement of ions through root cell membranes.

An increase in the ratio of stigmasterol to sitosterol with increasing salinity also affects ion permeability and, therefore, exclusion of Cl^- ions. The ratio of

stigmasterol to sitosterol increased in all three citrus rootstocks exposed to salt treatment (Douglas and Walker 1983). Furthermore, an increase in the ratio (cholesterol + campesterol)/ (stigmasterol +sitosterol) correlated well with salt exclusion capacity in these citrus rootstocks. A similar trend was observed in the most salt tolerant *Plantago* species, *P. maritima*, in which salt stress resulted in a decrease in sitosterol relative to cholesterol (Erdei et al. 1980). This change would also tend to shift membrane lipid composition towards free sterol components that are more effective in restricting passive ionic permeability in salt tolerant plants.

The concentration of polar lipids is generally maintained or increased in salt-tolerant plants exposed to salinity stress. Salt treatment resulted in an increase in phopholipid levels in the roots of the salt-tolerant sugar beet line FIA (Stuiver et al. 1981), as well as barley roots (Ferguson and Simon 1966), and halophytic *Plantago* species (Erdei et al 1980). The glycolipid level declined in the chloroplasts of alfalfa (Harzallah-Skhiri et al. 1980) as well as shoot of the salt-sensitive sugar beet line ADA (Stuiver et al. 1981), and in roots of *Plantago media* (Kuiper and Kuiper 1978) following salt treatment. In contrast, the glycolipid level of shoots either remained the same or increased in halophytic species and varieties.

Associated with an elevated total concentration of

polar lipids are changes in the fatty acid components. The individual fatty acid components of MGDG and DGDG, increased in unsaturation as a result of salinity stress. Linolenic acid is the predominate fatty acid of glycolipids and changes relative to linoleic acid under salinity stress. The ratio of linoleic to linolenic acid decreased in the shoots of salt-sensitive sugar beet line ADA (Stuiver et al. 1981) and *Plantago media* (Kuiper and Kuiper 1978) which suggests that a higher degree of unsaturation of the chloroplast glycolipids may be required for salt resistance.

Another aspect of salt tolerance involves an active regulatory mechanism. Active transport by ion-specific ATPases, regulates Cl- exclusion from root cells (Douglas and Walker 1984) as well as Na+ accumulation in vacuoles of leaves (Stuiver et al. 1981) Membrane-bound enzymes, like ATPase, are affected by membrane fluidity which, in turn, influences the conformational state and activation energy of the enzyme. Therefore, lipid-induced changes in membrane fluidity and permeability may affect the bulk lipid phase as well as the microenvironment of membrane-bound ATPases. For example, the phospholipid/sterol ratio of three citrus rootstocks was inversely related to the activation energy of the ATPase of the plasma membrane (Douglas and Walker 1984). The plasma membrane ATPase activity of root cells was highest in the

citrus rootstock exhibiting the best Cl- exclusion capacity. These observations suggest that changes induced by free sterols affect membrane fluidity which is correlated with ATPase activity. Thus, the free sterols influence both passive and active mechanisms of Cl^- exclusion.

Other lipid classes may also affect the microenvironment and activity of ATPases. High sulfolipid concentrations have been observed in the roots of several salt-resistant species such as sugar beet (Stuiver et al. 1978) and *Plantago* species (Kuiper and Kuiper 1978). In sugar beet, a high sulfolipid concentration has been correlated with (Na^+, K^+)-stimulated ATPase activity which is involved in sodium transport from the roots. The sodium is translocated to the shoots at a similar rate and amount as potassium and accumulates in the vacuoles of leaf cells (Stuiver et al. 1981). Comparison of two sugar beet inbred lines that differed in K^+/Na^+ uptake selectivity, FIA (low) and ADA (high), showed that the sulfolipid content in the roots of FIA was maintained at higher salt concentrations but was reduced in ADA (Stuiver et al. 1981). Therefore, a high sulfolipid content in the roots of salt resistant plants may be essential for the functionality of (Na^+ and K^+)-stimulated ATPases which are characteristic for halophytes (Hansson and Kuiper 1973).

Salinity stress and water-deficit stress are somewhat

interrelated since a salt-resistant plant must tolerate both salt or ion stress as well as salt-induced osmotic stress. Salinity may increase the water stress resistance in both halophytes (Gates 1972) and glycophytes (Kirkmam et al. 1974) due to an decrease in water content. However, alteration of the water status of salt-stressed plant cells does not necessarily induce the same changes in membrane lipids that are observed for water stress.

Water-deficit stress

Water-deficit stress has a profound impact on developmental and biochemical processes in plant cells. Water stress may lead to a reduction in cell elongation, photosynthesis, and respiration, decreases in the content of chlorophyll, protein, and RNA, and increases the polyamine concentration of plant cells (Levitt 1980). Dehydration may also damage cellular membranes which, in turn, affects membrane-bound proteins involved in numerous metabolic reactions. Thus, water stress has a profound impact on cellular processes associated with membrane systems and might be expected to alter lipid metabolism. However, relatively few studies have considered the impact of water-deficit stress on lipid metabolism. The stability of the membrane system upon exposure to water stress as well as the restoration of function after rehydration may contribute to tolerance or susceptibility to drought (Stewart and Bewley 1982).

Under water stress, there is a decrease in the polar lipid content that is more pronounced in the glycolipid than phospholipid fraction. Polar lipids decreased in *Spirodela* (Lechevallier 1977), roots of oat seedlings (Liljenberg and Kates 1985), cotton leaves (Pham Thi et al. 1985) and chloroplasts (Ferrari-Iliou et al. 1984), as well as in wheat and barley chloroplasts (Chetal et al. 1981, Chetal et al. 1983).

Glycolipid content of chloroplasts decreased in wheat and barley plants exposed to water stress during tillering, ear emergence and grain filling stages (Chetal et al. 1981) The levels of MGDG and DGDG decreased to a greater extent in the drought-sensitive than drought-tolerant cultivars of wheat and barley. Chloroplasts of the leaves of the drought-sensitive (Reba) cotton cultivar also exhibited a strong decrease in the DGDG content, whereas MGDG was less affected, when plants were stressed by witholding water (Ferrari-Iliou et al. 1984). The most striking change was the decrease in the percentage of the 18:3 component of DGDG, which is the major fatty acid component of the thylakoids. In addition, the stability of chloroplasts isolated from leaves during purification depended on the water stress level. The percentage of broken vs. intact chloroplasts increased at lower water potentials indicating a more fragile membrane system following water stress.

Phospholipid content decreased in cotton chloroplasts (Ferrari-Iliou et al. 1984) and leaves (Pham Thi et al. 1985) and in wheat and barley chloroplasts (Chetal et al. 1983) of plants exposed to water-deficit stress. Whole leaves of wheat and barley did not exhibit this same trend in that there was an increase, rather than a decrease, in the phospholipid content that was more pronounced in the drought-sensitive wheat and barley cultivars (Chetal et al. 1980). There was also a differential response of phospholipid components in some cases. For example, there was an increase in the PC content of chloroplasts (Chetal et al. 1981) and leaves (Chetal et al. 1980) for wheat and barley plants. At the same time, the amount of PG decreased, whereas, PI content did not change. Subsequent rewatering of water-stressed plants did not increase the phospholipids of chloroplasts to pre-stress levels, although recovery was greater in wheat than barley. In contrast, there was a decrease in PC in the leaves of drought-sensitive (Reba) and drought-tolerant (Mocosinho) cotton cultivars after exposure to water stress (Pham Thi et al. 1985). The incorporation of [1-^{14}C]acetate was markedly reduced in PC of water stressed cotton cultivars compared to controls.

PC is a precursor of glycinebetaine which is one of the betaines that accumulates in barley under water stress. Cotton leaves accumulate proline and amides rather than

betaines, hence the difference in lipid metabolism between these plants may reflect different mechanisms of drought resistance. Thus, the rate of turnover rather than the total content may be the most important factor in stress response (Giddings and Hanson 1982). PC is also a component of non-photosynthetic chloroplast membranes while PG is a major constituent of chloroplast membranes. Thus, PG may play a different role than PC during water stress. Possibly the glycerol backbone of PG serves as a biosynthetic pool of hexose precursors for photosynthesis. The decrease in PG, therefore, may indirectly contribute to the reduction in photosynthesis observed during water stress.

The degree of fatty acid unsaturation of glycolipids and phospholipids was also affected by water stress. An increase in the ratio of linoleic to linolenic acid was observed in the phospholipids and other acyl lipids of *Spirodela* (Lechevalier 1977). There was also a greater incorporation of [1-^{14}C]acetate into saturated relative to unsaturated fatty acids of glycolipids and phospholipids in cotton leaves (Pham Thi et al. 1985). This decrease in unsaturation was essentialy the the result of a reduction in linolenic acid synthesis. In other studies, no significant changes in the fatty acid composition were observed in either mosses following rapid or slow drying (Stewart and Bewley 1982) or in tissues of corn after

PEG-induced osmotic stress (Douglas and Paleg 1981). Rehydration of mosses, however, resulted in a transcient increase in unsaturated fatty acids which recovered to original levels in the desiccation-tolerant but not desiccation-sensitive moss.

Loss of unsaturated fatty acids from PC of the drought-tolerant moss did not correlate well with enhanced activity of degradative enzymes, or the incorporation of radiolabeled acetate and glycerol into the PC fraction, hence *de novo* synthesis probably does not play a role in recovery of unsaturated fatty acid content. Another explanation is that the reappearance of unsaturated acids is due to transacylation of fatty acids of the triglycerides found as oil droplets in the desiccation-tolerant moss. Lipid droplets also occur in the leaves (Trelease 1969) and roots (Nir et al. 1970) of corn plants exposed to water stress. These lipid bodies are composed mainly of triglycerides and small proportions of sterols, phospholipids and hydrocarbons (Trelease 1969). There was a 2-fold increase in triglycerides in the stems and leaves of corn plants that may have accumulated as lipid droplets in the cytoplasm when plants were water stressed (Douglas and Paleg 1981). Therefore, the transfer of lipids from cellular membranes to lipid droplets in the cytoplasm may occur during water stress. This process may be reversed upon release of water stress resulting in the

breakdown and utilization of lipid droplets for reassembly of membrane lipids.

Sterols are another lipid class modified by water stress. Sterol contents were altered in whole wheat seedlings (Biswas et al. 1983), and in specific tissues of oat (Liljenberg et al. 1985) and maize seedlings (Douglas and Paleg 1981). Both total free sterol and cholesterol concentrations increased in wheat seedlings exposed to PEG-induced osmotic stress (Biswas et al. 1983). Changes in free sterols were noted particuarly in the stems of maize seedlings exposed to PEG-induced osmotic stress at -1.5 MPa (Douglas and Paleg 1981). The trend was for an increase of individual sterol components resulting in a significantly higher total free sterol concentration in stressed seedlings. The most significant change was a nearly 2-fold increase of steryl esters in the stems of maize seedlings exposed to water stress. The fatty acids hydrolyzed from stem steryl esters were also significantly altered. Most noteworthy was the increase in 18:0 in the steryl esters of stems.

Sterols were also altered in the roots of oat seedlings that were exposed to two or four periods of water-deficit stress interspersed with rewatering periods (Liljenberg et al. 1985). The 4-desmethylsterols (free sterols) and the methylsterols (biosynthetic precursors of the former such as cycloartenol and cycloeuclenol) were identified in their

free, esterified and glycosylated forms following water stress. Two periods of water stress resulted in an insignificant decrease in the free, esterified and glycosylated 4-desmosterols or methylsterols of the roots. These findings are in agreement with previous results where only slight decreases in free sterol concentration were observed in the roots of water-stressed maize (Douglas and Paleg 1981) and soybeans (Grunwald 1978).

After four stress periods, the free sterol concentration increased by 25% while there was only a slight increase in the free methysterols. Slight changes were noted in the levels of glycosidic 4-desmethylsterols and methylsterols after four stress cylces. The glycosidic and acylated glycosides decreased 10% in the 4-desmethylsterols while the few methylsterols found in this form were unaffected by water stress. The most striking increase was in the esterified sterol and methylsterol fractions which increased 60% compared to controls. Although the most dramatic increase in esterified sterols of maize seedlings was in the stems rather than roots, in both cases this steryl fraction appears to be the most responsive to water stress.

The observed increase in steryl esters may be equivalent to triglyceride accumulation following water stress. Steryl esters are neutral lipids which do not compete for intracellular water and may possibly serve as

biosynthetic pools of sterol and fatty acid components for lipid synthesis upon recovery from water stress. In addition, possibly either a certain degree of water stress or an induction period is required for modification of sterol synthesis (Liljenberg et al. 1985). The imposition of water stress at -1.5 MPa for maize seedlings and in four stress periods for oat seedlings was sufficient to induce changes in the esterified steryl fractions in stems and roots, respectively.

Role of lipid metabolism in adaptation to water stress

An overall pattern of lipid metabolism emerges in which interconversions between lipids allows for the sequestering of lipids into neutral forms such as triglycerides and steryl esters, when lipid synthesis is curtailed and growth is limited (Parks 1984) by environmental stress. These extra-membrane lipid storage forms serve as a lipid reserve which may be utilized when growth is resumed upon release of stress. Triglycerides may be stored as oil droplets in the cytoplasm of cells and utilized as biosynthetic building blocks (glycerol, fatty acids) for synthesis of other lipid compounds upon release of stress. The adaptive significance of accumulation of triglyceride may be correlated with several properties of these molecules (Douglas and Paleg 1981). First, the triglyceride molecule represents a high energy storage form of greater calorific

value than proteins or carbohydrates. Secondly, the neutral, hydrophobic character of triglyceride molecules does not facilitate H-bonding to any extent so that intracellular water is available for the maintenance of protective hydration shells around proteins. Therefore, triglycerides represent a high energy, non-membrane storage form of lipid that does not compete with available water supplies.

Esterification of sterols may serve another function in terms of adjusting the lipid composition of membranes in response to a change in environmental conditions (Parks 1984). Sterols are partitioned into specific membrane structures depending on the composition of pre-existing membrane components (Wattenberg and Silbert 1983). Therefore, sterols may be adventitiously incorporated into membrane structures which are subsequently removed by the esterification process. The sterol and fatty acid components of steryl esters may also serve as a biosynthetic pool available for assembly into lipid structures upon release from stress.

Chapter III

Lipid Concentration and Composition of Five Maize Hybrids Grown in Nutrient Solution

Introduction

Lipids are important constituents of plant cell membranes and are involved in the regulation of membrane fluidity and permeability (Sitte 1977, Thompson 1983). Membrane lipid composition may influence permeability to water and small ions (Scarpa and De Grier 1971), fluidity and phase transition (Chapman 1975), and activity of membrane-bound enzymes (Robertson 1983) all of which may have pronounced influences on physiological processes in plants. Major lipid components that occur in plant cell membranes are phospholipids, galactolipids, free sterols and possibly steryl glycosides and esters (Kalinowska and Wojciechowski 1986). Phospholipids or galactolipids predominate in cellular membranes and are associated with sterols which intercalate with the fatty acid chains of the phospholipids (Mazliak 1977). Triglycerides, free fatty acids, and hydrocarbons are storage and membrane lipids that are also found in plant cells. The relative proportion of these lipid classes varies among representative plant parts, botanical levels of classification (species, genus, variety) and is moderated by the environment of the cell

and plant.

The distribution of lipids in higher plants may vary in representative tissues (Goodwin and Mercer 1983). For example, total free sterol concentrations were higher in roots than shoots for bean, barley and sugar beet (Stuiver et al. 1978). Tissue specificity in terms of lipid composition is also exhibited in other lipid classes. The fatty acids of phospholipids and triglycerides differed in composition between the leaf, stem and root tissues of 28-day-old maize seedlings (Douglas and Paleg 1981). These differences in the composition of lipid in the leaves, stems and roots may reflect differences in the physiological function of these tissues.

Genotypic differences in lipid composition have been reported in higher plants such as Mangrove species (Misra 1984), sugar beet inbred lines (Stuiver et al. 1981), citrus rootstocks (Douglas and Walker 1983), and most recently cotton varieties (Pham Thi et al. 1985). In some cases, such differences have been associated with the relative ability of varieties or species to maintain growth under limiting environments. Differences in lipid composition among hybrids of crop species could serve as selection criteria for evaluation of tolerance to environmentally-induced changes.

To date, comparative studies of lipid content of maize genotypes has focused on the fatty acid composition of oil

extracted from the kernal (Jellum 1966, Weber 1983). Comparison of the lipid content among maize hybrids has not been considered, and may be of importance in evaluation of the relative tolerance of hybrids to environmental stress.

The objectives of this study were to compare five maize hybrids quantitatively and qualitatively for several lipid classes (free sterols, steryl esters, steryl glycosides, free fatty acids) frequently cited as being important components of plant cell membranes. The distribution of these lipid components in the leaves, stems and roots of each hybrid was also of interest.

Abbreviations: Camp, campesterol; Chol, cholesterol; FFA, free fatty acid; FS, free sterol; R/S, root to shoot ratio; SE, steryl ester; SG, steryl glycoside; Sitos, sitosterol; Stig, stigmasterol; TL, total lipid.

Materials and Methods

Plant material

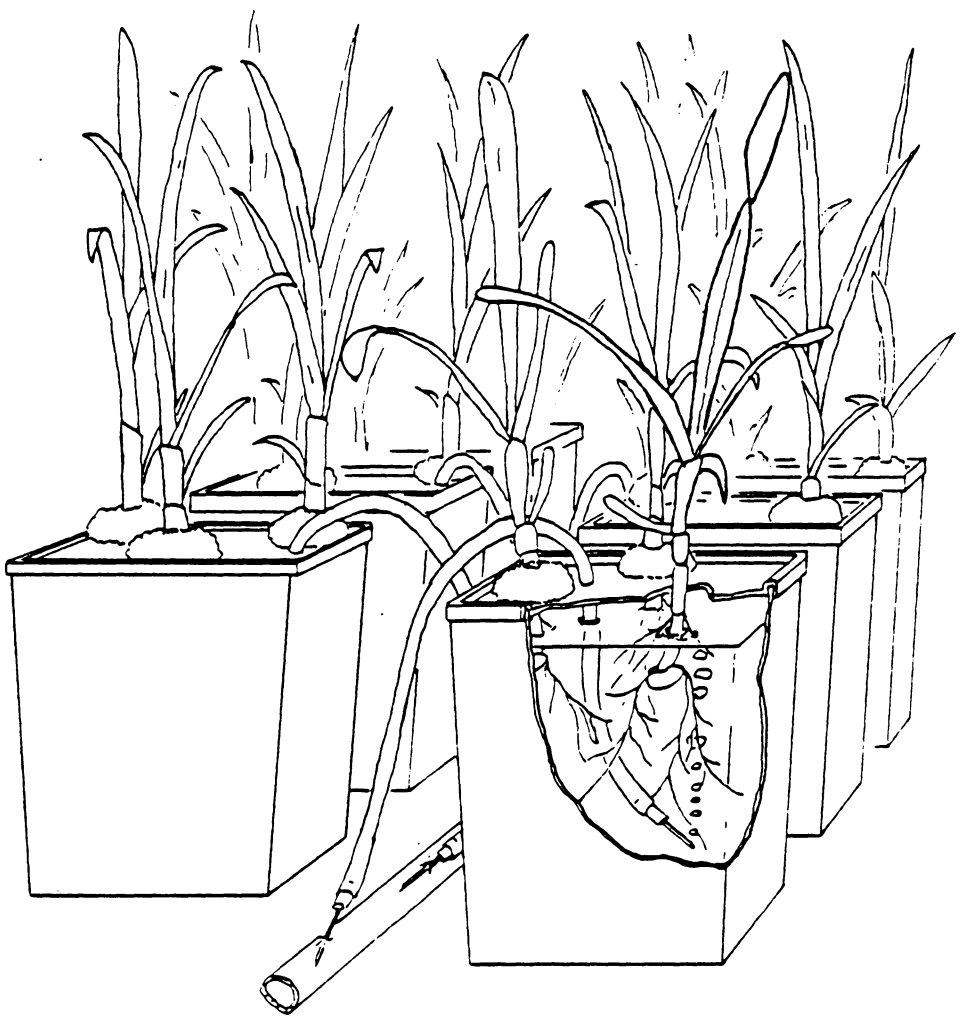
Maize seeds (*Zea mays* L. hybrids A619XH60, B73XVA17, B73XMO17, B73XPA91 and A632XH96) were germinated in vermiculite. At the two-leaf stage, three seedlings of each hybrid were transferred to each of three containers and grown hydroponically in 1 liter plastic containers

wrapped with aluminum foil (Fig. 3.1). Full strength Clark's nutrient solution (Clark 1982) was added to each container and replenished as necessary with distilled water. Aeration was provided using a hypodermic needle suspended in nutrient solution and connected by tubing to another hypodermic needle inserted in a central air hose. The plants were grown in a controlled environment room at 25 C under General Electric cool white fluorescent lamps (at a photosynthetically active radiation (PAR) of 150 μmol m^{-2} s^{-1}, continuous light) for 9 days prior to harvest. At the time of harvest the seedlings were 18-days-old. The experimental design was a completely randomized block with three replications of three plants each per treatment.

Harvest and lipid extraction

Maize seedlings were separated into leaf, stem and root tissues, placed in plastic bags and quick-frozen in dry ice and methanol. Tissue was freeze-dried, ground to pass a 40-mesh screen and stored in a dessicator at room temperature. An internal standard of n-heptadecanoic acid and cholestanol was added to freeze-dried tissue (200 mg) prior to extraction. Lipids were extracted from freeze-dried tissue (Nichols 1963) with isopropanol and filtered prior to refrigeration at 10 C for 18 h. Lipids were re-extracted from plant material with chloroform:isopropanol (1:1, v/v) for 18 h at 25 C

Figure 3.1. Diagram of hydroponic culture system used to grow maize seedlings in aerated Clark's nutrient solution.



utilizing a rotary shaker. The filtrates were combined, evaporated to dryness and the residue redissolved in chloroform:methanol (2:1, v/v). The extractant was washed using 88% KCl (Folch et al. 1957) and separated into two phases by centrifugation. The purified total lipid, collected from the lower phase, was stored in glass, teflon-capped, test tubes in chloroform under N_2 at 0 C.

Thin-layer chromatography of lipid extract

Lipids were separated into four classes using Silica gel G thin-layer chromatography (TLC) plates and a solvent system of n-hexane:diethyl ether:acetic acid (85:15:1, v/v/v). Lipid classes were viewed under a long wave UV lamp after spraying the TLC plates with 0.2% 2,7-dichlorofluorescein in 95% ethanol (Nichols 1964). Lipids corresponding to steryl esters (SE), free fatty acids (FFA), free sterols (FS) and steryl glycosides (SG) were identified by co-chromatography with known reference standards. All lipid classes, except SG, were eluted from the silica gel with 3 washings of diethyl ether through sintered glass funnels. SG were eluted with chloroform: methanol (2:1, v/v) followed by 3 washings with diethyl ether. Eluted fractions were stored in teflon-capped, glass test tubes under N_2 at 10 C.

Analysis of free fatty acids

FFA were methylated with 1.5 ml BCl_3-MeOH and heated for 5 minutes at 55 C in teflon-capped glass tubes. Fatty acid methyl esters were partitioned from the methanolic phase with hexane and analyzed using a gas liquid chromatograph (GLC, Bendix model 2600, Bendix Corp., Ronceverte, WV.) equipped with a flame ionization detector and a Model 3392A Hewlett-Packard integrator. Coiled glass columns (1m x 2mm i.d.) packed with 10% diethylene glycol succinate (DEGS) on 80/100 Chromosorb W AW were used for fatty acid separations. Nitrogen was used as a carrier gas and GLC operating conditions were: oven (150 C), detector (210 C) and inlet (190 C). Identification of plant fatty acids and sterols was by comparison of retention times with known standards.

Analysis of sterols

SE were saponified with 1 ml of 0.5N KOH in 90% methanol and heated for 2 hours at 65 C. An internal standard of cholestanol was added prior to heating. After heating, each sample was acidified using 6N HCl in 40% methanol, diluted with distilled water, and the sterols extracted into diethyl ether and rechromatographed. SG were hydrolyzed with 1 ml of 6N HCl in 40% methanol and heated for 4 hours at 65 C. An internal standard of β-cholestanol was added before heating. After heating the sterols were partitioned

with diethyl ether and rechromatographed using TLC as before.

FS, and sterols hydrolyzed from esters and glycosides, were converted to trimethylsilyl ether (TMS) derivatives with BSA [N,O-bis [trimethylsilyl)- acetamide] and heated for 1 hour at 55 C. TMS derivatives were analyzed by GLC using coiled, glass columns (2m x 2 mm i.d.) packed with 3% SE-30 on 80/100 Gas Chrom Q. Nitrogen was the carrier gas and GLC operating conditions were: oven (275 C), detector (315 C), and inlet (285 C).

Results

Dry weight analysis

Total dry weight differed significantly among the 18-day-old maize hybrids grown in Clark's nutrient solution (Tab. 3.1). Among the five maize hybrids, the dry weight of leaves from highest to lowest was as follows: A619xH60 > B73xMO17 > B73xPA91 > B73xVA17 > A632xH96. Stem and root dry weight did not vary significantly among hybrids.

The root/shoot ratio generally reflected the ranking of leaf dry weight in that the hybrid with the highest leaf dry weight (A619xH60) also had the lowest root/shoot ratio. The only exception to this trend was the root/shoot ratio

Table 3.1. Dry weight of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter in each column are not significantly different (P=0.05) by Duncan's Multiple Range Test.

Hybrid	Tissue DW (g/plant)				R/S
	Leaf	Stem	Root	Total	
A619xH60	1.14a	0.62a	0.91a	2.67a	0.52b
B73xMO17	0.91b	0.67a	0.88a	2.45ab	0.55b
B73xPA91	0.82bc	0.59a	0.87a	2.28b	0.61ab
B73xVA17	0.68cd	0.58a	0.93a	2.19bc	0.78a
A632xH96	0.57d	0.63a	0.71b	1.90c	0.59ab

of A632xH96. In this hybrid, the root dry weight was significantly lower than the other hybrids resulting in a lower root/shoot ratio.

Lipid classes

Total lipid (TL) concentration was highest in the leaves, compared to the stems and roots, of the the five maize hybrids (Tab. 3.2). In leaves and roots, the TL concentrations were similar in the hybrids, A619xH60, B73xMO17, and B73xPA91. The TL in the leaves of these three hybrids was nearly 2-fold greater than that observed for hybrids, B73xVA17 and A632xH96, which were similar to each other. A similar trend was observed in the roots except the magnitude of the differences among the hybrids was not as great. The only difference in the stem tissue was with A619xH60 which had significantly higher concentrations of TL than any of the other hybrids.

FFA concentration (Tab. 3.2) exhibited a similar pattern to that observed for TL, in that, concentrations were greatest in leaves followed by the stems and roots. The same hybrid groupings observed for TL can be made regarding the FFA concentration in leaves, stems and roots. Several significant differences were observed among hybrids in all tissues.

FS was present in a higher concentration than SG and SE in all three tissues (Tab. 3.2). The concentration of

Table 3.2. Lipid concentration of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter, in each column, within each tissue type, are not significantly different (P=0.05) by Duncan's Multiple Range Test.

Tissue	Hybrid	Lipid Class (mg/g DW)				
		TL	FFA	FS	SG	SE
Leaf	A619xH60	174.8a	13.5a	1.8a	0.25bc	0.039b
	B73XMO17	136.5b	11.4b	1.9a	0.45a	0.065a
	B73xPA91	147.5ab	13.6a	1.4a	0.35ab	0.036b
	B73xVA17	75.8c	7.2c	1.5a	0.45a	0.088a
	A632xH96	72.5c	7.3c	1.6a	0.36ab	0.086a
Stem	A619xH60	72.8a	6.8a	2.7a	0.79a	0.096a
	B73xMO17	46.2b	5.3b	2.4b	0.74a	0.046b
	B73xPA91	51.8ab	4.0c	2.0c	0.55b	0.047b
	B73xVA17	43.2b	3.2c	1.7c	0.56b	0.060ab
	A632xH96	41.5b	3.1c	2.3b	0.48b	0.087ab
Root	A619xH60	32.0ab	1.7a	2.2c	0.39b	0.308a
	B73xMO17	34.3a	1.6a	2.3c	0.36b	0.173b
	B73xPA91	32.0ab	1.8a	2.6bc	0.59a	0.196b
	B73xVA17	19.7c	0.7b	2.9b	0.36b	0.160b
	A632xH96	23.5bc	0.9b	3.3a	0.62a	0.223b

FS was similar in the stems and roots and higher than in the leaves. No significant differences were observed in the FS concentration in the leaves among any of the hybrids.

The concentration of SE was higher in the roots than the leaves or stems of all five hybrids (Tab. 3.2). SE concentrations were significantly lower in the leaves of A619xH60 and B73xPA91. In the stems, the SE concentration was highest in A619xH60, and lowest in B73xMO17 and B73xPA91. The roots of A619xH60 were significantly higher in SE compared to other hybrids.

Fatty acid components

Major free fatty acid components in the leaves, stems and roots of all corn hybrids were: lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3, Tab. 3.3). The FFA composition of maize hybrids differed between leaves, stems and roots with individual FFA components dominating in each tissue (Fig. 3.2). Generally, an unsaturated FFA (18:3 or 18:2) dominated in each tissue and a saturated acid (16:0) was found in second highest concentration.

Linolenic acid (18:3) was the dominant FFA component in leaves while the order of decreasing abundance of other FFA components was : 16:0 > 18:2 > 18:0 > 18:1 (except for B73xVA17) > 14:0 > 12:0 (Fig. 3.2). Significant

Table 3.3. Free fatty acid concentration of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter, in each column, within each tissue type, are not significantly different (P=0.05) by Duncan's Multiple Range Test.

Tissue	Hybrid	Fatty acid (µg x 10/g DW)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
Leaf	A619xH60	1.1a	4.0ab	159.7a	35.5b	31.9a	124.9a	988.8a
	B73xMO17	1.3a	2.6b	153.1ab	34.1b	26.1a	115.8a	804.2b
	B73xPA91	2.5a	4.9a	163.3a	41.1a	50.8a	130.2a	963.4a
	B73xVA17	1.2a	3.4ab	122.6b	25.7c	23.9a	88.8b	456.6c
	A632xH96	1.1a	3.1b	132.8b	27.8c	21.2a	68.1c	473.3c
Stem	A619xH60	1.0a	4.1a	152.3a	20.1ab	74.4a	269.6a	161.2a
	B73xMO17	0.5b	3.4a	137.4a	24.9a	42.9abc	205.4ab	113.7b
	B73xPA91	0.5b	2.4b	108.8a	24.5a	34.2bc	145.3bc	82.0bc
	B73xVA17	1.2a	2.5b	103.4a	18.1bc	62.6ab	82.1c	46.4c
	A632xH96	0.2b	2.0b	99.5a	14.1c	24.7c	111.6c	61.5c
Root	A619xH60	0.1a	1.0a	59.6a	18.6a	18.0a	65.6ab	7.3a
	B73xMO17	0.1a	0.8a	64.5a	14.0ab	8.1b	68.2ab	5.0a
	B73xPA91	0.1a	0.5a	52.6a	17.1a	13.5ab	86.1a	6.3a
	B73xVA17	0.1a	0.6a	32.0b	12.7ab	6.9b	13.4c	0.1b
	A632xH96	0.1a	n.d.	30.0b	9.8b	7.3b	38.9bc	0.1b

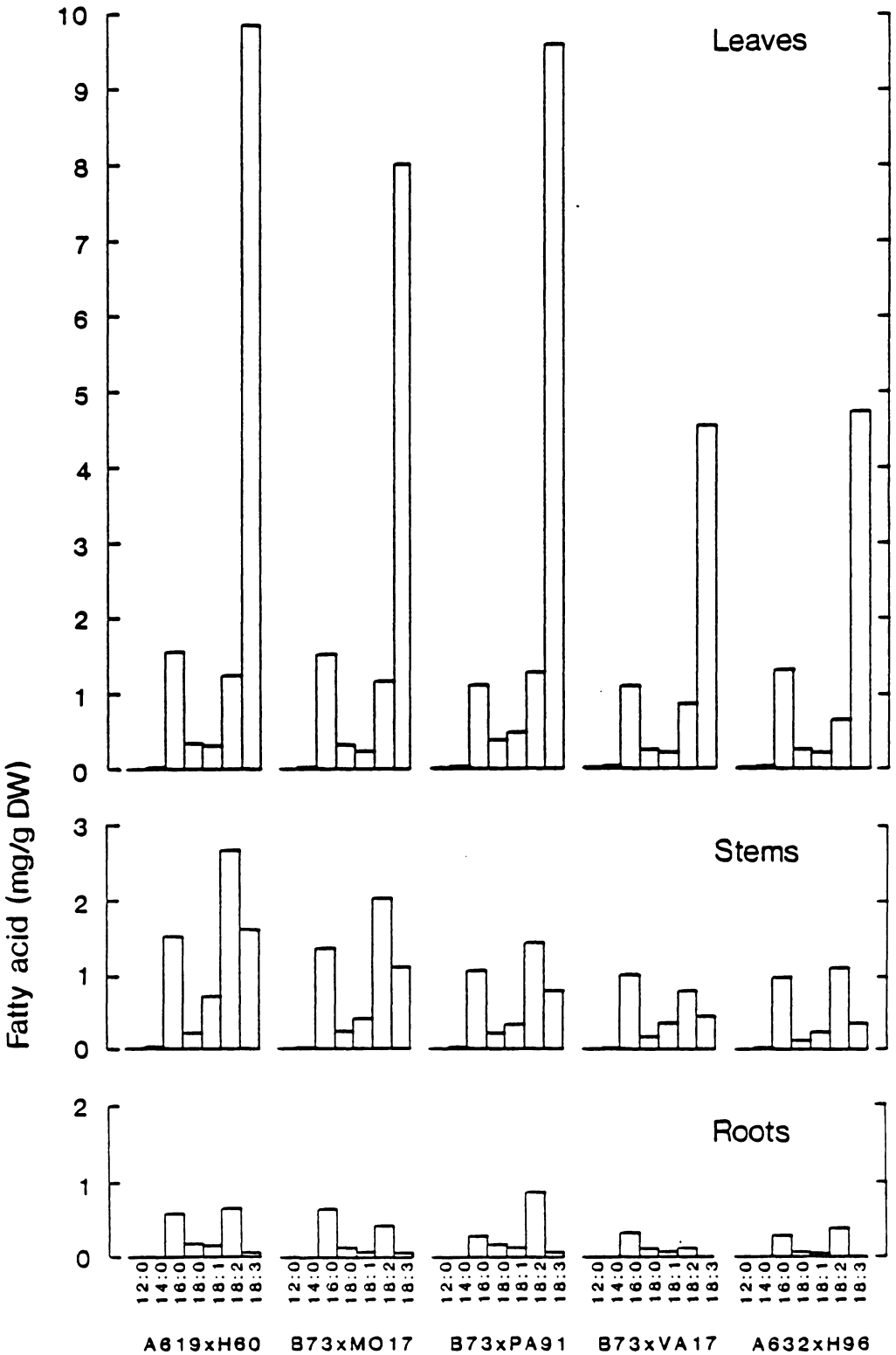
concentration differences were observed for most of these fatty acids in leaves when compared across hybrids except for 12:0 and 18:1 (Tab. 3.3).

The same FFA were observed in the stems of all the hybrids studied (Fig. 3.2). However, the relative abundance of these components was different from the leaves. Linoleic acid (18:2) was the dominant FFA in stems with the exception of one hybrid, B73xVA17. The order of decreasing abundance following 18:2 was : 16:0 (exception A619xH60) > 18:3 > 18:1 > 18:0 > 14:0 > 12:0. Significant differences were observed in all fatty acid components when compared across hybrids except for 16:0 (Tab. 3.3).

The concentrations of FFA were lowest in the root tissues (Fig. 3.2). The concentration of 12:0 was below the detectable limits of the GLC and integrator in all the hybrids. The concentration of 14:0 in A632xH96 and of 18:3 in B73xVA17 and A632xH96 were also below the detectable limits of the GLC and integrator. The dominant FFA in root tissues was 18:2 (exception B73xVA17). The relative concentration of the other fatty acids was generally in the order of 16:0 > 18:0 > 18:3 > 14:0. No significant differences were observed in concentration of 14:0 and 18:2 when hybrids were compared (Tab. 3.3).

As with total lipid and total FFA concentrations, individual FFA components in A619xH60, B73xMO17, and B73xPA91 were similar as were B73xVA17 and A632xH96 (Tab.

Figure 3.2. Free fatty acid composition of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution.


Leaves
Stems
Roots
Fatty acid (mg/g DW)
10
9
8
7
6
5
4
3
2
1
0
12:0
14:0
16:0
18:0
18:1
18:2
18:3
A619xH60
B73xMO17
B73xPA91
B73xVA17
A632xH96

3.3). However, the latter two hybrids exhibited lower concentrations of FFA components than did the other three hybrids.

Sterol components

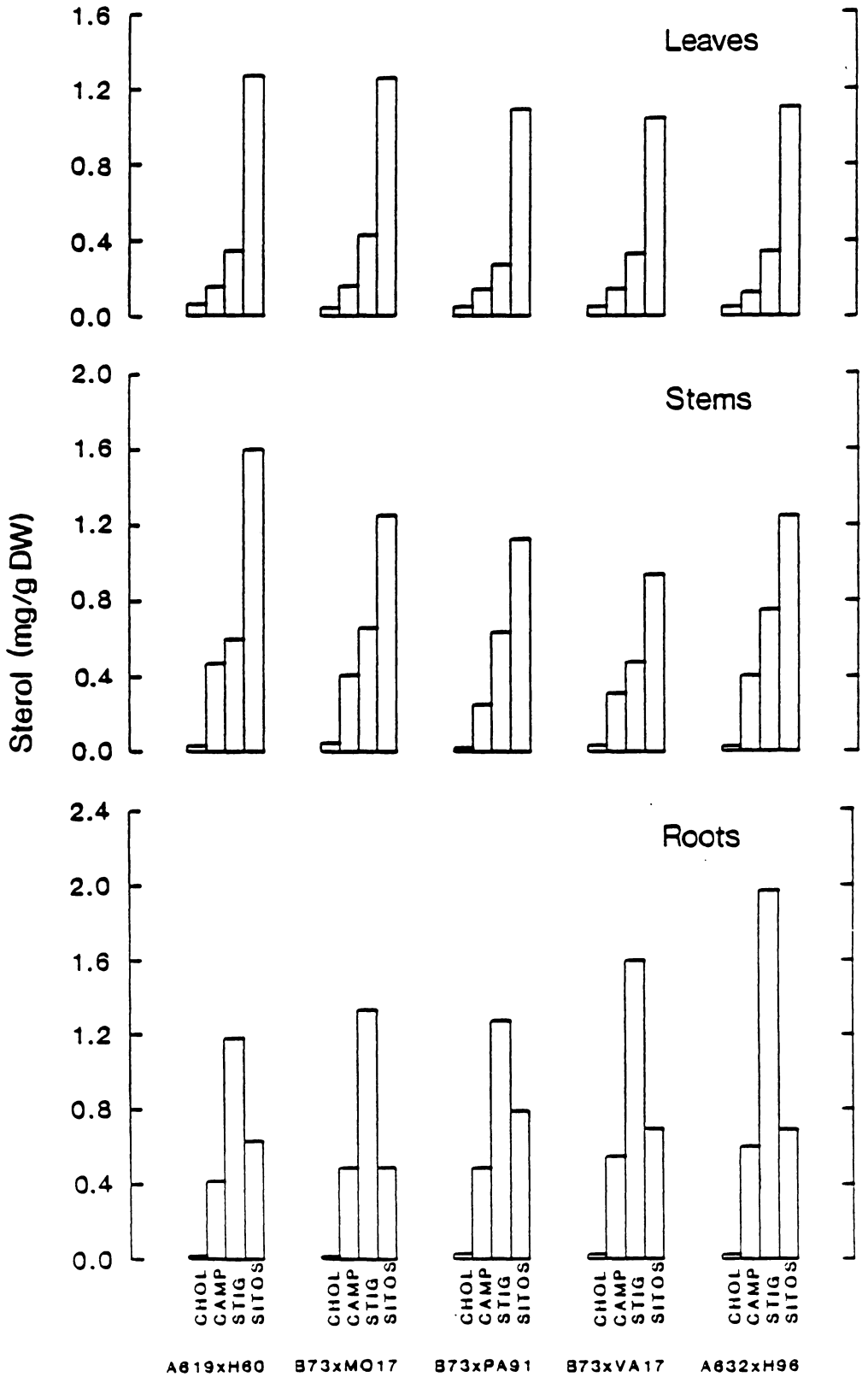
The major free sterols identified in the leaves, stems and roots of all the hybrids examined were cholesterol, campesterol, stigmasterol and sitosterol (Tab. 3.4). A difference in distribution occurs in the plant tissues with respect to the different sterol components. The cholesterol and sitosterol concentrations were highest in the leaves followed by the stems and roots. Campesterol and stigmasterol were lowest in the leaves, present in the stem and were highest in the roots. Thus, the relative proportions of individual sterols differ within specific plant tissues.

Hybrids differed with respect to the concentration of individual FS within respective plant tissues (Fig. 3.3). The individual FS composition of leaves and stems differed from roots in that sitosterol, rather than stigmasterol, was the dominant sterol component. In both the leaves and stems, the other sterols present in decreasing amounts were stigmasterol, campesterol and cholesterol. In the roots, sitosterol, campesterol and cholesterol occured in lower amounts. No significant differences among the hybrids were observed in concentrations of sitosterol and campesterol

Table 3.4. Free sterol concentration of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter, in each column, within each tissue type, are not significantly different (P=0.05) by Duncan's Multiple Range Test.

Tissue	Hybrid	Sterol (µg x 10/g DW)			
		Chol	Camp	Stig	Sitos
Leaf	A619xH60	6.4a	15.9a	34.4ab	127.7a
	B73xMO17	5.4ab	15.9a	42.6a	125.7a
	B73xPA91	4.4b	13.9a	27.9b	97.4a
	B73xVA17	4.8ab	14.1a	32.1ab	104.0a
	A632xH96	4.2b	13.0a	34.2ab	110.7a
Stem	A619xH60	3.0b	46.7a	59.9ab	160.2a
	B73xMO17	4.3a	41.4b	65.6a	125.3b
	B73xPA91	1.7c	34.6c	53.1bc	112.3bc
	B73xVA17	2.7b	30.9c	47.0c	93.8c
	A632xH96	3.6ab	40.5b	64.5a	124.1b
Root	A619xH60	2.3a	41.5c	117.5c	62.4b
	B73xMO17	2.4a	48.9bc	133.5c	48.5c
	B73xPA91	2.8a	48.4bc	127.7c	78.2a
	B73xH60	2.8a	54.7ab	159.4b	69.2ab
	A632xH96	2.1a	60.3a	196.6a	68.5ab

Figure 3.3. Free sterol composition of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution.


Leaves
Stems
Roots
Sterol (mg/g DW)
1.6
1.2
0.8
0.4
0.0
2.0
1.6
1.2
0.8
0.4
0.0
2.4
2.0
1.6
1.2
0.8
0.4
0.0
CHOL
CAMP
STIG
SITOS
A619xH60
B73xMO17
B73xPA91
B73xVA17
A632xH96

among hybrids in the leaves, although cholesterol and stigmasterol did vary (Tab. 3.4). Considerable differences were observed in concentrations of all sterols associated with the stem tissue analyzed for the five hybrids. The same was true for root tissues except that no significant differences were observed for cholesterol.

The same sterol components present as FS were also associated with the steryl glycoside·fraction (Tab. 3.5). Concentrations of SG were highest in the stems followed by the roots and leaves (Fig. 3.4). The major sterol glycosylated in the leaves and stems was sitosterol while in the roots campesterol was usually the dominant sterol. As in the free sterol fraction, a similar distribution pattern was observed for the various sterol components except the sitosterol concentration was highest in the stems followed by the leaves and roots. Significant differences in individual sterol concentrations were also observed among hybrids for specific plant tissues (Tab. 3.5). However, no significant differences were observed for cholesterol in the stems and roots, or for campesterol in the stems.

SE concentrations were highest in the roots followed by the stems and the leaves (Tab. 3.6). In the leaves, the concentration of cholesterol was below the detectable limits of the GLC and integrator. The same sterol components were present as in the FS and SG fractions.

Table 3.5. Sterol concentration of steryl glycosides of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter, in each column, within each tissue type, are not significantly different (P=0.05) by Duncan's Multiple Range Test.

Tissue	Hybrid	Sterol (μg/g DW)			
		Chol	Camp	Stig	Sitos
Leaf	A619xH60	7.90ab	36.9ab	30.2b	179.2bc
	B73xMO17	10.54a	66.9a	64.3a	310.0a
	B73xPA91	5.49ab	50.7ab	42.0ab	255.3ab
	B73xVA17	1.99b	32.2b	23.1b	106.7c
	A632xH96	4.47ab	42.4ab	39.1ab	276.8ab
Stem	A619xH60	4.00a	142.1a	87.0b	556.1a
	B73xMO17	3.91a	130.9a	112.5a	490.6ab
	B73xPA91	4.31a	132.0a	59.8cd	352.9b
	B73xVA17	2.92a	112.9a	80.7bc	361.1b
	A632xH96	3.49a	95.1a	55.9d	328.4b
Root	A619xH60	3.53a	126.1b	115.3b	149.4b
	B73xMO17	2.60a	134.3b	122.3b	104.2b
	B73xPA91	5.66a	229.5a	157.5ab	202.3a
	B73xVA17	4.38a	141.9b	99.4b	111.9b
	A632xH96	6.66a	251.4a	211.9a	154.2b

Figure 3.4. Sterol composition of steryl glycosides of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution.

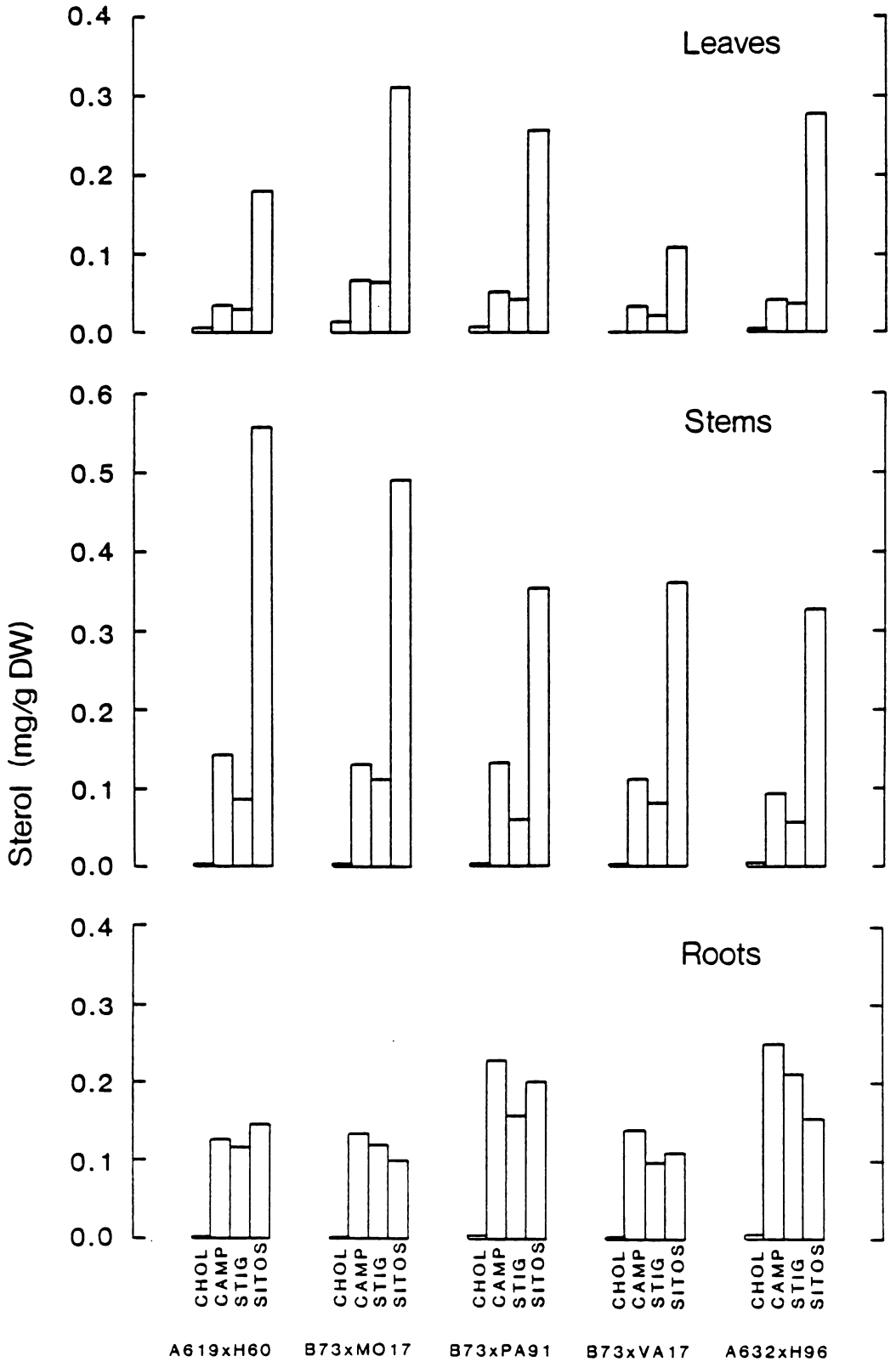

Leaves
Stems
Roots
Sterol (mg/g DW)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
CHOL
CAMP
STIG
SITOS
A619xH60
B73xMO17
B73xPA91
B73xVA17
A632xH96

Table 3.6. Sterol concentration of steryl esters of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution. Means followed by the same letter, in each column, within tissue type are not significantly different (P=0.05) by Duncan's Multiple Range Test.

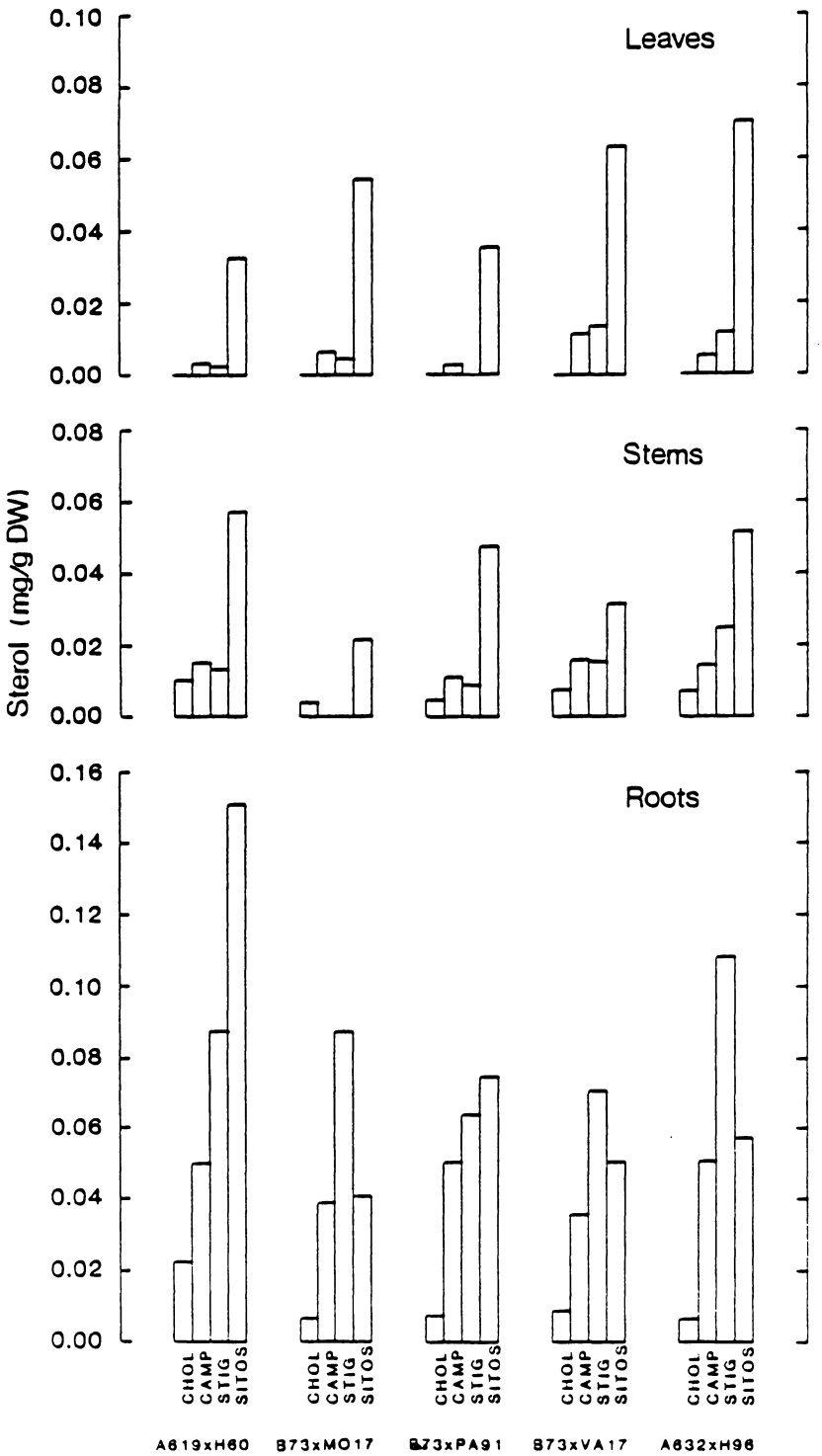
Tissue	Hybrid	Sterol (μg/g DW)			
		Chol	Camp	Stig	Sitos
Leaf	A619xH60	0.1a	3.7bc	2.4b	32.8b
	B73xMO17	0.1a	6.4b	4.6b	54.0ab
	B73xPA91	0.1a	2.9c	5.1b	63.6b
	B73xVA17	0.1a	10.6a	13.4a	87.6a
	A632xH96	0.1a	5.3bc	12.0a	69.1a
Stem	A619xH60	10.7a	15.4a	13.3ab	57.0a
	B73xMO17	3.4c	8.6a	11.5ab	22.2b
	B73xPA91	4.4bc	8.5a	4.9b	29.3ab
	B73xVA17	8.0ab	15.6a	15.6a	20.1b
	A632xH96	7.1abc	18.5a	18.5a	46.2ab
Root	A619xH60	22.0a	48.2a	87.7ab	150.8a
	B73xMO17	6.8b	39.0a	87.6ab	40.3b
	B73xPA91	7.4b	50.7a	63.7b	74.5b
	B73xVA17	8.5b	35.6a	71.1b	45.1b
	A632xH96	6.4b	50.6a	108.6a	57.1b

Sitosterol was the predomiant sterol esterified in the leaves and stems while stigmasterol was commonly esterified in the roots (exception A619xH60 and B73xM017). Similar distribution patterns were observed for FS and SG fractions except cholesterol was lowest in the leaves and increased in the stems and roots (Fig. 3.5). A distributional pattern for sitosterol was not as obvious.

Discussion

Dry weight analysis (Tab. 3.1) revealed significant differences among hybrids for leaf tissue only. Variablity of leaf dry weight may be related to rate of leaf appearance during early plant development. Rate of leaf appearance differs among maize hybrids that are grown at a constant temperature, depending on the relative maturity of hybrids (Tollenaar et al. 1984). It is possible that the maize hybrids considered in this study differed in maturity rate, which, in turn, determined rate of leaf appearance and leaf dry weight at harvest time. Root to shoot ratio also significantly differed among maize hybrids. Genetic differences for root to shoot ratio occur within species and thus could potentially contribute to differential response of hybrids to water stress (Parsons

Figure 3.5. Sterol composition of steryl esters of leaves, stems and roots of five maize hybrids grown in Clark's nutrient solution.


Leaves
Stems
Roots
Sterol (mg/g DW)
0.10
0.08
0.06
0.04
0.02
0.00
0.16
0.14
0.12
CHOL
CAMP
STIG
SITOS
A619xH60
B73xMO17
B73xPA91
B73xVA17
A632xH96

1979).

Inherent differences in the concentration of major lipid classes were observed among the maize hybrids (Tab. 3.2). These inherent differences in lipid concentration may reflect, principally, the genetic variability of maize hybrids. Hybrids of maize inbred lines represent genetically diverse backgrounds (Smith et al. 1985) and may exhibit differences in morphology (Jenison et al 1981), and physiological response (Ackerson 1983). The data presented here demonstrate inherent differences in lipid concentration of specific plant organs among maize hybrids. Most notable is the observation that the TL and FFA concentrations of leaves and roots of A619xH60, B73xMO17 and B73xPA91 were greater than those observed for hybrids B73xVA17 and A632xH96. Within each of these hybrid groupings the TL and FFA concentrations were similar. Comparable hybrid groupings were not apparent in the free, glycosidic and esterified steryl fractions. In addition, no particular maize hybrid exhibited consistently higher concentrations of FS, SG or SE than other hybrids. Genotypic differences in the concentration of the specific lipid classes considered in this study are confined to the TL and FFA.

Comparison of grape rootstocks (Kuiper 1968) and taxonomically unrelated species such as bean, barley, and sugar beet (Stuiver et al. 1978) revealed differences in

lipid content. Furthermore, these innate compositional differences were correlated with the relative salt tolerance of these species. Potentially, the inherent differences in lipid composition of maize hybrids could affect the ability of hybrids to adapt to environmental changes. Another factor to consider is that environmental stress may induce changes in lipids that may be more important in adaptation of plants than inherent differences. For example, the inherent differences in sterol concentration of citrus rootstocks was not directly related with salt resistance (Douglas and Walker 1983). However, salt-induced changes in sterol concentration were highly correlated with the salt-exclusion capacity of these rootstocks. Thus, inducible changes in the lipid composition of maize hybrids may provide insight as to the differential tolerance of these hybrids to environmental stress.

Tissue specificity with respect to the concentration of fatty acid (Fig. 3.2) components was also observed in the leaves, stems and roots of all hybrids. Each tissue exhibited a distinctive profile of FFA which may reflect, in part, the specific cellular membranes present in each plant organ. Palmitic or linoleic were the dominant FFA components in the roots of maize hybrids considered in this study. The fatty acid composition of total lipids isolated from a plasma membrane enriched fraction of maize roots was

linoleic (60%) and palmitic (30%) acids (Gronewald et al. 1982). Therefore, the FFA in the root tissue of maize hybrids may reflect the principal fatty acids of lipids of the plasma membrane.

Other FFA components were found in highest concentration in the leaves and stems of all hybrids (Fig. 3.2). Linolenic acid (18:3) clearly dominated the fatty acid profile of leaves while linoleic acid (18:2) occurred in the highest concentration in stem tissue. Linolenic acid is the principal fatty acid incorporated into glycolipids of leaf chloroplastic membranes (Mazliak 1977). Linolenic acid observed in maize leaves may therefore, serve as a biosynthetic pool which could be sequestered into glycolipids.

The total concentration of FS, SG and SE differed among the tissues of hybrids(Tab. 3.2). FS were proportionally higher in concentration in the leaves followed by the stems and roots of hybrids (Fig. 3.3). From what is known about sterol synthesis at this time (Grunwald 1975a) it is difficult to postulate why this type of FS distribution might occur in tissues.

In contrast, the SG concentration was higher in the stems than the roots and leaves and may be related to the role of SG as transport forms of sterols (Grunwald 1971). Sterols associated with the esterified fraction were found in the highest concentration in the roots and progressively

lower in the stems and leaves. The greatest amounts of SE appear to be localized in mitochondrial membranes of plant tissue (Kemp and Mercer 1968) although there is evidence that SE may also be involved in the transfer of sterols from one organelle to another (Kemp et al. 1967). Possibly the differences in concentration of SE among the different tissues in maize hybrids reflects the relative availability of free sterols for esterification in each of these tissues.

Differences in distribution of individual sterol components associated with each steryl fraction was also apparent (Tabs 3.4, 3.5, 3.6). Most striking was the distribution of the free sterols, cholesterol and sitosterol, which was highest in the roots and decreased in the stems and leaves, respectively (Fig. 3.3). Campesterol and stigmasterol concentrations increased in the reverse direction. Sitosterol was the dominant free sterol component in the leaves while stigmasterol was most abundant in the roots. A progressive increase in the proportion of stigmasterol relative to sitosterol has been reported to occur in roots during the germination of maize seedlings (Kemp et al. 1967) and with increasing age of etiolated bean tissue (Guens 1973). Therefore, the distribution of sitosterol and stigmasterol between leaves and roots may reflect differences in the relative age of plant organs. Possibly the synthesis of stigmasterol and

sitosterol either by conversion of sitosterol to stigmasterol (Navari-Izzo and Izzo 1985) or synthesis via a common precursor (Grunwald 1985) is related to the relative tissue age of stems and roots in maize hybrids.

Concentrations of SG and SE were also distributed among the plant organs of maize hybrids (Tabs 3.5, 3.6). As in the FS, sitosterol was the most abundant sterol component of SG and SE fractions of leaves and stems but no pattern of distribution was apparent (Figs 3.4, 3.5). Instead the campesterol and stigmasterol concentrations of these fractions exhibited the same trend as FS towards higher levels in the roots and diminished levels in the stems and leaves. However, unlike the FS and SG fractions, the cholesterol component of the SE fraction was highest in the root and was lower in the stem and leaves. Overall, individual sterols were partitioned into the sterol forms to different extents in leaf, stem and root tissue.

The relative ability of a cell or plant to sequester sterol (and fatty acid) components into other biosynthetic pools may be an important mechanism for survival of environmental stress (Parks 1984). Identification of patterns of lipid distribution in the tissues of hybrids will provide a basis for observation of stress-induced changes in lipid metabolism. In addition, genotypic differences in lipid composition among maize hybrids may suggest a differential response of hybrids to environmental stress.

Chapter IV

Lipid Concentration and Composition Following PEG-induced Osmotic Stress in Five Maize Hybrids

Introduction

Lipid metabolism in higher plants is affected by numerous environmental stresses including light (Bush et al. 1971, Bae and Mercer 1980), temperature (Davis and Finkner 1972, Sikorska and Farkas 1982, Chapman et al. 1983), salinity (Stuiver et al. 1978, Kuiper and Kuiper 1978, Douglas and Walker 1983), and water-deficit (Douglas and Paleg 1981, Chetal et al. 1981, 1983, Liljenberg et al. 1985). Environmental changes in lipid metabolism may represent consequential or degradative events without a significant role in adaptation to the environment (Kuiper 1985). However, changes in lipid metabolism may also confer some adaptive advantage that allows survival in a particular environmental niche. The lipid constituents of membranes can be modified, in both concentration and composition, by changes in the environment. Thus, membranes are dynamic structures in which components are synthesized, degraded and partitioned into various intracellular membranes (Robertson 1983, Wattenberg and Silbert 1983). This biosynthetic adaptability may represent an elegant system

by which the plant cell is able to buffer the impact of external changes (Park 1984), such as water stress.

Water stress has a profound impact on developmental and biochemical processes of higher plants. Water-deficit stress may inhibit cell elongation, reduce photosynthetic and respiratory activity, decrease the content of chlorophyll, protein and RNA and increase the polyamine content of plant cells (Levitt 1980). Ultrastructural changes, reflecting membrane damage, include a disruption of the mesophyll and bundle sheath cells of leaves (Giles et al. 1974) and alteration of mitochondria and loss of definition of cristae (Nir et al. 1979). Thus, water stress affects many cellular processes associated with membrane systems.

Water-deficit induced changes in lipid metabolism have been demonstrated in *Spirodela* (Lechevallier 1977), roots of oat seedlings (Liljenberg et al. 1985), tissues of maize seedlings (Douglas and Paleg 1981), cotton leaves (Pham Thi et al. 1985) and chloroplasts (Ferrari-Iliou et al. 1984), and in wheat and barley chloroplasts (Chetal 1981, 1983). Generally, severe water deficit results in a decrease in polar lipid content (phospholipids and glycolipids) and an increase in neutral lipid species such as triglycerides and steryl esters (Parks 1984). The free sterols either increase or decrease in concentration depending on the duration and severity of water stress

(Douglas and Paleg 1981, Liljenberg et al. 1985). Possibily several induction cycles or a critical degree of water stress is required for certain modifications of lipid metabolism (Liljenberg et al. 1985). Furthermore, the response of higher plants to mild water stress may involve early metabolic events in adjustment of membrane lipids that contribute to cell membrane stability during severe water stress.

The objectives of the present investigation were to determine the effects of a mild water stress of short duration on several lipid classes (free fatty acid, free sterol, steryl glycoside, steryl ester) in the leaves, stems and roots of five maize hybrids and to evaluate the electrolyte leakage from leaf discs as a measure of cell membrane stability.

Abbreviations: Camp, campesterol; Chol, cholesterol; FFA, free fatty acid; FS, free sterol; R/S, root to shoot ratio; SE, steryl ester; SG, steryl glycoside; Sitos, sitosterol; Stig, stigmasterol; TL, total lipid.

Materials and Methods

Plant material

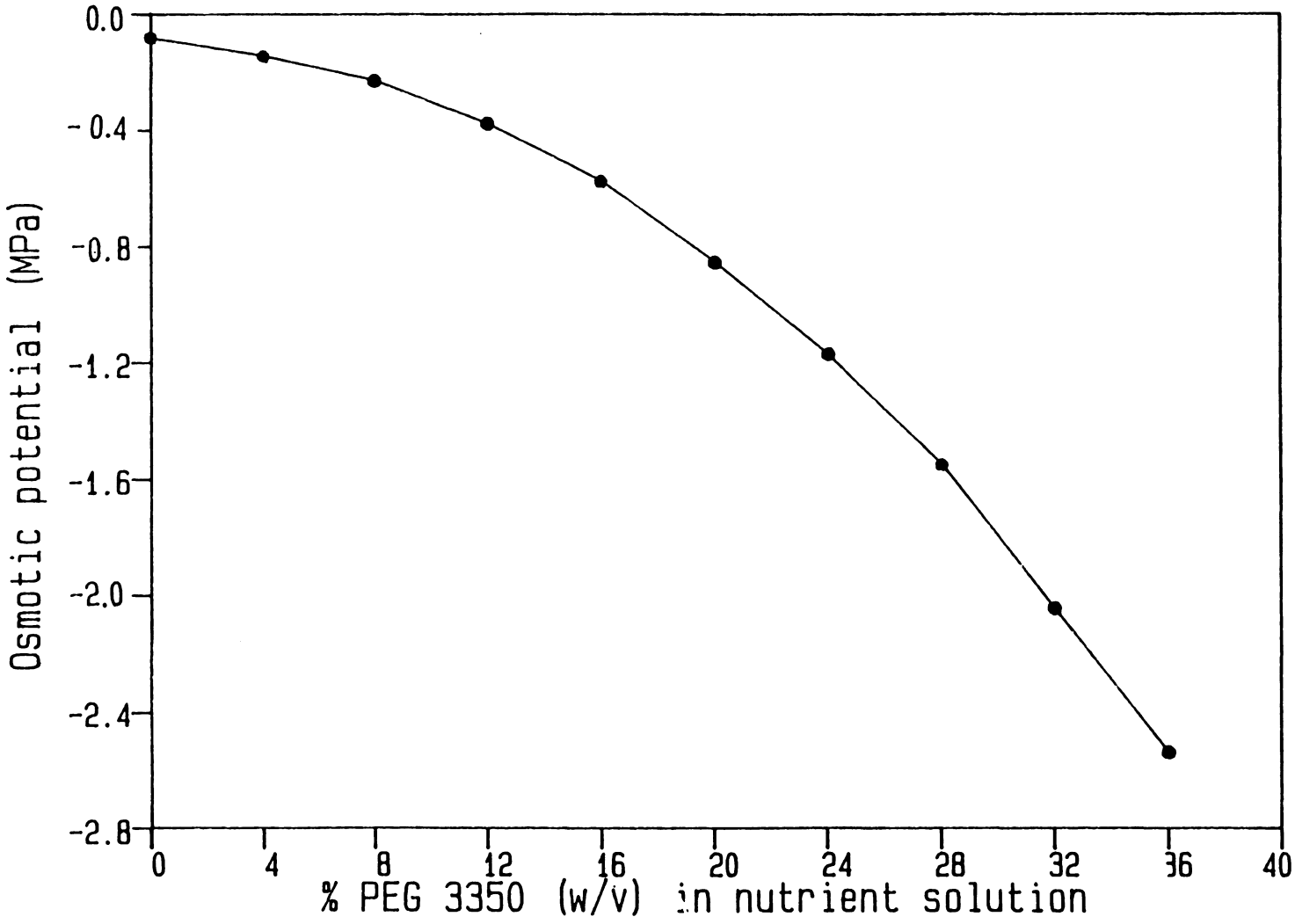
Maize seeds (*Zea mays* L. hybrids A619xH60, B73xVA17, B73xMO17, B73xPA91, and A632xH96) were germinated in vermiculite. At the two-leaf stage, maize seedlings were transferred to one-liter plastic containers and grown in full strength Clark's nutrient solution for 7 days as previously described.

Osmotic potential treatments

Maize seedlings were exposed to reduced osmotic potential for 48 hours by replacing the nutrient solution with nutrient solution supplemented with polyethylene glycol (PEG) 3350. The osmotic potential of PEG in Clark's nutrient solution was determined by freezing point depression using an osmometer (Fiske Co., Uxbridge, Mass; Fig. 4.1). Concentrations of 12 and 16% PEG 3350 (w/v) created osmotic potentials of about -0.4 and -0.6 MPa, respectively. Controls were grown in Clark's nutrient solution minus PEG (-0.1 MPa).

The experimental design was a completely randomized 5x3 factorial with 5 maize hybrids and 3 osmotic potential levels. Each value shown in the tables represents the mean of three triplicate analyses.

Figure 4.1. Osmotic potential of PEG 3350 in Clark's nutrient solution. Standard error is less than ±0.45.


0.0
-0.4
-0.8
-1.2
-1.6
-2.0
-2.4
-2.8
Osmotic potential (MPa)
0
4
8
12
16
20
24
28
32
36
40
% PEG 3350 (w/v) in nutrient solution

Lipid extraction and analysis

Lipid class (free fatty acid, free sterol, stery glycoside, steryl ester) extraction and analysis of leaf, stem and root tissue was conducted as previously described.

Plant material and electrolyte leakage

Maize seedlings were grown hydroponically in a controlled environment room at 25 C under Westinghouse warm and cool white fluorescent lamps at a photosynthetically active radiation (PAR) of 250 μmol m^{-2} s^{-1}, continuous light, for 7 days prior to harvest. Twenty leaf discs were punched from the third oldest leaf of every plant using a cork borer (5 mm diameter). Leaf discs were immediately floated on either deionized, distilled water or PEG solutions (8, 16, 24, 32, 40, 48 and 56% PEG 8000, formerly PEG 6000, w/v) in a glass petri dish for 24 hours in continuous light at 25 C. The osmotic potentials created by these PEG solutions were -0.02, -0.09, -0.20, -0.37, -0.61, -0.96 and -1.22 MPa, respectively. Leaf discs were rinsed three times and incubated with deionized, distilled water for an additional 24 hours at 25 C (Blum and Ebercon 1981). Electrolyte leakage of leaf discs was determined by measuring the electrical conductivity (mhos) of the incubation medium at 25 C using a linear conductivity meter (Arthur H. Thomas, Co., Philadelphia, PA). Leaf discs were autoclaved for 15 minutes, cooled to 25 C, and the total

electrolyte leakage was determined by measuring the electrical conductivity of the incubation medium. Percent electrolyte leakage was calculated as the initial electrical conductivity before autoclaving divided into the total electrical conductivity of the incubation medium after autoclaving, expressed as a percent.

Results

Dry weight analysis

Dry weight of the leaf, stem and root tissues (Tab. 4.1) of the five maize hybrids was altered by exposure to osmotic potentials of -0.4 and -0.6 MPa. Significant decreases in tissue dry weight were demonstrated by the hybrids, B73xVA17 and A632xH96. There was a significant decrease in the dry weight of all tissues of the hybrid, B73xVA17, at the -0.6 MPa osmotic potential. Stem and root dry weight in this hybrid also decreased at the -0.4 MPa compared to the -0.1 MPa osmotic potential. The only other significant decrease in dry weight was observed in the leaves of the hybrid, A632xH96, at an osmotic potential of -0.6 MPa. The dry weight of the stems and roots, of the same hybrid, exhibited a trend towards a reduction in dry weight at -0.6 MPa. Other significant differences include

Table 4.1. Dry weight of leaf, stem and root tissues of five maize hybrids as affected by PEG. *, significantly different from control (-0.1 MPa) at P=0.05; **, significantly different from control at (-0.1 MPa) at P=0.01.

Hybrid	Osmotic Potential (MPa)	Tissue DW (g/plant)				R/S
		Leaf	Stem	Root	Total	
A619xH60	-0.1	1.14	0.62	0.91	2.67	0.52
	-0.4	1.04	0.59	0.85	2.49	0.52
	-0.6	1.12	0.62	0.92	2.66	0.53
B73xMO17	-0.1	0.91	0.67	0.88	2.45	0.55
	-0.4	0.90	0.61	0.96*	2.47	0.64**
	-0.6	0.87	0.61	0.85	2.34	0.57
B73xPA91	-0.1	0.82	0.94	0.67	2.28	0.61
	-0.4	0.85	0.61	0.86	2.31	0.59
	-0.6	0.83	0.65	1.05**	2.53**	0.71*
B73xVA17	-0.1	0.68	0.58	0.93	2.19	0.78
	-0.4	0.47	0.37*	0.65*	1.49*	0.76
	-0.6	0.45*	0.34*	0.59**	1.38**	0.75
A632xH96	-0.1	0.57	0.63	0.71	1.90	0.59
	-0.4	0.57	0.69	0.77	2.03	0.62
	-0.6	0.46*	0.43	0.55	1.45	0.62

an increase in root and total dry weight of the hybrid B73xPA91, and in the root dry weight of B73xMO17. No significant change in tissue dry weight was demonstrated in the hybrid, A619xH60, although the dry weight declined slightly at the -0.4 MPa osmotic potential.

The root to shoot ratio of all hybrids increased with reduced osmotic potential, with the exception of B73xVA17. In this hybrid, the root to shoot ratio decreased with reduced osmotic potentials.

Total lipid

Total lipid (TL) concentration (Tab. 4.2) increased in the leaves, stems and roots of several maize hybrids exposed to -0.4 and -0.6 MPa osmotic potentials. In the roots, the TL concentration significantly increased ($P=0.01$) in all hybrids grown at reduced osmotic potentials and was 4 to 7-fold greater at the -0.6 MPa than the -0.1 MPa osmotic potential. Significant increases in the TL concentration in the leaves occurred at -0.6 MPa in the hybrid, B73xMO17, and at the -0.4 MPa in the hybrids, B73xVA17 and A632xH96. In the stems, there was a highly significant increase in TL concentration at the -0.6 MPa osmotic potential in the hybrids, A619xH60 and B73xPA91, and at -0.4 MPa in A619xH60 and B73xMO17.

Table 4.2. Lipid concentration of leaves, stems and roots of five maize hybrids as affected by PEG. *, significantly different from control (-0.1 MPa) at P=0.05; **, significantly different from control (-0.1 MPa) at P=0.01.

Tissue	Hybrid	Osmotic Potential (MPa)	Lipid class (mg/g DW)				
			TL	FFA	FS	SG	SE
	A619xH60	-0.1	174.8	13.5	1.8	0.25	0.039
		-0.4	198.8	11.6	1.8	0.23	0.033
		-0.6	214.3	12.4	1.7	0.37*	0.033
	B73xMO17	-0.1	136.5	11.4	1.9	0.45	0.065
		-0.4	155.7	12.0	1.8	0.58	0.061
		-0.6	205.5*	10.4	1.6	0.42	0.085
Leaf	B73xPA91	-0.1	147.5	13.6	1.4	0.35	0.036
		-0.4	162.8	15.2*	1.5	0.42	0.054*
		-0.6	163.2	13.5	1.5	0.32	0.056
	B73xVA17	-0.1	75.8	7.2	1.5	0.16	0.088
		-0.4	98.3*	7.7	2.0	0.19	0.082
		-0.6	89.5	7.3	2.3*	0.19	0.069
	A632xH96	-0.1	72.5	7.3	1.6	0.36	0.086
		-0.4	80.3*	9.8	1.7	0.44	0.100
		-0.6	75.0	11.4	1.9	0.41	0.025**
	A619xH60	-0.1	72.8	6.8	2.7	0.79	0.096
		-0.4	82.0*	4.8*	2.5	0.78	0.075
		-0.6	94.8**	3.0**	2.9	0.73	0.085
	B73xMO17	-0.1	46.2	5.3	2.4	0.74	0.046
		-0.4	72.5*	4.6	2.2	0.68	0.037
		-0.6	65.3	4.3	2.3	0.57	0.028**
Stem	B73xPA91	-0.1	51.8	4.0	2.0	0.55	0.047
		-0.4	54.2	3.6	2.6	0.52	0.043
		-0.6	76.5**	4.0	2.2	0.53	0.041
	B73xVA17	-0.1	43.2	3.2	1.7	0.56	0.060
		-0.4	39.5	2.9	1.9	0.51	0.040**
		-0.6	46.0	2.6	1.9	0.52	0.039**
	A632xH96	-0.1	41.5	3.1	2.3	0.48	0.087
		-0.4	52.7	2.7	2.1	0.34	0.044
		-0.6	58.7	2.9	1.9	0.42	0.023*
	A619xH60	-0.1	32.0	1.7	2.2	0.39	0.308
		-0.4	116.2**	1.4	2.6	0.34	0.160*
		-0.6	170.3**	1.5	2.5	0.29*	0.158*
	B73xMO17	-0.1	34.3	1.6	2.3	0.36	0.173
		-0.4	153.3**	1.9	2.1	0.41	0.151
		-0.6	130.2**	2.5**	2.3	0.38	0.145
Root	B73xPA91	-0.1	32.0	1.8	2.6	0.59	0.196
		-0.4	34.2	1.2	3.1	0.60	0.178
		-0.6	209.0**	1.3	3.2	0.62	0.127
	B73xVA17	-0.1	19.7	0.7	2.9	0.36	0.160
		-0.4	110.8**	0.6	2.7	0.37	0.160
		-0.6	112.7**	0.6	2.7	0.36	0.139
	A632xH96	-0.1	23.5	0.9	3.3	0.62	0.223
		-0.4	121.7**	0.6**	3.1	0.43	0.130*
		-0.6	178.0**	0.7	2.5*	0.42	0.127*

Free fatty acids

Few significant differences in the total free fatty acid (FFA) concentration (Tab. 4.2) were observed in tissues of maize hybrids at the -0.4 or -0.6 MPa osmotic potential. The only exceptions were the significant changes in the leaves of hybrid, B73xPA91 at -0.4 MPa, the stems of the hybrid, A619xH60 at both osmotic potentials, and the roots of B73xMO17 at -0.4 and A632xH96 at -0.6 MPa. Although only small changes occurred in the concentrations of FFA among treatments, there was a trend towards a decline in FFA with reduced osmotic potential.

The qualitative composition of the FFA varied with osmotic potential (Tab. 4.3). Although not always statistically significant, several trends were apparent. For example, the concentration of 18:3 increased in the leaves of all hybrids except, A619xH60, and decreased in the stems of all hybrids except B73xPA91. The remaining unsaturated fatty acids (18:1 and 18:2) generally decreased in the leaves and stems at reduced osmotic potentials although there were exceptions to this trend. Saturated fatty acids (12:0, 14:0, 16:0 and 18:0) tended to decrease or were unaffected by osmotic treatments. Quantitative changes in FFA concentrations were prevalent in the leaves of the hybrid, B73xPA91, and stems of the hybrid, A619xH60.

Table 4.3. Free fatty acid concentration of leaves, stems and roots of five maize hybrids as affected by PEG. *, significantly different from control (-0.1 MPa) at P=0.05; **, significantly different from control (-0.1 MPa) at P=0.01.

Tissue	Hybrid	Osmotic Potential (MPa)	Fatty acid (µg x 10/g DW)						
			12:0	14:0	16:0	18:0	18:1	18:2	18:3
	A619xH60	-0.1	1.1	4.0	159.7	35.5	31.9	124.9	988.8
		-0.4	1.3	3.3	127.5	30.9	19.6	110.6	866.9*
		-0.6	1.5	2.6	130.5	35.6	18.7	116.7	936.5
	B73xMO17	-0.1	1.3	2.6	153.1	34.1	26.1	115.8	804.2
		-0.4	1.1	2.9	162.4	35.7	25.4	113.5	854.6
		-0.6	1.0	3.1	155.1	37.9*	23.8	104.6	717.6
Leaf	B73xPA91	-0.1	2.5	4.9	163.3	41.1	50.8	130.2	963.4
		-0.4	1.0	4.8*	147.6	45.6*	33.1*	130.4	1160.3*
		-0.6	0.4	2.7**	141.9*	37.8*	20.7*	108.6	1038.6
	B73xVA17	-0.1	1.2	3.4	122.6	25.7	23.9	88.8	456.6
		-0.4	0.7	2.7	102.5	24.4	15.4	78.6	550.9
		-0.6	0.6	2.7	91.9*	24.3	14.1	75.4	522.1
	A632xH96	-0.1	1.1	3.1	132.8	27.8	21.2	68.1	473.3
		-0.4	1.4	3.8	123.6	32.5*	21.8	75.1	718.0
		-0.6	1.5	4.3*	116.3**	33.2*	20.5*	87.4	778.0
	A619xH60	-0.1	1.0	4.1	152.3	20.1	74.4	269.6	161.2
		-0.4	0.7	4.7	112.2*	17.2	39.5	179.2	129.8
		-0.6	0.3**	3.1	66.9**	14.1**	26.0*	106.9**	87.1*
	B73xMO17	-0.1	0.5	3.4	137.4	24.9	42.9	205.4	113.7
		-0.4	0.7	3.6	110.3	21.7	35.6	164.2	120.3
		-0.6	0.6	2.9	104.8	23.8*	36.0	151.9	107.3
Stem	B73xPA91	-0.1	0.5	2.4	108.8	24.5	34.2	145.3	82.0
		-0.4	0.1.	1.9	101.5	19.9	29.9	132.2	78.5
		-0.6	0.3	2.6	108.0	21.3	28.7	135.2	101.3
	B73xVA17	-0.1	1.2	2.5	103.4	18.1	62.6	82.1	46.4
		-0.4	0.2**	2.9	102.7	14.5	24.4**	101.2	44.3
		-0.6	0.5	2.9	89.2	16.5	25.0**	93.0	34.4*
	A632xH96	-0.1	0.2	2.0	99.5	14.1	24.7	111.6	61.5
		-0.4	0.2	2.2	102.2	17.6	24.1	80.2*	46.2
		-0.6	0.5**	3.5	100.6	17.7	41.2	81.9	44.1*
	A619xH60	-0.1	0.1	1.0	59.6	18.6	18.0	65.6	7.3
		-0.4	0.1	1.1	55.8	19.3	11.9	47.1	7.0
		-0.6	0.1	1.2	53.4	18.8	13.8	59.9	7.2
	B73xMO17	-0.1	0.1	0.8	64.5	14.0	8.1	68.2	5.0
		-0.4	0.1	0.9	62.3	13.3	12.9	95.3	6.4
		-0.6	0.1	1.0	89.1	16.0*	21.5*	116.3**	8.5
Root	B73xPA91	-0.1	0.1	0.5	52.6	17.1	13.5	86.1	6.3
		-0.4	0.1	0.9	44.9	20.3	11.9	42.2	2.8
		-0.6	0.1	1.0	45.1	17.2	12.7	46.6	4.1
	B73xVA17	-0.1	0.1	0.6	32.0	12.7	6.9	13.4	0.1
		-0.4	0.1	0.3	27.3	10.2	6.4	16.0*	0.1
		-0.6	0.1	0.5	30.9	9.7**	5.1*	14.2	0.1
	A632xH96	-0.1	0.1	0.1	30.0	9.8	7.3	38.9	0.1
		-0.4	0.1	0.1	28.0	8.5*	6.4	19.3*	0.1
		-0.6	0.1	0.1	31.8	7.5**	7.6	24.7	0.1

Free, glycosidic and esterified sterols

The total free desmethylsterol (FS), steryl glycoside (SG) and steryl ester (SE) concentrations (Tab. 4.2) of maize hybrids were relatively unchanged by reduced osmotic potential treatments. Significant changes in these steryl fractions were demonstrated in the leaves and roots, but not in stems, of all hybrids. Only a few significant differences in the concentration of FS were demonstrated in the tissues of hybrids. Total FS concentration significantly increased at -0.6 MPa in the leaves of B73xVA17 but was significantly lower at this osmotic potential in the roots of the hybrid, A632xH96. In both hybrids, the FS concentration changed in a similar manner in these tissues.

Total SG and SE concentrations of maize seedlings (Tab. 4.2) were also relatively unaffected by reduced osmotic potentials. The concentration of SE changed more than SG, although only a few significant differences were apparent. Almost all hybrids demonstrated a trend of reduced SG and SE concentrations in the stems and roots. This reduction was more apparent in the SE than SG fraction. In addition, an inverse relationship between the concentration of SG and SE was apparent in the leaves of all hybrids. For example, an increase in the total SG concentration in the hybrids, B73xVA17, A632xH96 and A619xH60, corresponded to a decrease in the total SE concentration in the leaves. The opposite

trend was demonstrated in the hybrids, B73xMO17 and B73xPA91, in which the SG concentration decreased and SE concentration increased in the leaves. A similar relationship was not apparent in the total SG and SE concentrations of the stems or roots of maize hybrids.

Few significant differences were apparent in the qualitative composition of the FS (Tab. 4.4), SE (Tab. 4.5) or SG (Tab. 4.6). The root tissues were generally more responsive to the osmotic treatments, than either the stems or leaves of maize hybrids, as evidenced by a greater number of significant differences in the sterols of these tissues. Of particular interest was an increase in the stigmasterol to sitosterol ratio in the FS, SG and SE fractions of the roots of all hybrids. The hybrids, B73xVA17 and A632xH96, demonstrated the greatest shift in these ratios in all three steryl fractions of the roots. In addition, significant differences in the ratios of these two sterols occurred primarily in the SG and SE fractions of several hybrids.

Electrolyte leakage

Electrolyte leakage of leaf discs (Fig. 4.2) revealed significant genotypic differences among the maize hybrids. The percent electrolyte leakage was markedly higher in the hybrid, A632xH96, compared to all other hybrids.

Table 4.4. Free sterol concentration of leaves,stems and roots of five maize hybrids as affected by PEG. *, significantly different from the control (-0.1 MPa) at P=0.05; **, significantly different from the control (-0.1 MPa) at P=0.01.

Tissue	Hybrid	Osmotic Potential (MPa)	Sterol (µg x 10/g DW)				Stig/Sitos
			Chol	Camp	Stig	Sitos	
Leaf	A619xH60	-0.1	6.4	15.9	34.4	127.7	0.27
		-0.4	5.5	16.6	34.4	123.1	0.28
		-0.6	5.9	14.7	34.0	119.4	0.29
	B73xMO17	-0.1	5.4	15.9	42.6	125.7	0.34
		-0.4	5.3	16.3	37.6	118.6	0.32
		-0.6	5.2	12.9	32.7	105.3	0.31
	B73xPA91	-0.1	4.4	13.9	27.9	97.4	0.29
		-0.4	4.3	16.0	28.9	99.5	0.29
		-0.6	4.5	16.1	30.3	100.2	0.30
	B73xVA17	-0.1	4.8	14.1	32.1	104.0	0.31
		-0.4	4.9	24.1	40.1	126.7	0.32
		-0.6	6.1	25.6	50.3*	144.8*	0.35
	A632xH96	-0.1	4.2	13.0	34.2	110.7	0.31
		-0.4	4.1	16.0	35.2	110.3	0.32
		-0.6	3.3	17.0	39.6	128.2	0.31
Stem	A619xH60	-0.1	3.0	46.7	59.9	160.2	0.37
		-0.4	2.3	44.0	57.3	141.8	0.40
		-0.6	3.0	50.4	68.3	173.6	0.39
	B73xMO17	-0.1	4.3	41.4	65.6	125.3	0.52
		-0.4	3.7	39.8	57.9	121.0	0.48
		-0.6	5.1	36.7	60.9	129.2	0.47
	B73xPA91	-0.1	1.7	34.6	53.1	112.3	0.47
		-0.4	2.1	45.5	63.3	145.4	0.43
		-0.6	1.4	37.1	56.0	123.7	0.45
	B73xVA17	-0.1	2.7	30.9	47.0	93.8	0.50
		-0.4	3.4*	32.4	47.8	103.6	0.46
		-0.6	3.7**	32.5	51.8	99.3	0.52
	A632xH96	-0.1	3.6	40.5	64.5	124.1	0.52
		-0.4	3.5	34.3*	58.5	108.8	0.54
		-0.6	3.1	32.9*	54.5	101.5	0.54
Root	A619xH60	-0.1	2.3	41.5	117.5	62.4	1.88
		-0.4	1.8	47.2	143.4	70.1	2.05
		-0.6	1.8	44.2	139.2	65.6	2.12
	B73xMO17	-0.1	2.4	48.9	133.5	48.5	2.75
		-0.4	1.5**	43.2	122.5	47.0	2.61
		-0.6	0.9**	42.4	131.6	50.7	2.59
	B73xPA91	-0.1	2.8	48.4	127.7	78.2	1.63
		-0.4	2.7	56.5	160.6	93.7	1.71
		-0.6	4.1	56.7	164.8	90.0	1.83*
	B73xVA17	-0.1	2.8	54.7	159.4	69.2	2.30
		-0.4	2.4	53.3	148.7	61.0	2.44
		-0.6	2.3	51.9	154.7	57.3*	2.70*
	A632xH96	-0.1	2.1	60.3	196.6	68.5	2.87
		-0.4	1.2	57.7	190.4	60.2	3.16
		-0.6	2.5	46.7*	152.7	50.5*	2.54

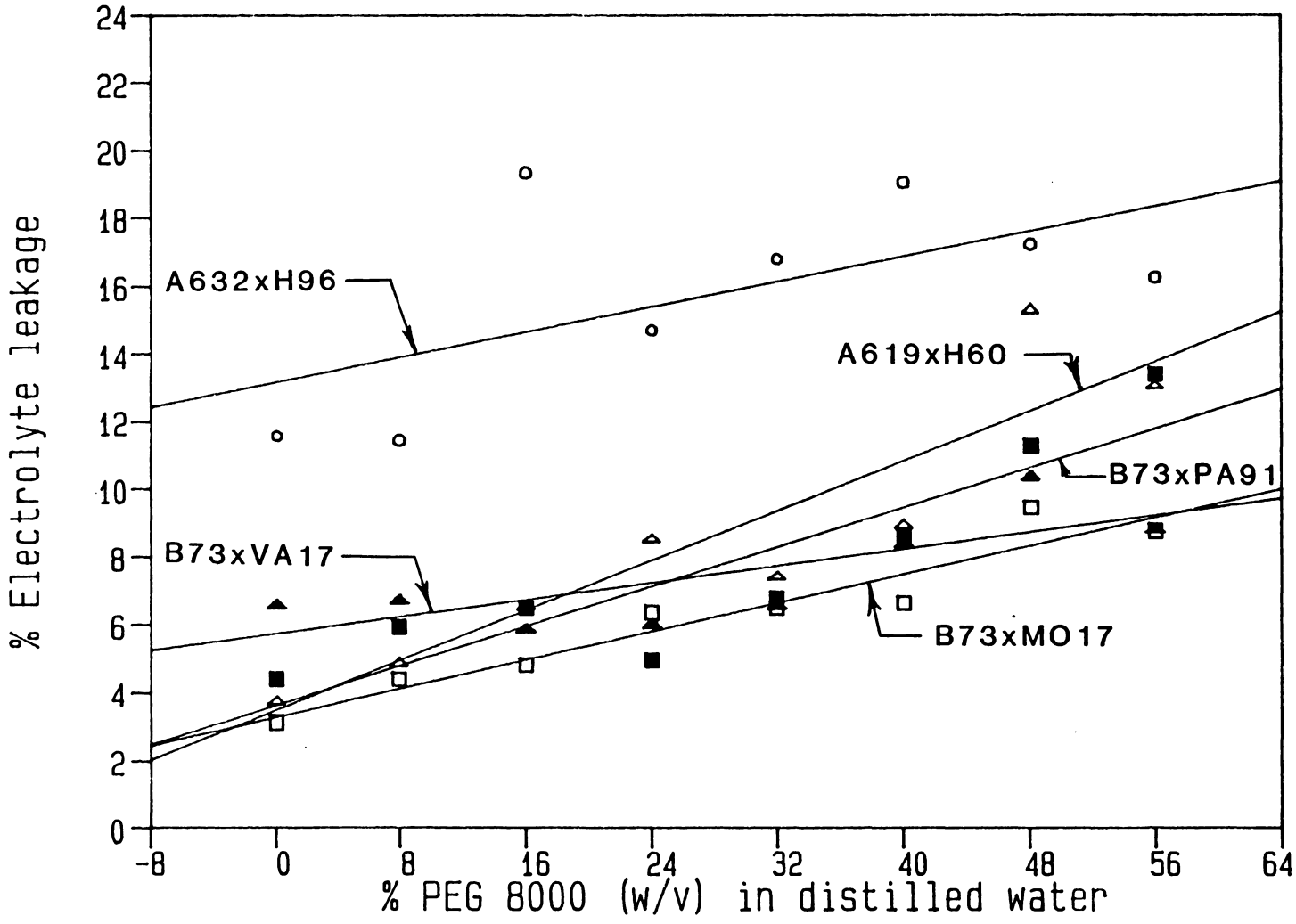
Table 4.5. Sterol concentration of steryl glycosides of leaves, stems and roots of five maize hybrids as affected by PEG. *, significantly different from the control (-0.1 MPa) at P=0.05; **, significantly different from the control (-0.1 MPa) at P=0.01.

Tissue	Hybrid	Osmotic Potential (MPa)	Sterol (µg/g DW)				Stig/Sitos
			Chol	Camp	Stig	Sitos	
	A619xH60	-0.1	7.9	36.9	30.2	179.2	0.17
		-0.4	3.5**	35.0	30.2	164.1	0.18
		-0.6	3.6**	52.8*	54.5**	255.2*	0.21
	B73xMO17	-0.1	10.5	66.9	64.3	310.0	0.21
		-0.4	7.5	88.3	83.4	398.1	0.21
		-0.6	3.8	60.9	64.4	288.9	0.22
Leaf	B73xPA91	-0.1	5.5	50.7	42.0	255.2	0.16
		-0.4	6.9	60.9	47.4	302.0	0.16
		-0.6	4.9	34.1	31.3	237.2	0.13
	B73xVA17	-0.1	2.0	32.1	23.1	106.7	0.22
		-0.4	1.9	37.4	28.7	125.2	0.23
		-0.6	2.7	35.9	28.4	123.9	0.23
	A632xH96	-0.1	4.5	42.3	39.1	276.8	0.14
		-0.4	4.5	55.6	54.3	321.5	0.17
		-0.6	5.1	49.8	59.2	295.0	0.20
	A619xH60	-0.1	4.0	142.1	87.0	556.1	0.16
		-0.4	6.1	143.2	92.3	538.4	0.17
		-0.6	3.5	135.9	102.4	490.7	0.21*
	B73xMO17	-0.1	3.9	130.9	112.5	490.6	0.23
		-0.4	4.0	120.1	100.8	451.1	0.22
		-0.6	5.1	96.1**	84.0**	389.4	0.22
Stem	B73xPA91	-0.1	4.3	132.0	59.8	352.9	0.17
		-0.4	4.1	93.7	56.9	362.9	0.16
		-0.6	4.5	110.4	69.7	347.4	0.20
	B73xVA17	-0.1	2.9	112.9	80.7	361.1	0.22
		-0.4	2.8	105.3	73.2	330.7	0.22
		-0.6	2.6	104.1	84.3	327.1	0.26**
	A632xH96	-0.1	3.5	95.1	55.9	328.4	0.17
		-0.4	6.7	76.3	42.2*	219.3	0.19
		-0.6	14.3*	88.5	51.6	265.6	0.19
	A619xH60	-0.1	3.5	126.1	115.3	149.4	0.77
		-0.4	5.0	110.8	104.6	120.9	0.87
		-0.6	4.2	96.3	97.4	96.9*	1.01
	B73xMO17	-0.1	2.6	134.3	122.3	104.2	1.17
		-0.4	3.5	139.6	155.2*	111.6	1.39*
		-0.6	3.6	135.4	140.4	103.5	1.36
Root	B73xPA91	-0.1	5.7	229.5	157.4	202.3	0.78
		-0.4	7.1	235.6	157.3	200.7	0.78
		-0.6	7.5	235.3	179.3	202.3	0.89**
	B73xVA17	-0.1	4.4	141.9	99.4	111.9	0.89
		-0.4	6.3	141.4	111.9	109.3	1.02
		-0.6	4.0	139.0	119.2*	97.4	1.22**
	A632xH96	-0.1	6.7	251.4	211.9	154.2	1.37
		-0.4	4.1*	162.3*	166.5	95.0*	1.75**
		-0.6	6.5	145.7*	166.2	101.4*	1.64*

Table 4.6. Sterol concentration of steryl esters of leaves, stems and roots of five maize hybrids as affected by PEG. *, significantly different from the control (-0.1 MPa) at P=0.05; **, significantly different from the control (-0.1 MPa) at P=0.01.

Tissue	Hybrid	Osmotic Potential (MPa)	Sterol (µg/g DW)				Stig/Sitos
			Chol	Camp	Stig	Sitos	
	A619xH60	-0.1	0.1	3.7	2.5	32.8	0.08
		-0.4	0.1	4.4	9.6**	18.7	0.51**
		-0.6	0.1	3.2	2.7	27.2	0.10
	B73xMO17	-0.1	0.1	6.4	4.6	54.0	0.09
		-0.4	0.1	5.6	4.7	50.4	0.09
		-0.6	0.1	8.9	7.0	69.3	0.10
Leaf	B73xPA91	-0.1	0.1	2.9	5.1	33.0	0.15
		-0.4	0.1	6.2	5.2	47.5	0.11
		-0.6	0.1	7.4	5.2	48.3	0.11
	B73xVA17	-0.1	0.1	10.6	13.4	63.6	0.21
		-0.4	0.1	10.2	12.3	59.2	0.21
		-0.6	0.1	8.7	10.6	49.7*	0.21
	A632xH96	-0.1	0.1	5.3	12.0	69.1	0.17
		-0.4	0.1	10.1**	15.9*	74.0	0.22
		-0.6	0.1	10.1**	10.9*	15.4**	0.71**
	A619xH60	-0.1	10.7	15.4	13.3	57.0	0.23
		-0.4	8.7	10.2	9.1	46.5	0.20
		-0.6	9.0**	6.0*	5.4	64.9	0.12*
	B73xMO17	-0.1	3.4	8.6.	11.5	22.2	0.64
		-0.4	5.4	6.4	7.5	13.0	0.51
		-0.6	7.6*	3.8*	4.5*	11.7	0.38
Stem	B73xPA91	-0.1	4.4	8.5	4.9	29.3	0.24
		-0.4	5.2	6.3	1.9*	29.2	0.07
		-0.6	6.0	4.2**	3.2	28.4	0.10
	B73xVA17	-0.1	8.0	16.5	15.6	20.1	1.02
		-0.4	10.8	9.3	8.5**	14.0	0.77
		-0.6	10.3.	6.1.	11.9*	7.6	0.96
	A632xH96	-0.1	7.1	14.7	18.5	46.2	0.40
		-0.4	6.0	8.3**	9.4	26.2	0.32
		-0.6	7.7	4.6**	2.8*	29.2	0.10**
	A619xH60	-0.1	22.0	48.2	87.7	150.7	0.58
		-0.4	11.8*	21.2*	55.6**	71.3*	0.78
		-0.6	16.3	22.5*	69.8	49.6**	1.41*
	B73xMO17	-0.1	6.8	39.0	87.6	40.3	2.17
		-0.4	4.3	30.1	80.7	36.3	2.22
		-0.6	1.5	26.9	78.1	28.6	2.73
Root	B73xPA91	-0.1	7.3	50.7	63.7	74.5	0.85
		-0.4	6.2	44.4	57.7	69.8	0.83
		-0.6	7.8	31.3	46.5	41.8	1.11**
	B73xVA17	-0.1	8.5	35.6	71.1	45.1	1.57
		-0.4	9.1	32.8	75.6	42.9	1.77
		-0.6	5.1	29.9	75.9	28.4	2.67
	A632xH96	-0.1	6.4	50.6	108.6	57.1	1.90
		-0.4	5.9	29.6*	70.0	24.6**	2.85**
		-0.6	8.0	27.2*	66.6*	25.3**	2.63*

Figure 4.2. Linear regression analysis of percent electrolyte leakage for PEG-treated leaf discs of maize hybrids, △, A619xH60, r^2=0.84; □, B73xM017, r^2=0.92; ○, A632xH96, r^2=0.36; ▲, B73xVA17, r^2=0.59; ■, B73xPA91, r^2=0.82.


% Electrolyte leakage
24
22
20
18
16
14
12
10
8
6
4
2
0
A632xH96
A619xH60
B73xPA91
B73xVA17
B73xMO17
-8
0
8
16
24
32
40
48
56
64
% PEG 8000 (w/v) in distilled water

Furthermore, the hybrids A632xH96 and B73xVA17, exhibited a higher and parallel electrolyte leakage compared to other hybrids. The initial percent electrolyte leakage was nearly identical in the hybrid grouping, A619xH60, B73xMO17 and B73xPA91, but varied at subsequent PEG concentrations. At the higher PEG concentrations, the order of percent electrolyte leakage among these hybrids was A619xH60 > B73xPA91 > B73xMO17. Maize hybrids appear to represent two distinct groupings in which the hybrids, A632xH96 and B73xVA17, differ from the other hybrid grouping, A619xH60, B73xMO17 and B73xPA91. A further distinction between these two groupings of hybrids, was the degree of positive correlation between the percent PEG and percent electrolyte leakage. There was a weak correlation (r^2=0.36, 0.59) between the percent PEG and the percent electrolyte leakage in the hybrids, A632xH96 and B73xVA17, compared to a reasonable degree of correlation (r^2=0.84, 0.92, 0.82) in the hybrids, A619xH96, B73xMO17 and B73xPA91.

Discussion

Dry weight of the leaves, stems and roots of the five maize hybrids (Tab. 4.1) changed following exposure to -0.4 and -0.6 MPa osmotic potentials. The hybrids, B73xVA17 and

A632xH96, were more sensitive to reduced osmotic potential compared to other hybrids based on the decrease in tissue dry weight. Leaf dry weight significantly decreased in both hybrids, whereas stem and root dry weight significantly decreased only in the hybrid, B73xVA17. Leaf elongation rate, among members of the Graminaceae, is very sensitive to plant water status (Van Volkenburg and Boyer 1985) and small reduction in water potential rapidly affect cell enlargement, and therefore dry matter accumulation (Acevedo et al. 1971). The water potential required to inhibit the expansion growth of plant organs of maize was -0.50, -1.00 and -1.40 MPa for stems, leaves and roots, respectively (Westgate and Boyer 1985). Therefore, osmotic potentials of -0.4 and -0.6 MPa, probably exerted a mild water stress on maize hybrids in the present study. The significant decrease in the dry weight in all tissues of B73xVA17, suggest that mild water stress inhibited growth to the greatest extent in this hybrid.

Dry weight also increased following mild water stress in the tissues of all hybrids, except B73xVA17. The root dry weight and root to shoot ratio significantly increased in the hybrids, B73xMO17 and B73xPA91, and slightly increased in the hybrids, A619xH60 and A632xH96. Enhanced root growth contributes to the increase in the root to shoot ratio observed in water-limiting conditions (Parsons 1979), and may represent an important adaptive

characteristic in some plants (Levitt 1980). Maize seedlings exposed to mild water stress in other studies, exhibited a net increase in root growth, in spite of lowered water potential, due to differential maintenance of turgor by osmotic adjustment (Sharp and Davies 1979, Westgate and Boyer 1985). It is possible that increased root dry weight in the hybrids, B73xMO17 and B73xPA91, reflects the differential abilities of these hybrids for osmotic adjustment of root tissue. Slight increases in the leaf and stem dry weight were also demonstrated by the hybrids, B73xPA91 and A632xH96, with reduced osmotic potential. A drought-induced osmotic regulation in the leaves was both diurnal and seasonal in field-grown maize and sorghum (Fereres et al. 1978). The slight increase in leaf and stem dry weight of the hybrid B73xPA91 at both osmotic potentials, and of the hybrid A632xH96 at the -0.4 MPa osmotic potential, may represent osmotic adjustment of these tissues. Therefore, the increase in dry weight in leaf, stem or root tissues of all hybrids, except B73xVA17, suggests the maintenance of growth by osmotic adjustment.

Mild water stress induced a striking increase in the TL concentration of roots, and a slight increase in the TL concentration of the leaves and stems of all maize hybrids (Tab. 4.2). TL concentration of roots was significantly increased ($P=0.01$) by mild water stress in all maize hybrids, except B73xPA91, in which the TL concentration

significantly increased only at -0.6 MPa. The sudden exposure of maize seedlings to PEG-induced osmotic stress may induce the greatest changes in the roots which are directly exposed to the reduced osmotic potential of the surrounding medium. However, root lipid concentration was not significantly altered in 28-day-old maize seedlings exposed to PEG-induced osmotic stress at -1.5 MPa in the lipid classes analyzed (triglycerides, phospholipids, free fatty acid, free sterol and steryl ester; Douglas and Paleg 1981). Therefore, the significant increase in TL concentration of roots of the five maize hybrids may reflect an increase in lipid classes not analyzed in this study or may represent the inclusion of PEG retained on the roots after washing and carried over to the lipid after extraction. Minor changes in lipid classes suggest that total FFA, FS, SG and SE concentrations (Tab. 4.2) were not significantly altered by osmotic treatments of -0.4 or -0.6 MPa. FFA concentration decreased slightly in all tissues at -0.6 MPa with the exception of leaves and roots, of the hybrids A632xH96 and B73xM017, respectively. The concentrations of total FS, SG and SE (Tab. 4.2) exhibited significant changes in the leaves and roots, but not stems, of only a few hybrids. The generalized decrease in FFA concentration, and the minor changes in the FS, SG and SE fractions suggest that either the intensity or duration of water stress was insufficent to stimulate significant

changes in lipid metabolism of maize hybrids.

Severe water stress, imposed for 48 hours using PEG in nutrient solution at -1.5 MPa, resulted in a significant increase in total FS in the stems and leaves, but only slight increases in the roots of 28-day-old maize seedlings (Douglas and Paleg 1981). Furthermore, four inductive periods of water stress were required to increase the free desmethyl- and methylsterol concentrations in roots of 5-day-old oat seedlings (Liljenberg et al. 1985). Two periods of water stress with interjacent rewatering, did not significantly alter the composition or concentration of free, glycosylated, or esterified sterols. However, after four periods of water stress, either a threshold level of stress or a cumulative stress history was attained, resulting in a 60% increase in both the methylsterols and steryl esters. Thus, slight initial shifts in the concentration of lipid classes of the five maize hybrids may not directly reflect the pattern of lipid changes under severe water stress. However, shifts in lipid metabolism observed under mild water stress may represent adaptation to water stress rather than degradative lipid changes associated with senescence of tissue and increased lipolytic activity under severe water stress.

A consistent change common to all maize hybrids was the increase in the stigmasterol to sitosterol ratio of the free, glycosidic and esterified sterols (Tabs 4.4, 4.5,

4.6). Environmental factors such as increased temperature (Davis and Finkner 1973), light (Bush et al. 1976), and salinity (Douglas and Walker 1983) induce shifts in this ratio either by conversion of sitosterol to stigmasterol (Navarri-Izzo and Izzo 1985) or synthesis via a common precursor (Grunwald 1985). In addition, the stigmasterol to sitosterol ratio increased in the roots of germinating maize seedlings (Kemp et al. 1967) and with increasing tissue age (Guens 1973, Grunwald 1975b). In the present study, a significant increase in the stigmasterol to sitosterol ratio was demonstrated in the root tissue of the hybrids, B73xVA17 and A632xH96. It is possible that an increase in the ratio of stigmasterol to sitosterol, following mild water stress, is indicative of reduced growth rate or enhanced senescence of root tissue. Stigmasterol to sitosterol ratio was also increased in citrus rootstocks exposed to salinity treatment and was correlated with a salt-induced reduction in growth rate of the rootstocks (Douglas and Walker 1983).

Genotypic differences were expressed in maize hybrids in response to mild water stress. No consistent differences in the lipid classes analyzed (FFA, FS, SG, and SE) were expressed in all tissues of maize hybrids. However, two general groupings emerged in which the hybrids, B73xVA17 and A632xH96, differed from the other hybrid grouping which included the hybrids, A619xH60,

B73xMO17, and B73xPA91. A similar growth response was expressed in the two hybrids, B73xVA17 and A632xH96, compared to other hybrids. Growth was inhibited to the greatest extent in these two hybrids based on the reduction in tissue dry weight. Similar changes in lipid concentration were also demonstrated in several lipid classes in the tissues of the hybrids, B73xVA17 and A632xH96. A further distinction can be made based on differences in the electrolye leakage of leaf discs following PEG-induced osmotic stress (Fig. 4.2). The hybrids, B73xVA17 and A632xH96, exhibited a greater percent electrolyte leakage than the hybrids, A619xH60, B73xMO17, and B73xPA91, especially at the higher PEG concentrations. Electrolyte leakage of leaf discs provides a measure of cell membrane stability and has been used as a measure of drought and heat tolerance in crop species (Blum and Ebercon 1981, Trapani and Gentinetta 1984, Trapani and Motto 1984). Maize hybrids, in the present study, differed in the adjustment of cellular membranes in leaves following osmotic shock. Leakage of solute increased upon rehydration in a desiccation-intolerant moss due to a greater structural disruption of the membrane systems (Stewart and Bewley 1982). The difference in maize hybrids may reflect both the ability to modify membrane lipids during water stress, and the stability of membranes following rehydration.

The complex response of plant lipid metabolism to water stress may involve a number of integrated changes in the cellular membranes. Genotypic differences in lipid content, and stress-induced changes during water-deficit and rehydration may be an important facet of drought tolerance in some crop species. This suggests that water-deficit-induced changes in lipid metabolism, in addition to genotypic differences, may play an important role in growth under water stress.

Chapter V

SUMMARY

Genotypic differences in dry weight and concentrations of free fatty acid (FFA), free sterol (FS), steryl glycoside (SG) and steryl ester (SE) were exhibited in the tissues of five maize hybrids (A619xH60, B73xMO17, B17xPA91, B73xVA17, A632xH96). Leaf dry weight was lower in B73xVA17 and A632xH96 compared to other hybrids, but only slight differences were observed in the stem or root tissue. Consequently, the root to shoot ratios were higher in B73xVA17, A632xH96, and B73xPA91 than in the other hybrids. Inherent differences in the total lipid (TL) and FFA concentrations were observed among the five hybrids. The TL and FFA concentrations in the leaves and roots of A619xH60, B73xMO17 and B73xPA91 were greater than those observed for either B73xVA17 or A632xH96. These differences were not apparent in the free, glycosidic or esterified steryl fractions.

The distributional pattern of fatty acid and sterol components was similar among the five maize hybrids in leaves, stems and roots. The FFA components, palmitic (16:0) or linoleic (18:2), were dominant in the roots whereas, linolenic (18:3) and linoleic (18:2), were dominant in the leaves and stems, respectively. Sterol

components associated with the free, glycosylated and esterified steryl fractions were also distributed in a distinctive manner in maize tissues. Sitosterol was the dominant sterol in the leaves and stems of all three steryl fractions. Stigmasterol was commonly esterified or found in the free form in the roots, while campesterol was usually glycosylated in this tissue. The total concentrations of FS and SE were proportionally higher in the leaves than the stems or roots. In contrast, the SG concentration was highest in the stems, followed by the roots and leaves.

Osmotic potentials of -0.4 and -0.6 MPa altered the dry weight, and FFA, FS, SG, and SE concentrations in the tissues of several maize hybrids. Leaf dry weight significantly decreased in B73xVA17 and A632xH96, but a significant reduction in stem and root dry weight occurred only in B73xVA17. Lowered osmotic potentials also increased the root dry weight of B73xMO17 and B73xPA91, and the root to shoot ratio of all hybrids except B73xVA17. Osmotically-induced increases in the TL concentration occurred primarily in the roots although slight increases were observed in the stems and leaves of all hybrids. In contrast, relatively small changes occurred in the FFA, FS, SG, and SE classes. FFA concentrations declined slightly in nearly all hybrid tissues. The total FS, SG and SE fractions were relatively unchanged by reduced osmotic potentials and the few significant changes were confined to

the leaves or roots. The qualitative composition of these steryl fractions changed to a greater extent in the root tissues, than either the stems or leaves, of maize hybrids. Of particular interest was an increase in the stigmasterol to sitosterol ratio in the hybrids, B73xVA17 and A632xH96.

Electrolyte leakage of leaf discs also differed among the five maize hybrids. B73xVA17 and A632xH96, exhibited a greater percent electrolyte leakage than A619xH60, B73xMO17 and B73xPA91, especially at high PEG concentrations. In addition, the degree of positive correlation between the percent PEG and percent electrolyte leakage differed among hybrids. There was a weak positive correlation in B73xVA17 and A632xH96, compared to a reasonable degree of correlation in the other hybrids. Electrolyte leakage provides a measure of cell membrane stability and increased leakage is a characteristic of drought sensitive crop species.

In the current study, maize hybrids differed inherently in maturity rate, relative development of root and shoot, and in the physiological and biochemical response of plant organs to mild osmotic stress. Two distinct groups were discernable in this study relative to growth characteristics, response to osmotic stress and electrolyte leakage. Hybrids B73xVA17 and A632xH96 were inherently slower maturing plants, were more responsive to osmotic stress, as indicated by greater reductions in dry matter

accumulation and changes in lipid components, and exhibited a higher percentage of electrolyte leakage of leaf discs than the hybrids, A619xH60, B73xMO17 and B73xPA91. Of particular interest in the two sensitive hybrids was the significant increase in the stigmasterol to sitosterol ratio in the root tissue of stressed plants. These shifts have often been correlated with reduced growth and senescence of plant tissues. However, another conceivable explanation is that shifts in these sterol ratios may confer some adaptive advantage to stress conditions. The hybrids B73xVA17 and A632xH96 may inherently be unable to withstand or adjust to the imposed osmotic stresses as readily as the more tolerant hybrids (A619xH60, B73xMO17 and B73xPA91). This lack of adjustment may be related to the relatively slow developmental rate of the sensitive hybrids compared to the tolerant hybrids and the time of imposition of the stress.

LITERATURE CITED

Acevedo, E., Fereres, E., Hsiao, T.C. & Henderson, D.W. 1979. Diurnal growth trends, water potential, and osmotic adjustment of maize and sorghum leaves in the field. - Plant Physiol. 64: 470-480.

Ackerson, R.C. 1983. Comparative physiology and water relations of two corn hybrids during water stress. - Crop Sci. 23: 278-283.

Armond, P.A., Bjorkman, O. & Staehelin, L.A. 1980. Dissociation of supramolecular complexes in chloroplast membranes. A manifestation of heat damage to the photosynthetic apparatus. - Biochim. Biophys. Acta 601: 433-442.

Bae, M. & Mercer, E.I. 1970. The effect of long- and short-day photoperiods on the sterol levels in the leaves of *Solanum andigena*. - Phytochemistry 9: 63-68.

Bell, F.P. 1975. Cholesterol exchange between the microsomal, mitochondrial and erthyrocyte membranes and its enhancement by cytosol. - Biochim. Biophys. Acta 398: 18-27.

Biswas, A.K., Sarkar, U. & Chaudhuri, M.A. 1983. Water-stress induced changes in total steroids and cholesterol contents in wheat seedlings. - Sci. Cult. 49: 109-110.

Blum, A. & Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. - Crop Sci. 21: 43-47.

Bush, P.B., Grunwald, C. & Davis, D.L. 1971. Changes in sterol composition during greening of etiolated barley shoots. - Plant Physiol. 47: 745-749.

Chapman, D. 1975. Phase transitions and fluidity characteristics of lipids and cell membranes. - Q. Rev. Biophys. 8: 185-235.

- , De-Felice, J. & Barber, J. 1983. Influence of winter and summer growth conditions on leaf membrane lipids of *Pisum sativum* L. - Planta 157: 218-223.

Chetal, S., Wagle, D.S. & Nainwatee, H.S. 1981. Glycolipid changes in wheat and barley chloroplast under water stress. - Plant Sci. Lett. 20: 225-230.

- , Wagle, D.S. & Nainwatee, H.S. 1983. Phospholipid changes in wheat and barley chloroplast under water stress. - Plant Sci. Lett. 29: 273-278.

Clark, R.B. 1982. Nutrient solution growth of sorghum and corn in mineral nutrition studies. - J. Plant Nut. 5: 1039-1057.

Cocucci, M. & Ballarin-Denti, A. 1981. Effect of polar lipids on ATPase activity of membrane preparations from germinating radish seeds. - Plant Sci. Lett. 17: 391-400.

Cossins, A.R. 1980. The adaptation of membrane dynamic structure to temperature. -*In* Membrane Fluidity in Biology (R. C. Aloia, ed.), pp. 83- 106. Academic Press, New York.

Davis, D.L. & Finkner, V.C. 1972. Influence of temperature on sterol biosynthesis in *Triticum aestivum*. - Plant Physiol. 52: 324-326.

De La Roche, I.A., Andrews, G.J. & Kates, M. 1973. Changes in phospholipid composition of a winter wheat cultivar during germination at 2 C and 24 C. - Plant Physiol. 51: 468-473.

Demel, R.A. & De Kruyff, B. 1976. The function of sterols in membranes. - Biochim. Biophys. Acta 457: 109-132.

Douce, R. & Joyard, J. 1980. Plant galactolipids. - *In* The Biochemistry of Plants (P.K. Stumpf, ed.), vol. 4, pp. 321-362. Academic Press, New York. ISBN 0-12-675404-7.

Douglas, T.J. & Paleg, L.G. 1981. Lipid composition of *Zea mays* seedlings and water stress-induced changes. - J. Exp. Bot. 32: 499-508.

- & Walker, R.R. 1983. 4-Desmethylsterol composition of citrus rootstocks of different salt exclusion capacity. - Physiol. Plant. 58: 69-74.

- & Walker,R.R. 1984. Phospholipids, free sterols and adenosine triphosphatase of plasma membrane-enriched preparations from roots of citrus genotypes differing in chloride exclusion ability. - Physiol. Plant. 62: 51-58.

Eichenburger, W. 1977. Steryl glycosides and acylated steryl glycosides. - *In* Lipids and Lipid Polymers in Higher Plants (M. Tevini and H.K. Lichtenthaler, eds), pp. 169-182. Springer-Verlag, Berlin. ISBN 3-540-082-1-8.

Erdei, L., Stuiver, C.E.E. & Kuiper, P.J.C. 1980. The effect of salinity on lipid composition and on activity of Ca^{2+}- and Mg^{2+}-stimulated ATPase in salt-sensitive and salt tolerant *Plantago* species. - Physiol. Plant. 49: 315-319.

Fereres, E., Acevedo, E., Henderson, D.W. & Hsiao, T.C. 1978. Seasonal changes in water potential and turgor maintenance in sorghum and maize under water stress. - Physiol. Plant. 44: 261-267.

Ferguson, C.H.R. & Simon, E.W. 1966. Salt-induced changes in the composition of lipid classes in barley roots. - Can. J. Plant Sci. 46: 639-646.

Ferrari-Iliou, R., Pham Thi, A.T. & Viera da Silva, J. 1984. Effect of water stress on the lipid and fatty acid composition of cotton (*Gossypum hirsutum*) chloroplasts. - Physiol. Plant. 62: 219-224.

Folch, J., Lees, M. & Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. - J. Biol. Chem. 226: 497-509.

Ford, R.C. & Barber, J. 1983. Incorporation of sterol into chloroplast thylakoid membranes and its effect on fluidity and function. - Planta 158: 35-41.

Gates, C.T. 1972. Ecological response of the Australian native species *Acacia harpophylla* and *Atriplex nummularia* to soil salinity: Effects on water content, leaf area, and transpiration rate. - Aust. J. Bot. 20: 261-272.

Gaunt, J.K. & Stowe, L.M. 1967. Analysis and distribution of tocopherols and quinones in the pea plant. - Plant Physiol. 42: 851-858.

Giddings Jr, T.H. & Hanson, A.D. 1982. Water stress provokes a generalized increase in phosphatidylcholine turnover in barley leaves. - Planta 155: 493-501.

Giles, K.L., Beardsell, M.F. & Cohen, D. 1974. Cellular and ultrastructural changes in mesophyll and bundle sheath cells of maize in response to water stress. - Plant Physiol. 54: 208-212.

Goodwin, T.W. & Mercer, E.I. 1983. Terpenes and Terpenoids. - *In* Introduction to Plant Biochemistry, pp. 400-464. Pergamon Press, Oxford. ISBN 0-08-024921-3.

Gronewald, J.W., Abou-Khalil, W., Weber, E.J. & Hanson, J.B. 1982. Lipid composition of a plasma membrane enriched fraction of maize roots. - Phytochemistry 21: 859-862.

Grossman, K., Weiler, E.W. & Jung, J. 1985. Effects of different sterols on the inhibition of cell culture growth caused by the growth retardant tetracyclais. -Planta 164: 370-375.

Grünwald, C. 1968. Effect of free sterols on the permeability of alcohol-treated red beet tissue. - Plant Physiol. 43: 484-488.

- 1971. Effects of free sterols, steryl ester, and steryl glycoside on membrane permeability. - Plant Physiol. 48: 653-655.

- 1974. Sterol molecular modifications influencing membrane permeability. - Plant Physiol. 54: 624-628.

- 1975a. Plant sterols. - Ann. Rev. Plant Physiol. 26: 209-236.

- 1975b. Phytosterols in tobacco leaves at various stages of physiological maturity. - Phytochemistry 14: 79-82.

- 1978. Shading influence on the sterol balance of *Nicotiana tabacum* L. - Plant Physiol. 61: 76-79.

- 1985. *In vivo* synthesis of stigmasterol in *Nicotiana tabacum*. - Phytochemistry 24: 2915-2918.

Guens, J.M.C. 1973. Variations in sterol composition in etiolated mung bean seedlings. - Phytochemistry 13: 474-488.

Hanson, A.D., Nelsen, C.E. & Everson, E.H. 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. - Crop Sci. 17: 724-726.

Hansson, G., Kuiper, P.J.C. & Kylin, A. 1973. Effect of preparation methods on the induction of (sodium and potassium)-activated adenosine triphosphatase from sugar beet root and its lipid composition. - Physiol. Plant. 28: 430-435.

Harwood, J.L. 1980. Plant acyl lipids: structure, distribution, and analysis. - *In* The Biochemisty of Plants (P.K. Stumpf, ed.), vol. 4, pp. 1-55. Academic Press, New York. ISBN 0-12-675404-7.

Harzallah-Skhiri, F., Guillot-Salamon, T. & Signol, M. 1980. Lipid changes in plastids isolated from alfalfa seedlings grown under salt stress. - *In* Biogenesis and Function of Plant Lipids (P. Mazliak, P. Benvensiste, C. Costes and R. Douce, eds), pp. 99-102. Elsevier/ North-Holland Biomedical Press, Amsterdam. ISBN 0-444-80273-8.

Henry, M.J. 1985. Plant growth regulating activity of sterol and gibberellin biosynthesis inhibitors. - Plant Growth Reg. Bull. 13: 9-11.

Hirayama, O. & Nushida, T. 1978. Effects of lipids and their related compounds on chloroplast functions. - Agric. Biol. Chem. 42: 141-146.

Jackson, P.C. & St. John, J.B. 1984. Anesthetics alter the lipid composition of barley root membranes. - Planta 162: 415-421.

Jellum, M.D. 1966. Fatty acid composition of corn oil of parental inbreds and reciprocal crosses. - J. Hered. 57: 243-248.

Jenison, J.R., Shank, D.B. & Penny, L.H. 1981. Root characteristics of 44 maize inbreds evaluated in four environments. - Crop Sci. 21: 235-237.

Jones, M.M. & Turner, N.C. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. - Plant Physiol. 61: 122-126.

Kalinowska, M. & Wojciechowski, Z.A. 1986. Modulation of activities of steryl glucoside hydrolase and UDPG:sterol glucosyltransferase from *Sinapis alba* by detergents and lipids. - Phytochemistry 25: 45-49.

Kemp, R.J., Goad, L.J. & Mercer, E.I. 1967. Changes in the levels and composition of the esterified and unesterified sterols of maize seedlings during germination. - Phytochemistry 6: 1609-1615.

- & Mercer, E.I. 1968. Studies on the sterols and sterol esters of the intracellular organelles of maize shoots. - Biochem. J. 110: 119-125.

Ketchie, D.O. & Kuiper, P.J.C. 1979. Fatty acid levels in apple leaves of different age as affected by temperature. - Physiol. Plant. 46: 93-96.

Kirkman, M.B., Gardner, W.R. & Gerloff, G.C. 1974. Internal water status of kinetin-treated salt-stressed plants. - Plant Physiol. 53: 241-243.

Kuiper, D. & Kuiper, P.J.C. 1978. Lipid composition of the roots of *Plantago* species: Response to alteration of the level of mineral nutrition and ecological significance. - Physiol. Plant. 44: 81-86.

Kuiper, P.J.C. 1968. Lipids in grape roots in relation to chloride transport. - Plant Physiol. 43: 1367-1371.

- 1980. Lipid metabolism as a factor in environmental adaptation. - *In* Biogenesis and Function of Plant Lipids (P. Mazliak, P. Benveniste, C. Costes and R. Douce, eds), pp. 169-176. Elsevier/North-Holland Biomedical Press. ISBN 0-444-80273-8.

- 1985. Environmental changes and lipid metabolism of higher plants. - Physiol. Plant. 64: 118-122.

Lechevalier, D. 1977. Effets du polyethylene glycol sur les lipids et les lipochromes des colonies de Spirodele. - Physiol. Veg. 15: 387-402.

Lehoczki, E., Polos, E., Laskay, G., & Farkas, T. 1985. Chemical compositions and physical states of chloroplast lipids related to atrazine resistance in *Conyza canadensis* L. -Plant Sci. 42: 19-24.

Le Rudulier, D., Strom, A.R., Dandekar, A.M., Smith, L.T. & Valentine, R.C. 1984. Molecular biology of osmoregulation. Science 224: 1064-1068.

Levitt, J. 1980. Water stress. - *In* Responses of Plants to Environmental Stress, Vol. 2, pp. 25-211. Academic Press, New York. ISBN 0-12-445502-6.

Li, P.H. 1985. Potato cold hardiness and freezing stress. - *In* Cellular and Molecular Biology of Plant Stress (J.L. Key and T. Kosuge, eds), pp. 201-216. Alan R. Liss. ISBN 0-8451-2621-0.

Liljenberg, C. Karunen, P. & Ekman, R. 1985. Changes in steryl lipids of oat cells as a function of water-deficit stress. - Physiol. Plant. 63: 253-257.

- & Kates, M. 1985. Changes in lipid composition of oat root membranes as a function of water-deficit stress. - Can. J. Cell Biol. In Press.

Lyons, J. M. & Raison, J.K. 1970. Oxidation activity of mitochondria isolated from plant tissue sensitive and resistant to chilling injury. - Plant Physiol. 45: 386-389.

- & Breidenbach, R.W. 1979. Strategies for altering chilling sensitivity as a limiting factor in crop production. - *In* Stress Physiology in Crop Plants (H. Mussel and R.C. Staples, eds), pp. 179-196. John Wiley and Sons, New York. ISBN 0-471-03809-1.

Mazliak, P. 1977. Glyco- and Phospholipids of Biomembranes - *In* Lipids and Lipid Polymers in Higher Plants (M. Tevini and H.C. Lichtenthaler, eds.), pp. 48-74. Springer-Verlag, New York. ISBN 3-540-08201-8.

McClane, J.M. 1986. A diallel study of flowering and of ear components of yield in corn belt maize and their interactions with population density. Ph.D. thesis, Virginia Poly. Tech. Inst. and State Univ., Blacksburg, VA.

McKersie, B.D. & Thompson, J.E. 1979. Influence of plant sterols on the phase properties of phospholipid bilayers. - Plant Physiol. 63: 802-805.

McMurchie, E.J. & Raison, J.K. 1979. Membrane fluidity and its effect on the activation energy of membrane-associated enzymes. - Biochim. Biopys. Acta 554: 364-374.

Misra, S., Choudhury, A. Dutta, A.K. & Ghosh, A. 1984. Sterols and fatty acids from three species of Mangrove. - Phytochemistry 23: 2823-2827.

Moore, L.D. & Orcutt, D.M. 1982. Free sterol and total lipids in stems of susceptible and resistant tobacco cultivars colonized by *Phytophthora parasitica* var. *nicotianae*. - Phytopathology 72: 1048-1051.

Morris, G.J. & McGrath, J.J. 1981. The response of multicellular liposomes to freezing and thawing. Cryobiology 18: 390-398.

Müller, M. & Santarius, K.A. 1978. Changes in chloroplast membrane lipids during adaptation of barley to extreme salinity. - Plant Physiol. 62: 326-329.

Navari-Izzo, F. & Izzo, R. 1984. *In vivo* conversion of [4-^{14}C]sitosterol and [22,23-^{3}H]sitosterol to stigmasterol in barley seedlings. - Phytochemistry 23: 769-772.

Nichols, B.W. 1963. Separation of the lipids of photosynthetic tissues: Improvement in the analysis by thin-layer chromatography. - Biochim. Biophys. Acta 70: 417-422.

- 1964. Separation of plant phospholipids and glycolipids. - *In* New Biochemical Separations (A.T. Jones and L.J. Morris, eds.), pp. 321-337. Van Norstand, New York.

Nir, I., Poljakoff-Mayber, A. & Klein, S. 1970. The effect of water stress on mitochondria of root cells. - Plant Physiol. 45:173-177.

Orcutt, D.M. & Patterson, G.W. 1974. Effect of light intensity upon lipid composition of *Nitzschia closterium* (*Cylindroteca fusiformis*). - Lipids 9: 1000-1003.

Parks, L.W., Bottema, C.D.K. & Rodriguez, R.J. 1984. Physical and enzymic studies on the function of sterols in fungal membranes. - *In* Isoprenoids in Plants: Biochemistry and Function (W.D. Nes, G. Fuller, and L-S Tsai, eds.), pp. 433-452. Marcel Dekker, New York.

Parsons, L.R. 1979. Breeding for drought resistance: What plant characteristics impart resistance? - HortScience 14: 590-593.

Pham Thi, A.T., Borrel-Flood, C., Viera da Silva, J., Justin, A.M. & Maziak, P. 1985. Effects of water stress on lipid metabolism in cotton leaves. - Phytochemistry 24: 723-727.

Quinn, P.J. & Williams, W.P. 1978. Plant lipids and their role in membrane function. - Prog. Biophys. Mol. Biol. 34: 109-173.

1983. The structural role of lipids in photosynthetic membranes. - Biochim. Biophys. Acta 737: 223-266.

Robertson, R.N. 1983. The Lively Membranes. pp. 206. Cambridge University Press, Cambridge. ISBN 0-521-23747-5.

Scarpa, A. & De Grier, J. 1971. Cation permeability of liposomes as a function of the chemical composition of the lipid bilayers. - Biochim. Biophys. Acta 241: 789-796.

Sharp, R.E. & Davies, W.J. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. - Planta 147: 43-49.

Sikorska, E. & Kacperska-Palacz, A. 1979. Phospholipid involvement in frost tolerance. - Physiol. Plant. 47: 144-150.

- & Farkas, T. 1982. Sterols and frost hardening of winter rape. - Physiol. Plant. 56: 349-352.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. - New Phytol. 73: 377-420.

Sitte, P. 1977. Functional organization of biomembranes. - *In* Lipids and Lipid Polymers in Higher Plants (M. Tevini and H.C. Lichtenthaler eds.), pp. 1-27. Springer-Verlag, New York. ISBN 3-540-08201-8.

Smith, J.S.C., Goodman, M.M. & Stuber, C.W. 1985. Genetic variability within U.S. maize germplasm: Widely-used inbred lines 1970-1979. - Crop Sci. 25: 681-685.

Smolenska, G. & Kuiper, P.J.C. 1977. Effect of low temperature upon lipid and fatty acid composition of roots and leaves of winter rape plant. - Physiol. Plant. 41: 29-35.

Stewart, R.R.C. & Bewley, J.D. 1982. Stability and synthesis of phospholipids during desiccation and rehydration of a desiccation-tolerant and desiccation-intolerant moss. - Plant Physiol. 69: 724-727.

Stuiver, C.E.E., Kuiper, P.J.C. & Marschner, H. 1978. Lipids from bean, barley and sugar beet in relation to salt resistance. - Physiol. Plant. 42: 124-128.

- , Kuiper, P.J.C., Marschner, H. & Kylin, A. 1981. Effects of salinity and replacement of K^+ by Na^+ on lipid composition in two sugar beet inbred lines. - Physiol. Plant. 52: 77-82.

Tevini, M. 1977. Light, function and lipids during plastid development. - *In* Lipids and Lipid Polymers in Higher Plants (M. Tevini and H.K. Lichtenthaler eds), pp. 121-145. Springer-Verlag, New York. ISBN 3-540-08201-8.

Thompson, G.A. 1983. Molecular responses of membranes to stress. *In* Molecular and Physiological Aspecits of Stress in Plants (A.C. Purvis, ed.), pp. 7-27. Proc. South. Sec. Am. Soc. Plant Physiol., Knoxville, TN.

Tollenaar, M., Muldoon, J.F. & Daynard, T.B. 1984. Differences in rates of leaf appearance among maize hybrids and phases of development. - Can. J. Plant Sci. 64: 759-763.

Trapani, N. & Gentinetta, E. 1984. Screening of maize genotypes using drought tolerance tests. - Maydica 29: 89-100.

- & Motto, M. 1984. Drought tolerance tests in maize. - Maydica 29: 325-334.

Trelease, R.N. 1969. Ultrastructural characteristics, compostion, and utilization of lipid bodies in the maize shoot apex during postgerminative development (abstr.) - J. Cell Biol. 43: 147a.

Van Volkenburgh, E. & Boyer, J.S. 1985. Inhibitory effects of water deficit on maize leaf elongation. - Plant Physiol. 77: 190-194.

Wattenberg, B.W. & Silbert, D.F. 1983. Sterol partitioning among intracellular membranes. - J. Biol. Chem. 258: 2284-2289.

Weber, E.J. 1983. Variation in corn (*Zea mays* L.) for fatty acid compositions of triglycerides and phospholipids. - Biochem. Genet. 21: 1-13.

Werb, Z. & Cohn, Z.A. 1971. Cholesterol metabolism in the macrophage. - J. Exp. Med. 134: 1545-1569.

Westgate, M.E. & Boyer, J.S. 1985. Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. - Planta 164: 540-549.

Wood, A. & Paleg, L.G. 1972. The influence of gibberillic acid on the permeability of model membrane systems. - Plant Physiol. 50: 103-108.

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