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# Membrane stabilization and desiccation tolerance during seed corn (Zea mays L.) drying

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**Membrane stabilization and desiccation tolerance during seed corn (*Zea mays* L.) drying**

Chen, Yuguang, Ph.D.

Iowa State University, 1990

Membrane stabilization and desiccation tolerance

during seed corn (Zea mays L.) drying

by

Yuguang Chen

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

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# INTRODUCTION

**Significance and objectives** Seed corn ears are
normally harvested at seed moisture contents as high as 400 g
$H_2O$ $kg^{-1}$ fresh weight and then mechanically dried to 110-120 g
$H_2O$ $kg^{-1}$ moisture in order to minimize deterioration due to
microorganisms, to avoid possible freezing injury and to
facilitate early harvest, conditioning and storage of the
seed. However, high-temperature (>45°C) drying of the high-
moisture seed has been associated with reduced seed quality.
In general, reductions in seed quality are often accompanied
by increased leaching of electrolytes and sugars from the
seed when soaked in water. This greater permeability upon
rehydration is likely an expression of decreased membrane
integrity. To improve the seed quality, it is common to
precondition (expose to ambient air following harvest) the
ears of high-moisture corn prior to high-temperature drying.
Previous studies (Herter and Burris, 1989b) have shown that
preconditioning can improve the seed quality following
subsequent drying. Preconditioning does not require a
substantial loss of moisture but appears to be a metabolic
process. Thus, the preconditioning treatment should be a
good system in which to study the induction of high-
temperature desiccation tolerance of maturing maize seed.
Understanding the physiological and biochemical basis for
this process should provide a much better understanding of

both desiccation and membrane performance during seed corn
drying. This understanding will allow the consistent
production of high quality seed and give some background
information on stress tolerance in seed production. The
research investigates the preconditioning phenomena with the
objective of determining the effects of preconditioning on
the membrane stabilization and the induction of desiccation
tolerance during seed drying in maize. The specific
objectives are:

1. To determine the effects of preconditioning methods
on seed quality and membrane integrity.

2. To determine the metabolic changes related to the
membrane stabilization during preconditioning in order to
understand the mechanism of preconditioning.

3. To study the relationship of ABA and protein
accumulation to the induction of desiccation tolerance.


· **Background information** High drying temperatures of 45
and 50°C adversely affect seed germination and seedling vigor
of corn (Navratil and Burris, 1984). The genetic variation
in susceptibility to injury by the high drying temperature
has also been reported (Seyedin et al., 1984; Herter, 1987).
Certain maternal inbreds, such as B73 and Mo17, are
intolerant of high drying temperatures, whereas others, such
as A632, are relatively tolerant. The effects of drying

temperature on membrane integrity indicated by sugar leakage and conductivity were studied (Navratil and Burris, 1984; Seyedin et al., 1984). Seed harvested over a range of 490-520 g $H_2O$ $kg^{-1}$ moisture and dried at 50°C leached significantly more sugars into distilled water than did those dried at 35°C. The comparison of the electrical conductivity of the leachate from low (35°C) and high (50°C) temperature dried seed measured at 25°C indicated statistical differences at the 1% level (Seyedin et al., 1984). Significant differences were also noted for drying temperatures, harvest moisture, and sample intervals, averaged across inbreds (Herter, 1987). The conductivity increased with progressive sample intervals, but decreased with a reduction in seed moisture content. The reductions in seed vigor correlated with an increase in electrolyte leakage during drying, which indicated an alteration in cellular membrane permeability (Herter and Burris, 1989a). Dehydration enhances the leakage of cytoplasmic electrolytes by inducing one of two possible types of membrane damage (Simon, 1977). The first type of membrane damage assumes that the membrane is repulsed during cellular collapse to form large discontinuities in the lipid bilayer. An alternative model is that dehydration induces an alteration in the hydrophobic-hydrophilic interaction within the membrane so that the structure of the cellular membranes below 200 g $H_2O$ $kg^{-1}$ hydration is different than that above

290 g $H_2O$ $kg^{-1}$ hydration. No direct evidence, however, has proven the above hypothesis. McKersie and Stinson (1980) reported that dehydration resulted in a reorganization of the cellular membranes in dehydration-sensitive seed. This reorganization did not involve a lipid phase change, but may result in changes in membrane integrity.

Nevertheless, membrane integrity appears to play a role in post-harvest drying of seed (Herter and Burris, 1989ab), and in the desiccation of other organelles such as pollen (Hoekstra and Roekel, 1988; Hoekstra et al., 1989). What may be critical features of desiccation tolerance are the abilities: (a) to limit damage during desiccation; (b) to maintain physiological integrity of membranes in the dry state so that metabolism can be reactivated quickly upon rehydration; and (c) to provide a repair mechanism upon rehydration, in particular, to regain integrity of membranes and membrane-bound organelles. Thus, protection against extensive damage may be attributed to a membrane active agent such as sugars and/or certain ions, and the capacity to repair elicited damage is also important. Therefore, a central question related to high-temperature desiccation tolerance concerns the nature of the tolerance mechanism.

Several compounds are synthesized in tolerant organisms during drying (Clegg, 1986; Crowe and Crowe, 1982, 1986a; Panek et al., 1986). Compounds such as glycerol, glycogen,

trehalose were observed to accumulate in various tolerant organisms (Bewley, 1979; Crowe and Crowe, 1986a; Crowe et al., 1987). When enzymes, structural proteins, nucleic acid, macromolecular complexes, etc., are desiccated in their native state, the integrity of the molecules can be retained if some water remains associated with them to prevent the formation of unfavorable conformations or fragmentation. Hence, the production or availability of substances (e.g., sugar, polyols, amino acid, anions) to maintain bound water content could be an important feature of membrane stabilization. Crowe and Crowe (1982, 1986a) reported that in anhydrobiotic organisms, tolerance to dehydration is associated with stabilization of the lipid membranes by the disaccharide, trehalose. They suggested that the trehalose acts as a spacer preventing contact between the drying membrane thus inhibiting fusion which could result in a membrane lipid phase change. Further, Leopold and Vertucci (1986) and Koster and Leopold (1988) working on imbibing soybean seed suggested that the desiccation tolerant state may be one in which some tissue components are able to resist crystallization. Polyols or sugars may provide this protective effect only if they do not become crystallized. Membrane stabilization under these conditions requires that: (a) some polyol or sugar must be present to protect the hydrophilic membrane components during drying, and (b) the

sugar components must be resistant to crystallization during water loss. Above all, it appears reasonable to presume that membranes are the sensitive site in desiccation damage and that tolerance to desiccation involves the provision for some membrane protection.

The role of membranes themselves in stress-inducing environments has been well studied (Quinn, 1983; Raison et al., 1982; Raison and Wright, 1983; Raison, 1985; Raison and Orr, 1986; Thompson, 1985). Membrane-mediated processes are often particularly sensitive to stress, and membranes are the only structural part of cells capable of substantial modification in response to an imposed stress (Thompson, 1985). Specific interests focus on the membrane phospholipids because of their major structural roles in most membranes. Many organisms can acclimate to environmental stresses by altering their membrane lipid composition. The proportion of different lipid classes in a given membrane can be significantly different in cells exposed to different environmental conditions (Thompson, 1983). Based on the model proposed by Quinn (1983), the adaptive changes in cell membranes upon environmental perturbation are related to the stability of the membranes which depend on an appropriate balance of bilayer/nonbilayer forming lipids. It is known that phosphatidylcholine (PC) tends to form a bilayer configuration under physiological conditions, whereas

phosphatidylethanolamine (PE) orients into an inverted

hexagonal ($H_{II}$) configuration (Quinn, 1983). Moreover, the

nature of the polar moiety of lipids can affect the physical

properties such as thermal responses of the membrane (Chapman

and Wallach, 1968; Chapman et al., 1986). A relatively

direct method of detecting lipid thermal properties (phase

transition) is by differential scanning calorimetry (DSC)

(McElhaney, 1982). Although there is no precise physical

description of the molecular events accompanying a phase

transition in biological membranes, the thermal response of

the membrane has been related to the sensitivity of a plant

to changes in environmental temperature (Crowe and Crowe,

1986b; Crowe et al., 1989; Orr and Raison, 1987; O'Neill and

Leopold, 1982; Raison et al., 1982; Raison and Wright, 1983;

Raison, 1985; Raison and Orr, 1986). Increases in PC/PE

ratio result in a more fluid lipid matrix which tends to

lower the phase transition temperature of the membrane

fraction under drought stress (Liljenberg and Kales, 1985;

Vigh et al., 1986). In membranes with uniform fatty acid

composition, PC forms a less tightly packed structure than PE

(McElhaney, 1982). Therefore, the changes in phospholipid

composition and thermal properties may be important to high-

temperature desiccation tolerance and other acclimation

processes.

The application of molecular biology techniques to

studying plasma membrane functions in higher plants is only now beginning (Sussman and Harper, 1989). An important aspect of the induction of desiccation tolerance has been related to the induction of specific proteins. Environmental stress may cause alterations in gene expression resulting in the induction of new proteins and repression of at least some normally expressed proteins (Sachs and Ho, 1986). It is believed that these stress-induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with the stress conditions. Kermode et al. (1985, 1989) reported that seed drying during development plays an important role in redirecting metabolism from a developmental to a germinative mode, i.e., from desiccation sensitive to insensitive. This change from development to germination may be induced by premature desiccation and is mirrored by a change in the pattern of soluble protein synthesis. Seeds desiccated during the desiccation-tolerant stage, however, resume protein synthesis at almost the control levels. Recently, stress-induced proteins, such as heat shock proteins, were correlated to stress tolerance in plants (Cooper and Ho, 1983; Cooper et al., 1984; Heuss-LaRose et al., 1987; Sachs and Ho, 1986). However, the specific function of these stress-induced proteins is the focal point of current research (Altschuler and Mascarenhas, 1982; Bonham-Smith et al., 1987, 1988). Other specific proteins

during seed development such as globulin (Kriz and Schwartz, 1986; Kriz, 1990) and late embryogenesis-abundant (Lea) proteins (Baker et al., 1988; Harada et al., 1989) might have some function in the induction of desiccation tolerance and membrane stabilization based on their unusual sequences (Baker et al., 1988). Morever, abscisic acid (ABA), a hormone known to inhibit the germination of mature embryos, was also found to have a role in regulation of the protein accumulation during seed development as related to precocious germination (Eisenberg and Mascarenhas, 1985; Kriz, 1990; Quatrano, 1986). The ABA regulation of storage protein synthesis is at both transcriptional and translational level (Eisenberg and Mascarenhas, 1985). This ABA effect may be related to physiological events, such as seed desiccation, during the late stage of seed development (Kermode et al., 1989). Therefore, it is suggested that ABA (synthesized within the surrounding seed tissues or supplied by mother plant through the vascular supply in the seed coat) maintains embryo metabolism in a developmental, i.e., largely anabolic mode (Kermode and Bewley, 1985ab). Changes in endogenous ABA levels might also provide a signal for synthesis of specific proteins in the induction of desiccation tolerance during seed corn preconditioning.

Currently, researchers use the preconditioning process in maturing maize seed as a system to study membrane

stabilization and desiccation tolerance during seed corn drying. The role of soluble sugars in membrane stabilization, and changes in membrane lipid composition and thermal properties as related to desiccation tolerance will be presented. Further, the role of ABA and desiccation-induced proteins in the induction of high-temperature desiccation tolerance and their relationship to membrane stabilization during post-harvest drying of seed corn will be discussed.

# PART I.

# THE ROLE OF CARBOHYDRATES IN DESICCATION TOLERANCE AND MEMBRANE BEHAVIOR IN MATURING MAIZE SEED

## ABSTRACT

Seed corn (_Zea mays_ L.) ears harvested at seed
moisture contents greater than 400 g $H_2O$ $kg^{-1}$ fresh weight
(fw) are sensitive to rapid, high-temperature (45 to 50°C)
drying, but tolerant to low-temperature drying. A
preconditioning process that precludes this injury without
major moisture loss was used to study the nature of the
drying damage and the role of soluble sugars in membrane
stabilization during drying of two seed corn hybrids. Ears
were harvested at moisture contents of 550, 450, and 400 g
$H_2O$ $kg^{-1}$ fw, and preconditioned at 35°C for 6 to 48 h before
drying at 50°C. Seed germination was correlated with
leachate conductivity (r=-0.79) and sugar leakage (r=-0.80)
after different times of preconditioning indicating the
involvement of membrane function in the damage caused by
high-temperature drying. Total soluble-sugar concentration
decreased during preconditioning with no significant change
in individual monosaccharide content. The percentage
composition of sucrose and a larger oligosaccharide,
raffinose, increased significantly during preconditioning.
The high correlations between raffinose/sucrose and warm
germination, conductivity, and sugar leakage (r=0.829, -
0.801, and -0.707 for A632, and 0.887, -0.782 and -0.787 for
B73, respectively) indicates the added effect of raffinose on
induced protection. These results suggest that soluble-sugar

compositional relationships rather than absolute content may play an important role in membrane stabilization. The presence of raffinose at certain levels may also be a key factor in protecting maturing seeds from high-temperature drying damage. The results also indicate that the transition from desiccation intolerance to tolerance is metabolic and not necessarily related to moisture loss.

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## INTRODUCTION

Seeds are intolerant of rapid drying rates or high drying temperatures during the early stage of their development. Kermode and Bewley (1985a) reported that seeds may undergo a transition from a desiccation-intolerance to a desiccation-tolerance state at a particular time in the course of their development. This change occurs at different stages of development for different species. Corn seed often is harvested on the ear at seed moisture as high as 400 g $H_2O$ $kg^{-1}$ fw. Thus, ears must be dried mechanically with heated air before shelling and safe storage. Fortunately, corn seed at this stage appears to be tolerant to moderate-temperature desiccation, although intolerant to high-temperature or excessively slow drying (Herter and Burris, 1989b). High drying temperatures of 45 and 50°C adversely affect seed germination and especially seedling vigor of corn at this stage (Navratil and Burris, 1984).

The role of membranes in reduction in seed quality associated with drying was demonstrated by measuring the conductivity and composition of the leached electrolytes and sugars from the seed (Herter and Burris, 1989a; McKersie and Stinson, 1980). In various desiccation-tolerant organisms, several compounds such as glycogen, glycerol, and trehalose were found to be synthesized and accumulated during preparation for the drying phase (Bewley, 1979; Crowe and

Crowe, 1986a; Crowe et al., 1987). Crowe and Crowe (1982, 1984, 1986a) reported that, in anhydrobiotic organisms, tolerance to dehydration is associated with stabilization of the lipid membranes by the disaccharide, trehalose. They suggested that trehalose acts as a spacer, preventing contact between the collapsing membrane during drying. This spacer inhibits lipid fusions that result in membrane lipid-phase changes. A similar stabilizing reaction may be operative in the desiccation processes of dehydrating seed (Koster and Leopold, 1988) and pollen (Hoekstra and Roekel, 1988; Hoekstra at el., 1989). The changes in soluble sugars in imbibing seed seem, however, to be a common phenomenon during seed germination (Bewley and Black, 1985; Gould and Rees, 1964; Wahab and Burris, 1975).

The present study investigates harvest maturity and seed drying as related to membrane behavior and carbohydrate metabolism. A preconditioning process that can induce high-temperature drying tolerance without a substantial loss in moisture was used to study these metabolic processes. Understanding the physiological basis for this process would provide an understanding of both desiccation and membrane performance in maturing seed.

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## MATERIALS AND METHODS

**Plant material**  The hybrid corn seed used in all experiments was produced at Iowa State University. The inbred lines A632 and B73 were used as seed parents, and H99xH95 was the common pollen parent. Seeds were harvested at moisture contents of 550, 450, and 400 g $H_2O$ $kg^{-1}$ fw (oven method). Physiological maturity for the inbreds used in these studies occurs at approximately 350 to 380 g $H_2O$ $kg^{-1}$ fw, which corresponds to a milk line of approximately 40%. On the basis of moisture samples, it was estimated that at least 90% of the ears were within a range of ± 20 g $H_2O$ $kg^{-1}$ fw from the mean harvest moisture. A split-plot design was used with six replicates.

**Preconditioning of corn seed**  Small-scale thin-layer experimental driers as described by Navratil and Burris (1982) were used to induce preconditioning. Samples were either dried directly at 50°C, relative humidity (RH) 17% as a control or preconditioned at 35°C, RH 22% for 6, 12, 24, 36, and 48 h before transfer to 50°C for the completion of the drying process down to 120 g $H_2O$ $kg^{-1}$ fw moisture content. Kernel samples were removed at each transfer time as the seed was preconditioned for moisture and sugar analysis. The dried seeds with different times of preconditioning and subsequent 50°C drying were used for seed quality and membrane behavior evaluation.

**Evaluation of seed quality and membrane integrity** Seed
quality was measured by the standard germination and a soil-
free cold test (Loeffler et al., 1985). The germination test
was evaluated after 7 d at 25°C (AOSA, 1985) and, the cold
test, after 14 d, (the first 7 d at 10°C followed by 7 d at
25°C). Individual seed conductivity and sugar leakage were
determined by soaking 100 seeds of each sample in soaking
trays for 24 h in high performance liquid chromatography
(HPLC)-grade water. Conductivity was measured by ASA-610
automatic seed analyzer (Agro Science Inc., Ann Arbor, MI) as
microamps (Herter and Burris, 1989a). Soaking-water
subsamples were used to measure the sugar leakage by the
phenol-sulfuric method (Dubois, 1956).

**Soluble sugar analysis** Sugars were extracted by
homogenizing 40 isolated corn embryos in 10 mL of 800-mL L$^{-1}$
ethanol with a Brinkmann homogenizer (Brinkmann Instruments
Co., Westbury, NY) and centrifuging at 20,000 x g for 10 min.
The pellet was washed with 5-mL ethanol and recentrifuged.
The supernatants were combined, and the ethanol was removed
by vacuum evaporation. Water was added to a final volume of
5-mL extract. The extract was cleared by centrifuging at
20,000 x g for 10 min and filtration through a 0.2-$\mu$m filter.
The sugars were separated by HPLC with a refractive index
detector (Shimadzu Corporation, Kyoto, Japan). Composition
was determined by comparing peak areas of interest with those

of the individual sugar standards by integration. A
SupelCosil LC-NH$_2$ column (Supelco Inc. Bellefonte, PA) was
used with a mobile phase:  acetonitrile:water 75:25 and flow
rate 1-mL min$^{-1}$.

## RESULTS

As normal maturation progressed and seed moisture content declined (Fig. 1), the tolerance to high-temperature drying increased (Figs. 2 and 3). High-moisture seed (>400 g $H_2O$ $kg^{-1}$ fw) was intolerant of high-temperature (>45°C) drying without preconditioning. Preconditioning these high-moisture seeds at 35°C induced high-temperature (50°C) desiccation tolerance (Figs. 2 and 3) without the moisture loss associated with a similar response under field conditions (Fig. 1). Genotypes differed in sensitivity to high-temperature drying, but they exhibited a similar response to preconditioning.

Membrane involvement in the high-temperature desiccation damage is indicated by increased leachate conductivity and sugar leakage (Figs. 2 and 3: B and C). Improvements in seed quality (germination) are accompanied by a decreased conductivity and sugar leakage from the seed soaked in water. Seed vigor, as indicated by cold germination, was improved by preconditioning for 24 h. During the same period, the conductivity and the leachate of sugars from dried seed decreased after preconditioning (Figs. 2 and 3). The correlation between cold germination and conductivity or sugar leakage reached a significant level (r=-0.79 and -0.80, respectively) through preconditioning.

To study the role of soluble sugar in improving seed

quality and membrane behavior, total and major soluble sugars were analyzed (Table 1). Total soluble sugars in embryos harvested at 530 to 550 g $H_2O$ $kg^{-1}$ fw decreased during preconditioning, but not in embryos harvested at 390 to 400 g $H_2O$ $kg^{-1}$ fw. Sucrose and monosaccharide content changed as did the total sugar content during preconditioning. The linear regression of total sugar and sucrose content over preconditioning time in either genotype was not significant ($R^2$=0.094 and 0.281 for total and sucrose in A632 of harvest 1, and 0.568 and 0.425 in B73, respectively). However, raffinose content in desiccation sensitive embryos (harvest 1 of A632 and both harvests in B73) increased during preconditioning, resulting in an increased ratio of raffinose/sucrose. Therefore, sugar compositional changes with time of preconditioning were calculated and shown in Figs. 4 and 5. Sucrose and raffinose percentage increased significantly after 6 h of preconditioning which was associated with the induction of high-temperature desiccation tolerance (Figs. 2 and 3). In addition, soluble sugar composition, especially the ratio of raffinose to sucrose, was well correlated with induced desiccation tolerance and membrane integrity (Table 2).
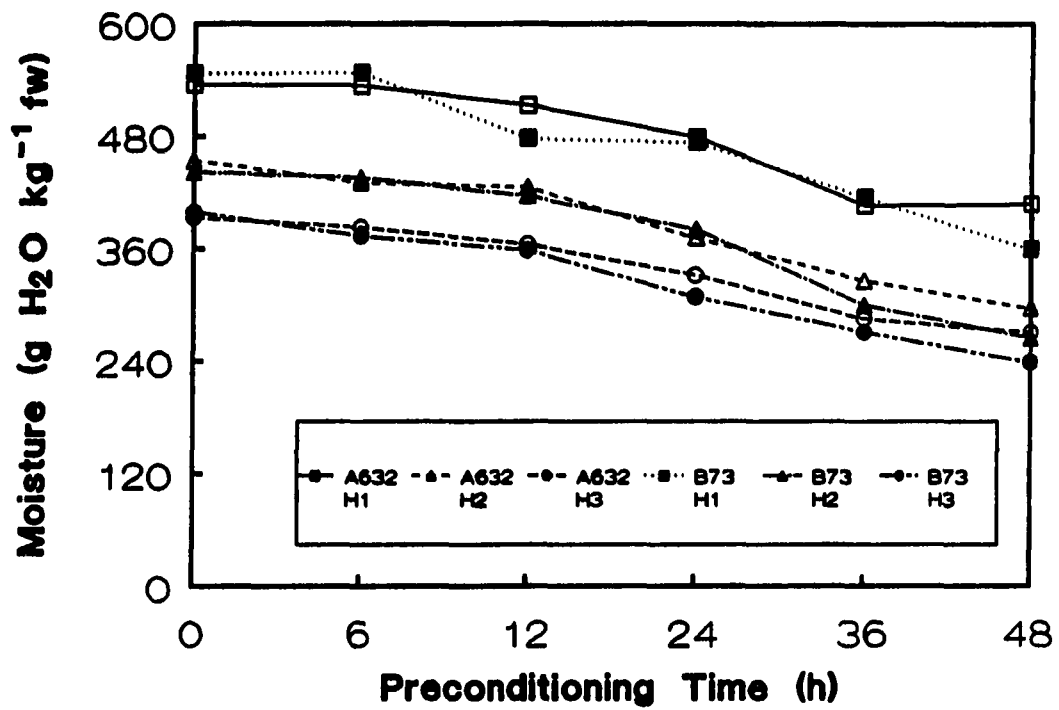
Fig. 1.   Changes of seed moisture content during
          preconditioning of A632 and B73.  Harvest moisture
          in $H_2O$ $kg^{-1}$ fw for A632 at H1=535, at H2=454, and at
          H3=393 and for B73 at H1=547, at H2=442g, and at
          H3=399
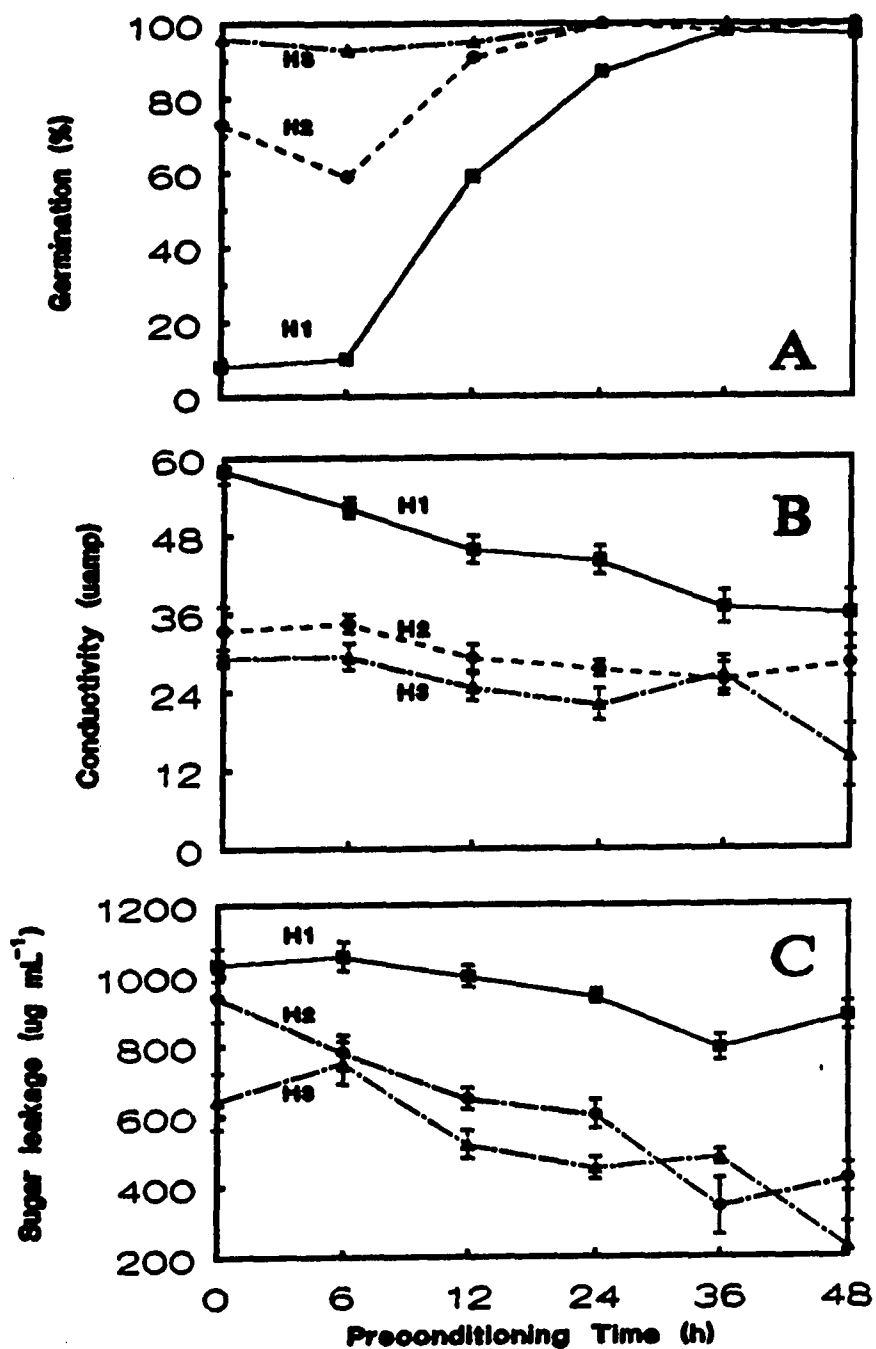
Fig. 2. Induction of high-temperature desiccation tolerance
by preconditioning of A632 high-moisture seed.
Membrane involvement of drying damage is indicated
by leachate conductivity (B) and sugar leakage (C).
Harvest moisture in $H_2O$ kg$^{-1}$ fw at H1=535, at H2=454,
and at H3=393
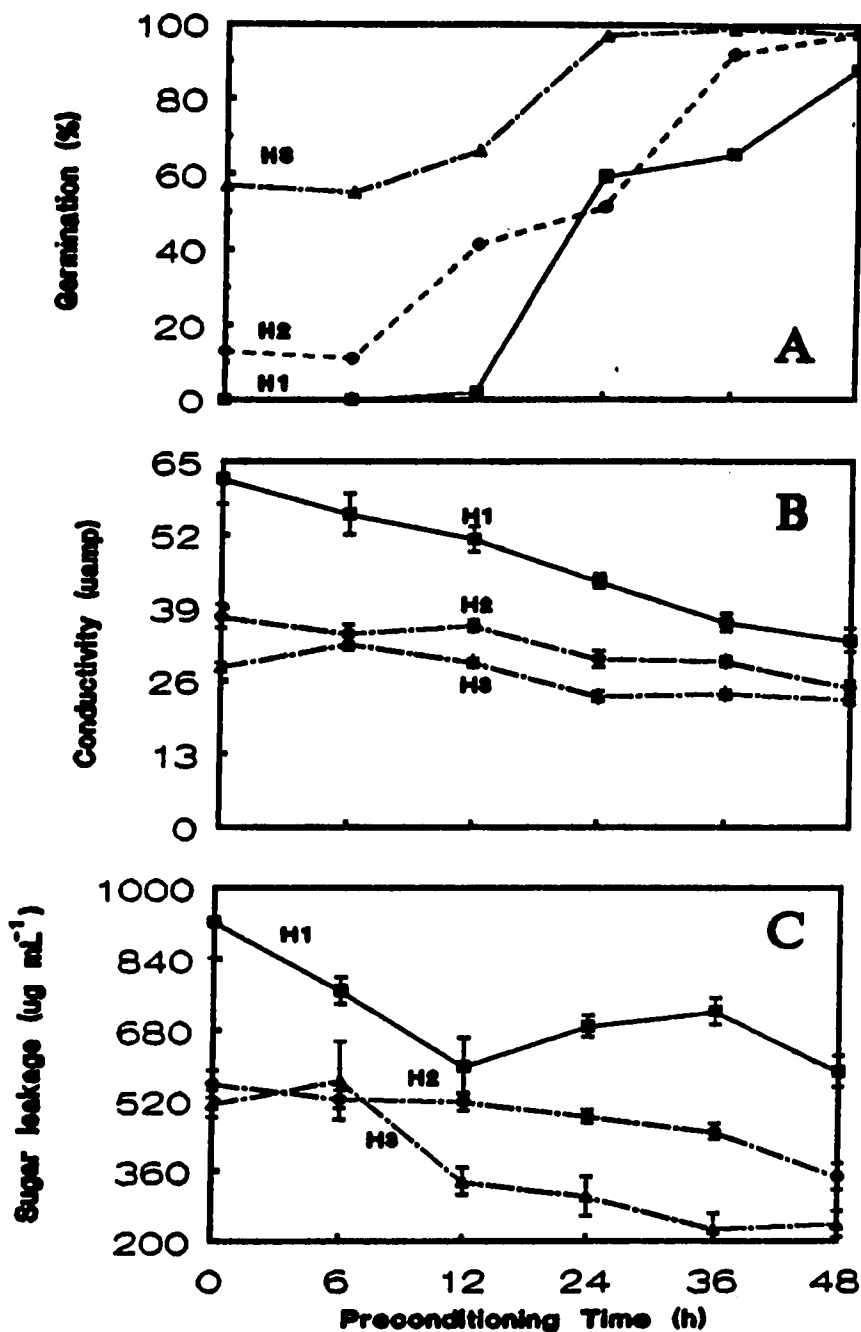
Fig. 3. Induction of high-temperature desiccation tolerance
by preconditioning of B73 high-moisture seed.
Membrane involvement of drying damage is indicated
by leachate conductivity (B) and sugar leakage (C).
Harvest moisture in $H_2O$ $kg^{-1}$ fw at H1=547, at
H2=442g, and at H3=399

Table 1.  Changes of total and major soluble-sugar content in
embryo of different maturing corn hybrids during
preconditioning at 35°C

| Harvest moisture | Precondition time | Soluble sugar content | | | |
|---|---|---|---|---|---|
| | | Mono[a] | Suc | Raf | Total[b] |
| g $H_2O$ $kg^{-1}$ fw | h | ------------mg $g^{-1}$ dw------------- | | | |
| | | A632 x (H99xH95) | | | |
| 535 | 0 | 28.9ab[c] | 56.5ab | 2.8c | 126.2a |
| | 6 | 24.5ab | 47.6bc | 1.2d | 81.2c |
| | 12 | 36.0a | 47.7c | 2.1cd | 81.8c |
| | 24 | 32.0a | 42.4c | 5.3b | 82.1c |
| | 36 | 24.5ab | 58.6a | 5.5b | 89.0b |
| | 48 | 19.6b | 65.3a | 7.6a | 93.7b |
| 393 | 0 | 15.6b | 38.4ab | 5.2ab | 70.0b |
| | 6 | 16.3ab | 41.6ab | 7.0a | 72.9b |
| | 12 | 16.8ab | 34.1cb | 4.0b | 61.6c |
| | 24 | 17.3ab | 36.7abc | 3.5b | 63.7c |
| | 36 | 19.0a | 33.1c | 6.8a | 69.2b |
| | 48 | 16.6ab | 43.3a | 6.1a | 80.1a |
| | | B73 x (H99xH95) | | | |
| 547 | 0 | 49.0a | 106.8a | 4.2bc | 218.9a |
| | 6 | 29.3ab | 86.2ab | 2.7c | 150.0ab |
| | 12 | 36.0a | 89.2ab | 4.3abc | 146.5ab |
| | 24 | 15.0b | 47.9b | 1.4c | 91.6b |
| | 36 | 15.4b | 70.1ab | 5.9ab | 97.1b |
| | 48 | 12.9b | 55.0b | 6.4a | 93.5b |
| 399 | 0 | 24.0ab | 62.4a | 7.4a | 132.2a |
| | 6 | 22.4ab | 78.8a | 8.5a | 129.5a |
| | 12 | 31.8a | 74.6a | 10.6a | 132.5a |
| | 24 | 16.1bc | 83.1a | 16.1a | 142.4a |
| | 36 | 12.5bc | 74.1a | 15.7a | 118.4a |
| | 48 | 6.6c | 51.1b | 13.1a | 77.9b |

[a]Abbreviations:  Suc:  sucrose, Raf:  raffinose, Mono:
monosaccharides.
[b]Total sugar content including unidentified sugars.
[c]Means within a column, genotype, and harvest moisture
not followed by the same letter are significantly different
from each other at P=0.05 level.
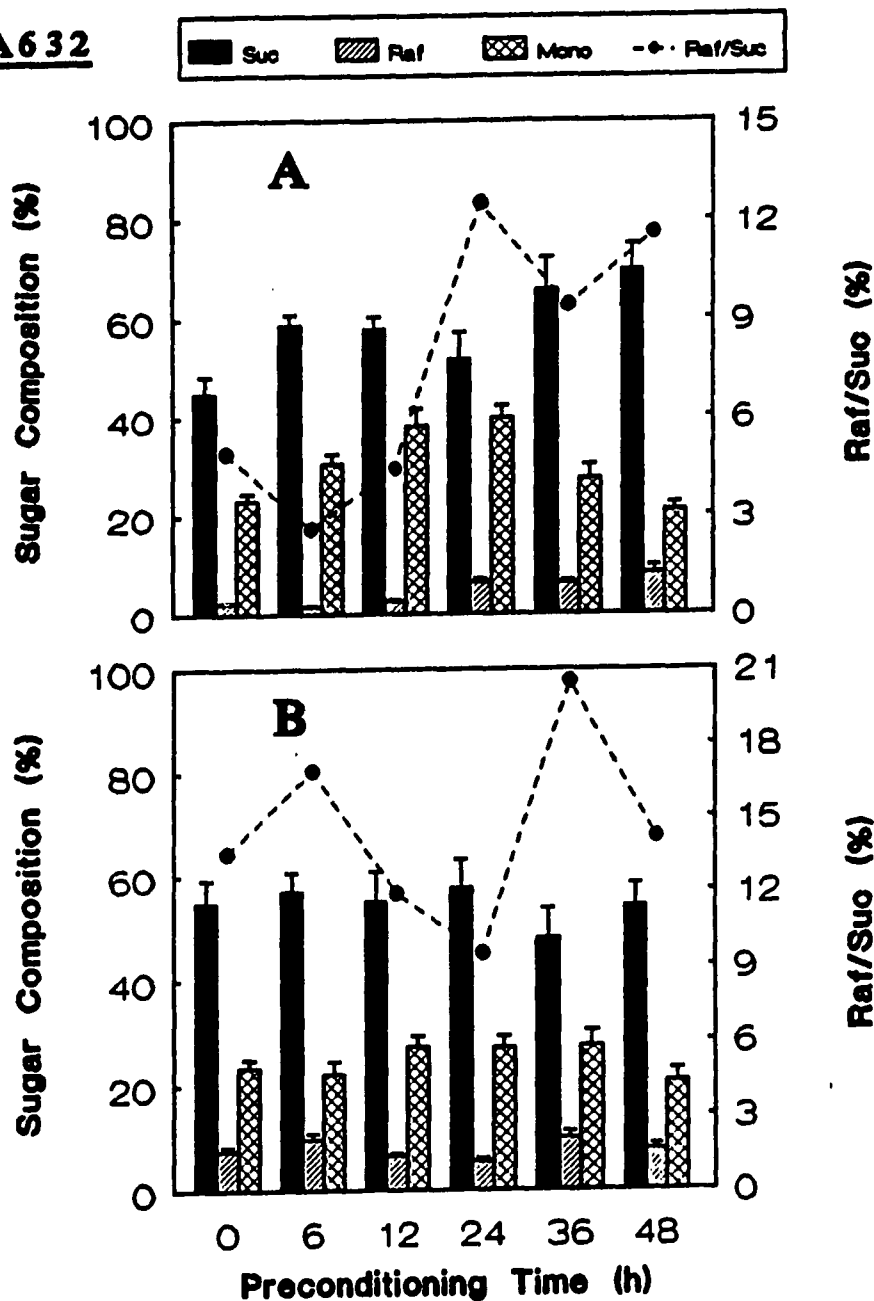
Fig. 4. Changes of sugar composition in A632 embryo of different maturity during preconditioning at 35°C. A: harvest moisture, 535 g $H_2O$ $kg^{-1}$ fw; B: harvest moisture 393 g $H_2O$ $kg^{-1}$ fw.; Abbreviations: Suc: sucrose, Raf: raffinose, Mono: monosaccharides, and Raf/Su: percentage of Raf in Suc
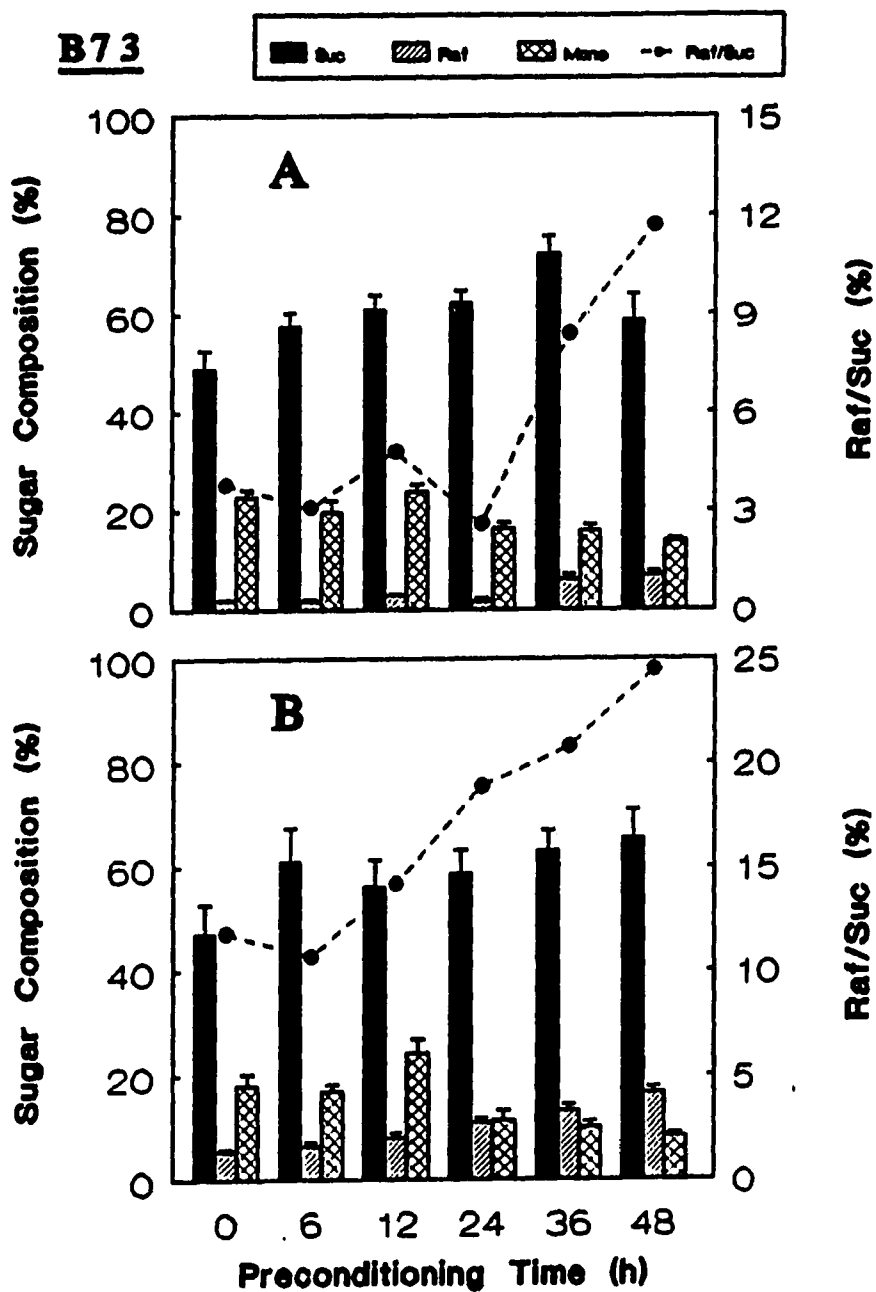
Fig. 5.  Changes of sugar composition in B73 embryo of
different maturity during preconditioning at 35°C.
A:  harvest moisture, 547 g $H_2O$ kg$^{-1}$ fw; B:  harvest
moisture, 399 g $H_2O$ kg$^{-1}$ fw.; Abbreviations:  Suc:
sucrose, Raf: raffinose, Mono:  monosaccharide, and
Raf/Su:  percentage of Raf in Suc

Table 2. Coefficients of linear correlation (r) between seed germination, sugar leakage, and conductivity with soluble-sugar composition of embryos

| Sugar composition (%) | Germination (%) | | Sugar leakage ($\mu$ ml$^{-1}$) | Conductivity ($\mu$amp) |
|---|---|---|---|---|
| | Warm | Cold | | |
| | A632 x (H99xH95) | | | |
| Glucose | -0.78 | -0.92** | 0.85* | 0.79 |
| Sucrose | 0.67* | 0.71* | -0.81* | -0.40 |
| Raffinose | 0.79* | 0.91** | -0.78* | -0.63 |
| Raf/Suc | 0.83** | 0.95** | -0.80* | -0.71* |
| | B73 x (H99xH95) | | | |
| Glucose | -0.68** | -0.79** | 0.41* | 0.45* |
| Sucrose | 0.27 | 0.31* | -0.36* | -0.49* |
| Raffinose | 0.88** | 0.92** | -0.78** | -0.77** |
| Raf/Suc | 0.89** | 0.91** | -0.78** | -0.79** |

*$P < 0.05$.
**$P < 0.01$.

## DISCUSSION

Seed corn when harvested above 400 g $H_2O$ $kg^{-1}$ fw is
intolerant to high drying temperatures above 45°C. This
drying damage is related to membrane disruption in terms of
increasing leachate conductivity and sugar leakage.
Preconditioning at 35°C can induce high-temperature drying
tolerance by improving seed germination and membrane
integrity (Figs. 2 and 3). Previous studies (Herter and
Burris, 1989b) and current research show that the
preconditioning occurs without a substantial loss in moisture
(Fig. 1). It appears to be a metabolic process which is
related to induced high-temperature drying tolerance. After
24 h of preconditioning, moisture content of the high-
moisture harvest samples was still greater than the 0 h
sample of the second harvest samples for either genotype.
Meanwhile, the tolerance to high-temperature drying
increased, as indicated by increasing seed germination and
reduced conductivity and sugar leakage. The high correlation
between seed germination and conductivity or sugar leakage
suggests a relationship between membrane integrity and seed
quality induced by drying damage. These results also suggest
the role of membrane stabilization in high-temperature
desiccation tolerance.

Membrane involvement in desiccation damage also was
observed in imbibing seed (Koster and Leopold, 1988), pollen

(Hoekstra and Roekel, 1988; Hoekstra et al., 1989), and other
anhydrobiotic organisms (Crowe and Crowe, 1982) by measuring
the membrane integrity. The production of certain sugars
that may maintain bound water content could be an important
feature of membrane stabilization and the induction of
desiccation tolerance (Clegg, 1986; Crowe and Crowe, 1986ab).
In higher plants, sucrose seems to be an effective membrane
protectant (Bewley, 1979; Hoekstra and Roekel, 1988; Koster
and Leopold, 1988). Sucrose also has been shown to protect
isolated thylakoids from desiccation damage (Santarius,
1973). The hydroxyl groups of sucrose may replace water by
hydrogen-binding to the phospholipid head groups of the
membrane (Straauss and Hauser, 1986). Recently, Hoekstra and
Roekel (1988) and Hoekstra et al. (1989) suggested that
sucrose was a key factor in preserving membranes in dry
pollen. By using a liposome model, they reported that when
liposomes were dried in the presence of sucrose, fusion and
leakage could largely be prevented at mass ratios of sugar to
lipids of >4. Koster and Leopold (1988) measured soluble
sugars in imbibing seed, and found that sucrose and larger
oligosaccharides were consistently present during the
tolerant stage and that oligosaccharides disappeared as
desiccation tolerance was lost. They suggested that sucrose
may function in the desiccation tolerance during imbibition,
and that the larger oligosaccharides serve to keep the

sucrose from crystallizing (Koster and Leopold, 1988;
Williams and Leopold, 1989). However, the reported changes
in soluble-sugar content may simply be a common phenomenon
associated with seed imbibition (Bewley, 1979; Gould and
Rees, 1964; Wahab and Burris, 1975).

In maturing cereal seed, soluble sugars usually decline
with increasing seed maturation and increased desiccation
tolerance (Bewley and Black, 1985). Based on our results in
preconditioned maturing seed, sucrose content was not well
correlated with the induction of high-temperature desiccation
tolerance (Table 1). Sucrose content changed as a fraction
of total soluble-sugar content rather than as preconditioning
treatment. No significant linear regression of sucrose
content over preconditioning time indicated that sucrose
content per se may not be acting in membrane stabilization
and desiccation tolerance, although a higher sucrose content
may be needed in imbibing seed and drying pollen for
desiccation tolerance (Hoekstra and Roekel, 1988; Koster and
Leopold, 1988). However, raffinose content increased
dramatically with preconditioning as seeds increased in
tolerance to high-temperature drying. The concentration of
raffinose is low as compared with other soluble sugars;
therefore, it may have a role other than acting directly as a
'spacer' as proposed by Crowe and Crowe (1984, 1986ab). The
combined percentage of sucrose and raffinose increased with

the induction of high-temperature tolerance through preconditioning (Figs. 4 and 5). A ratio of 9% raffinose to sucrose seems to be important to the induction of high-temperature desiccation tolerance. Therefore, sugar percentage composition rather than total content may be related to the stabilization of membranes with the induction of high-temperature drying tolerance (Table 2). The correlations between the ratio of raffinose/sucrose and seed germination, conductivity, and sugar leakage indicate an association of raffinose, in conjunction with sucrose, in the protection of high-moisture seed. The raffinose may act directly or may restrict crystallization of sucrose, thus providing better membrane protection (Koster and Leopold, 1988; Williams and Leopold, 1989).

Our results suggest that the changes in soluble-sugar composition that accompany successful desiccation during seed maturation seem to be similar to the roles of sugars in desiccation tolerance of imbibing seed and other organisms (Bewley, 1979; Hoekstra and Roekel, 1988; Koster and Leopold, 1988). The high quality of carefully dried seed harvested at 550-g $H_2O$ kg$^{-1}$ fw moisture content indicates that desiccation tolerance is controlled by both seed maturation and the temperature at which the drying occurs. Increased percentages of sucrose and raffinose after preconditioning are associated with protection of the high-moisture corn seed

from high-temperature desiccation damage. We conclude that preconditioning-induced desiccation tolerance is a metabolic process. The process may involve membrane stabilization by soluble sugars which would normally occur in the maturing seed. Changes in soluble sugar composition rather than content, especially the ratio of raffinose/sucrose, is highly correlated with membrane stability during high-temperature drying. The interaction of soluble carbohydrate and membrane lipid composition and function is under continued investigation (Part II of this dissertation).

# PART II.

## MEMBRANE PHOSPHOLIPID COMPOSITION AND THERMAL PROPERTIES
## AS RELATED TO DESICCATION TOLERANCE IN MATURING MAIZE SEED

**ABSTRACT**

Membrane phospholipid composition and thermal properties in maturing maize seed were studied to relate high-temperature desiccation tolerance to membrane stabilization. A preconditioning process precluding drying injury without major moisture loss was used to induce the high-temperature desiccation tolerance. Phosphatidylcholine (PC) accumulated, resulting in an increase in the PC/phosphatidylethanolamine (PE) ratio from 3.6 to 8 within 48 h as the high-temperature desiccation tolerance was induced during preconditioning. The increase in PC/PE ratio coincided with a decrease in both phase transition temperature and enthalpy of transition, indicating more stable membranes. The improved stability could be related to the high-temperature desiccation tolerance and membrane function after preconditioning. A shift in the fatty acid composition of the membrane lipids from linoleic acid (18:2) to oleic acid (18:1) during preconditioning indicates a more saturated fatty acid composition. This shift in fatty acids may result in membranes which more easily cope with high-temperature desiccation, as contrasted to a low-temperature effect. The results suggest that alterations in phospholipid molecular species and changes in fatty acid composition to a more saturated composition in maize seed during preconditioning

and maturation could be common mechanisms in high-temperature desiccation tolerance.

## INTRODUCTION

Corn seed, when harvested at seed moisture levels higher than 400 g $H_2O$ $kg^{-1}$ fresh weight (fw), are intolerant of high-temperature (>40°C) drying (Part I of this dissertation). High moisture seed, however, may be acclimated to high-temperature (50°C) drying by preconditioning the seed at 35°C for 12 to 36 hours. The membrane function of the reduced-quality seed resulting from high-temperature drying was demonstrated by measuring the conductivity and composition of leached sugars (Herter and Burris, 1989a). Recent studies in plant growth and temperature acclimation have identified several molecular mechanisms for membrane lipid alteration, in addition to the familiar regulation of fatty acid saturation related to cold hardiness (Thompson, 1985). The changes in fatty acid composition resulting from high-temperature desiccation, however, may be different from the cold hardiness response (Cheesbrough, 1989; Pearcy, 1978). Thompson (1983) reported that the proportions of different membrane lipid classes (such as PC and PE) in a given membrane can be significantly different in cells exposed to different environmental conditions (such as temperature and salinity). The nature of polar moiety of phospholipids can effect the thermal properties of both the membrane lipid and the parent membrane (Gennis, 1989; Michaelson et al., 1974; Raison and Wright, 1983). Therefore, the changes in polar-

head-group phospholipids and their thermal properties are of academic interest and practical importance in the different acclimation processes.

This research uses a preconditioning process as a model system for acclimating seed to high-temperature desiccation to study the role of membrane phospholipid composition and thermal properties in membrane stabilization and high-temperature desiccation tolerance. The relation between properties of the membrane phospholipids and high-temperature desiccation tolerance will be discussed.

## MATERIALS AND METHODS

**Plant material** The hybrid corn seed was produced at Iowa State University, Ames, IA. The inbred line B73 was used as seed parent, and H99xH95 was the common pollen parent. Seeds were harvested at moisture contents of 450 to 500 g $H_2O$ kg$^{-1}$ fw (oven method).

**Preconditioning of corn seed** A small-scale thin-layer experimental drier was used to induce preconditioning (Navratil and Burris, 1982). Samples were preconditioned at 35°C with a relative humidity (RH) of 22% for 0, 6, 12, 24, 36, and 48 h before transfer to 50°C with an RH of 17% for the completion of the drying process down to 120 g $H_2O$ kg$^{-1}$ fw moisture content. Samples were removed at each transfer time as the seed was preconditioned for phospholipid and thermal property analysis.

**Preparation of membrane fraction** Cellular membranes were isolated by homogenizing 60 corn embryos in 15-mL of 50 mM $NaHCO_3$ containing 300 mM sucrose, pH 7.0. The homogenate was filtered through four layers of cheesecloth and centrifuged at 48,000 x g for 1 h. The membrane pellet was washed in 50 mM $NaHCO_3$ and isolated by centrifugation at 48,000 x g for 1 h. Total lipid of the membrane fraction was extracted with chloroform:methanol (2:1).

**Phospholipid analysis** Polar lipids were separated from the neutral lipids in the total membrane lipid extract using acetone precipitation (Kates, 1986). The total lipid extract was dried to a small volume under nitrogen. Twenty volumes of cold acetone were added, mixed, and cooled on ice for one h. The precipitated phosphatides were dried under nitrogen and then weighed. The phospholipids were resuspended in chloroform:methanol (2:1) solution, separated, and determined by high performance liquid chromatography (HPLC) with a UV detector at 203 nm (Shimadzu Corporation, Kyoto, Japan). A SupelCosil Lc-Si (5$\mu$m) column (Supelco Inc. Bellefonte, PA) was used with a mobile phase of acetonitrile:methanol:85% phosphoric acid 130:5:1.5 v/v and a flow rate of 1-mL min$^{-1}$ (Chen and Kou, 1982). Fatty acid methyl esters of phospholipids were prepared by treating the lipids with boron fluoride-methanol (Morrison and Smith, 1964). Fatty acid composition was determined by a gas chromatograph (GC) with a flame ionization detector (Varin Associate, Sugar Land, TX). A Supelcowax 10 column (30m, 0.75 mm, 10 $\mu$m) was used (Supelco Inc., Bellefonte, PA).

**Measurement of thermal properties of membrane lipids** Phase transition changes of phospholipids were measured by differential scanning calorimetry (Perkin-Elmer Corp., Model DSC-7, Norwalk, CT). Phospholipid samples were dispersed in 20 mM Tris buffer, pH7.2 containing 2 mM EDTA. The samples

were then scanned at 10°k/min, and the reference pan
contained the same quantity of the buffer. The energy
involved in the phase transition was calculated by computer
integration.

# RESULTS

Membrane involvement in high-temperature desiccation damage was indicated by the decrease in membrane integrity. Improvements in seed quality were accompanied by decreased conductivity and by sugar leakage from seed soaked in water (r=-0.903 and -0.801, respectively) (Fig. 6). During the preconditioning, phospholipid composition of the membranes changed as high-temperature desiccation tolerance level was induced (Table 3).

Total phospholipid content increased during preconditioning. Generally, three major phospholipids, phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) are found in corn embryo membranes. The level of both PC and PE appear to increase as PI decreases during preconditioning. The increasing levels of PC and PE differ, however, which results in an increased ratio of PC/PE from 3.6 to 8 within 48 h of preconditioning.

To study the relation between phospholipid composition and the thermal properties of the membrane, phospholipid phase transitions were measured by differential scanning calorimetry (Fig. 7). At temperatures above 35°C, one transition was found tending to shift downward as preconditioning time increased. Phase transition temperature and the energy involved in the transition were calculated (Fig. 8). Alterations in polar head-group composition of the

membrane lipids achieved during preconditioning caused a
shift in the thermotropic behavior of the membrane lipids.
Increases in PC/PE ratio may cause a more
fluid lipid matrix, which tends to lower the phase transition
temperature of membrane fraction and the energy involved in
the transition.

Fatty acid composition of membrane lipid fractions is
shown in Table 4. No change in total saturated fatty acid
(16:0 or 18:0) composition was found. A shift, however, in
the unsaturated fatty acid composition of the membrane lipids
from linoleic acid (18:2) to oleic acid (18:1) suggests that
as seeds become tolerant to high-temperature desiccation
fatty acid composition becomes more saturated.
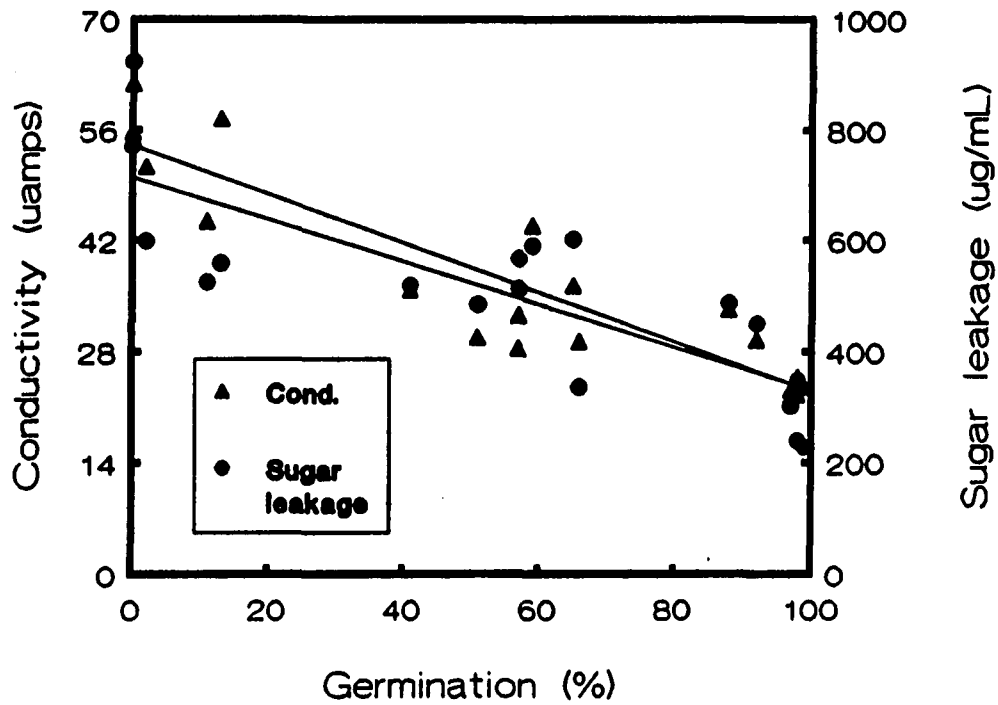
Fig. 6.  Correlation between seed germination and membrane
         integrity indicated by leachate conductivity and
         sugar leakage in seed, resulting from different pre
         conditioning times.  Results were analyzed using a
         linear regression ($r^2$ = 0.82 and 0.64 for conduc
         tivity and sugar leakage, respectively)

Table 3.  Changes in membrane phospholipids of maturing corn
embryos during preconditioning

| Preconditioning Time (hours) | Phospholipids ($\mu$g/g.dw) | | | | |
|---|---|---|---|---|---|
| | PI[a] | PS | PE | PC | PC/PE |
| 0 | 207.1 | 13.7 | 70.8 | 259.8 | 3.65 |
| 6 | 220.3 | 45.8 | 88.8 | 296.6 | 3.34 |
| 12 | 189.7 | 50.1 | 80.6 | 309.1 | 3.84 |
| 24 | 186.9 | 37.4 | 62.3 | 371.9 | 5.97 |
| 36 | 177.1 | 41.4 | 92.1 | 483.4 | 5.25 |
| 48 | 161.2 | 33.2 | 104.6 | 839.9 | 8.02 |
| $LSD_{0.05}$ | 63.9 | 22.3 | 35.2 | 89.3 | 1.05 |

[a]PI, phosphatidylinositol;  PS, phosphatidylserine;
PE, phosphatidylethanolamine;  PC, phophatidylcholine.
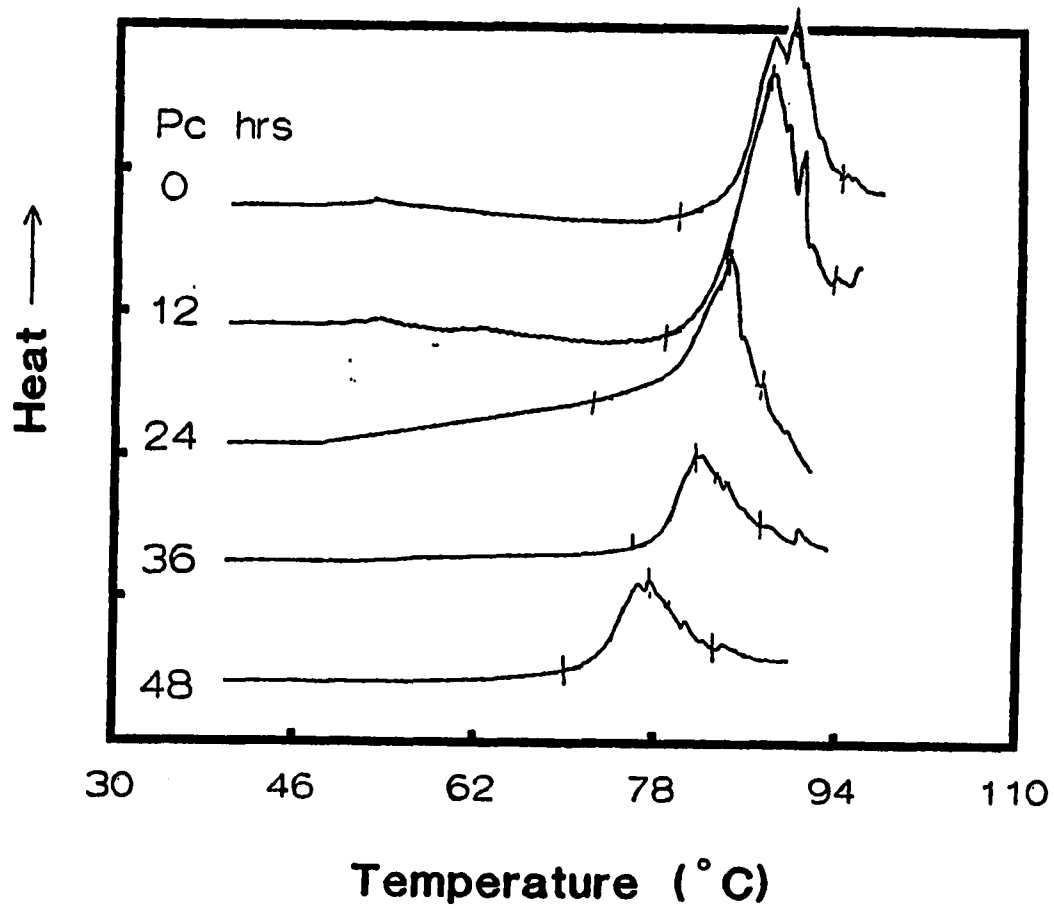
Fig. 7. DSC traces showing the thermal behavior of membrane
phospholipids of preconditioned seed. The traces
were obtained at a heating rate of 10°K/min
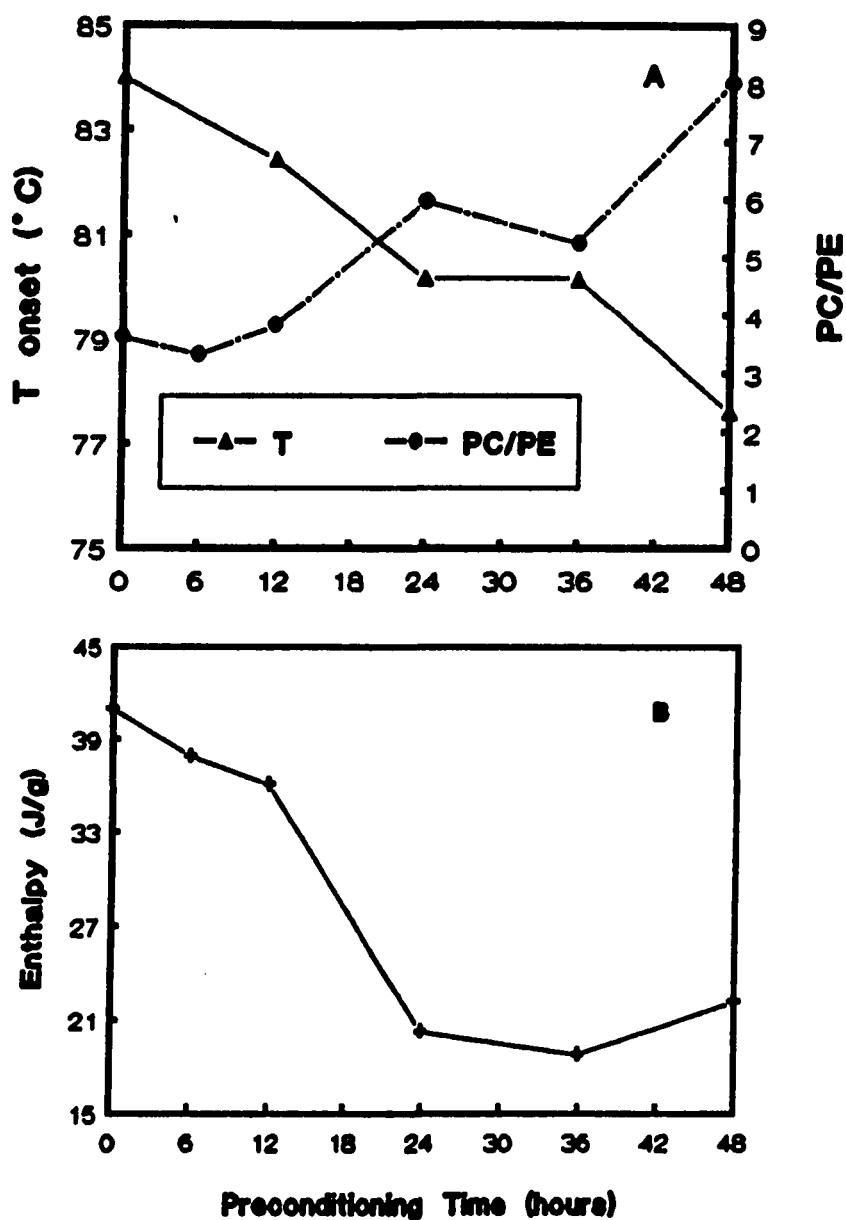
Fig. 8. Thermal response of membrane phospholipids of
preconditioned seed. A: Changes in phase
transition onset temperature and PC/PE ratio of
phospholipid fractions; B: Changes in enthalpy
indicating the energy required for phase
transitions

Table 4. Fatty acid composition of membrane phospholipids in preconditioned corn embryo

| Preconditioning Time (hour) | Fatty acid (%) | | | | |
|---|---|---|---|---|---|
| | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 |
| 0 | 20.9ab[a] | 3.58a | 15.1d | 59.3a | 2.75a |
| 6 | 18.0b | 3.61a | 17.1cd | 58.8a | 2.21a |
| 12 | 20.3ab | 4.58a | 14.3d | 57.7a | 2.68a |
| 24 | 21.6ab | 5.56a | 18.6bc | 51.4b | 2.05a |
| 36 | 22.7a | 4.44a | 20.7b | 49.5b | 1.84a |
| 48 | 21.7ab | 5.04a | 24.1a | 47.0b | 1.65a |

[a]Means within a column not followed by the same letter are significantly different from each other at P=0.05 level.

## DISCUSSION

Special attention has been focused on the role of
membranes in stress-inducing environments such as those
necessitating acclimation to low and high temperatures.
Cellular membrane systems appear to be one of the primary
sites of desiccation injury (Chen and Burris, 1990; Hoekstra
et al., 1989; Senaratna et al., 1984, 1987). The role of
membrane function in reductions in seed vigor associated with
high-temperature drying of high-moisture seed was
demonstrated by measuring the conductivity and composition of
the leached sugar (Herter and Burris, 1989a). Proportions of
different membrane lipid classes (such as PC and PE) in a
given membrane can be significantly different in cells
exposed to different environmental conditions (Thompson,
1983, 1985). The acclimation of high-moisture seed to high-
temperature drying was achieved by preconditioning the
maturing seed at 35°C for 12 to 36 h before it could be
safely dried at 50°C (Chen and Burris, 1990). During
preconditioning, total phospholipid content of the membrane
fractions increased. Compositional changes in individual
phospholipids also occurred (Table 3). Among the
phospholipids, the PC/PE ratio increased from 3.6 to 8 as
membrane stabilization to high-temperature desiccation was
induced. Under drought stress, PE appears to decrease and PC
to increase in wheat and oat seedlings, resulting in

increased frost resistance (Liljenberg and Kales, 1985; Vigh et al., 1986). As a result of this reorganization, the ratio of PC to PE almost doubled in the root tissues of stressed oat seedlings (Liljenberg and Kales, 1985). Seed stored at elevated temperatures and at high relative humidities exhibited a decrease in the total phospholipid content. Particularly, decreases in PC and PE associated with membrane destruction resulted in decreased seed viability (Pukacka and Kuiper, 1988). Investigations using soybean seed axes also indicates that the physical and compositional properties of cellular membranes are altered after desiccation stress. These changes occur only in axes of soybean seeds in the desiccation sensitive state (36 h of imbibition) (Senaratna et al., 1987). According to the model proposed by Quinn (1983), the adaptive changes in cell membranes upon environmental perturbation are related to the stability of the biomembranes, which depend upon an appropriate balance of bilayer/non-bilayer forming lipids. It is known that PC tends to form a bilayer configuration under physiological conditions, whereas PE forms an inverted hexagonal ($H_{II}$) configuration (Gagne et al., 1985). Therefore, increases in PC/PE ratio may play a role in membrane stabilization during the preconditioning process of the maturing maize seed.

Further analysis of the phase transition of membrane lipids indicates that alterations in polar head-group

composition of the membrane lipids achieved during

preconditioning correlated with a shift in the thermotropic

behavior of the membrane lipids (Fig. 6). In addition, the

enthalpy of transition decreased as phase transition

temperatures decreased during preconditioning (Fig. 8, B).

This finding indicates a more stable membrane system may be

induced by preconditioning. Drying has been shown to

increase the gel to the liquid crystalline transition

temperature (Tc) of the membrane phospholipids in intact

Typha pollen, which leads to gel phase domains at

physiological temperatures (Crowe et al., 1989). In our

membrane phospholipid model system, however, a phase

transition region was found at temperatures near 80°C, which

is beyond the physiological range. This high transition

temperature may have been caused by the phospholipid model

system used. The lipid phase transition temperatures are

affected by many factors such as the length of the fatty acyl

chain, the degree of saturation, the nature of the polar head

groups, and the hydration state of the membrane lipids

(Gennis, 1989; Warren, 1987). The dehydrated phospholipids

have a higher transition temperature than their fully

hydrated countparts do. Transition temperatures of 70 to

80°C for PC were found at water contents of 200 g kg$^{-1}$ and

below (Warren, 1987). In most instances, the phase

transition temperature depends on the fatty acyl chain of a

given phospholipid. At a chainlength of C-18, the saturated diacylphospholipid phase transition temperature may reach as high as 70 and 60°C for PE and PC, respectively (Blume, 1983). Because all these model results are informative as to the miscibility of different kinds of lipids, the thermodynamics and structure of such mixtures may be relevant to biomembranes. Although the significance of these model studies for the distribution of lipids in real biomembranes is not known, the preconditioning of high moisture seed decreases the phase transition temperature and the enthalpy of the transition and maintains membrane phospholipids in a more fluid and stable phase, even in the absence of water. These phenomena may be well correlated with the changes in phospholipid composition, especially with the increased of PC/PE ratio (Fig. 8). Increases in the PC/PE ratio result in a more fluid lipid matrix, which tends to lower the phase transition temperature of the membrane fraction in oat seedlings under drought stress (Liljenberg and Kales, 1985). In membranes with uniform fatty acid composition, PC forms less tightly packed structures than PE does (McElhaney, 1982). Biophysical studies have shown that when fatty acyl chains are identical, the temperature of transition from a gel to liquid crystalline state of a membrane is about 25°C higher in an artificial membrane of PE than of PC (Chapman and Wallach, 1968). Therefore, increases in both the PC to

PE ratio and the level of unsaturation of the fatty acyl chains result in a more fluid lipid matrix, indicated by a lower phase transition temperature of the membrane fraction.

A shift, however, in the fatty acid composition of the membrane lipids from linoleic acid (18:2) to oleic acid (18:1) during preconditioning (Table 4) suggests a more saturated fatty acid composition. This finding seems contrary to what would be expected of cells which are trying to cope with high temperatures by decreasing the phase transition temperature during preconditioning. In many prokaryotes, plants, and animals, organisms adapt to low temperatures by increasing the level of unsaturation in the fatty acids of membrane diacylglycerols (Francis and Coolbear, 1988; Orr and Raison, 1987; Raison and Wright, 1983; Raison, 1985; Raison and Orr, 1986). Less attention has been paid, however, to the effect of membrane fatty acid composition on the high-temperature adaptation of living organisms. Wide variations in the fatty acids of membrane lipids are found in different plant species, as well as in the same species under changing growth conditions (Cheesbrough, 1989; Francis and Coolbear, 1988; Low and Parks, 1987). Such changes have been assumed necessary for adjusting the "fluidity" or "microviscosity" of cell membranes. Lowering the growth temperature of plants invariably increases the proportion of unsaturated fatty

acids in the membrane lipids. When plants respond to high temperatures; however, the results may differ (Williams et al., 1988). Growth of plants at suboptimal temperatures increases the percentage of polyunsaturated fatty acid levels, whereas growth at elevated temperatures induces the opposite effect (Raison, 1985; Thompson, 1985). Pearcy (1978) determined the fatty acid composition of leaf chloroplasts and found at high growth temperatures a decrease in linoleic acid (18:2) with a concurrent increase in the more saturated fatty acids. The involvement of desaturase has been found during acclimation of developing soybean seeds altered by higher growth temperatures (Cheesbrough, 1989). Therefore, a compensating mechanism may occur in plants acclimating to high temperature desiccation. We found a more saturated fatty acid composition in the membrane lipids of high-temperature desiccation tolerant seed than intolerant one during the preconditioning process. These may cope more easily with high-temperature desiccation by adjusting microviscosity although any cause and effect relationship is uncertain.

It seems likely, therefore, that the increase in PC in membranes after preconditioning could be part of a common protective mechanism during either drought or cold hardening. This process decreases membrane lipid phase transition temperature and the enthalpy of the transition, and favors

the formation of more stable and functional membranes. A
more saturated fatty acid composition, however, may be needed
for an organism to acclimate to high-temperature desiccation.
Thus, we suggest that modifications in the phospholipid
molecular species and their fatty acid composition may play a
crucial role in preserving membrane functionality during and
following high-temperature desiccation.

# PART III.

## ABA LEVELS AND DESICCATION INDUCED PROTEINS

## AS RELATED TO THE INDUCTION OF DESICCATION TOLERANCE

## ABSTRACT

Preconditioning the ears of high-moisture seed corn prior to high-temperature drying induced desiccation tolerance. The role of ABA and desiccation-induced proteins in the induction of high-temperature desiccation tolerance during post-harvest drying of seed corn was studied using the preconditioning system. Seed vigor as indicated by cold test germination increased by 20% through 24 hours of preconditioning. ABA content remained at a high level through 24 hours of preconditioning and then dropped dramatically after 36 hours as seed becomes tolerant to high-temperature drying. Meanwhile, SDS-polyacrylamide gel electrophoresis showed a new protein bands (68K) appearing after preconditioning and reaching their highest quantity after 36 hours. The results indicate that the high level of ABA prior to 24 hours of preconditioning and the newly-induced protein may be related to the transfer from desiccation intolerance to tolerance during post-harvest drying of high-moisture seed corn. The role of ABA in the protein synthesis and the relationship of ABA and protein synthesis with high-temperature desiccation tolerance and membrane stabilization will be discussed.

## INTRODUCTION

Drying high moisture seed corn (>40%) at high
temperatures (45-50°C) may adversely affect seed germination
and seedling vigor (Navratil and Burris, 1984). This
desiccation intolerance is related to the metabolic
intolerance of seed to the high temperature drying.
Maturation drying during seed development has a role in the
transition from seed development to germination, i.e., from
desiccation intolerance to desiccation tolerance (Dasgupta et
al., 1982; Kermode and Bewley, 1985ab). Therefore, the
induction of desiccation tolerance may account for some
metabolic change during seed development and/or post harvest
development (Parts I and II). Critical features of
desiccation tolerance of maturing maize seeds are the ability
to reduce their sensitivity to the high-temperature drying,
i.e., to quickly induce a mechanism to cope with the high-
temperature desiccation.

Seed drying during development plays an important role
in redirecting metabolism from a developmental to a
germinative mode (Kermode and Bewley, 1985ab). Maturing seed
changes from a desiccation sensitive to a stage where they
are insensitive. This change may be elicited by premature
desiccation, and also results in a change in the pattern of
soluble protein synthesis (Misra and Bewley, 1985). Seed
desiccated during the desiccation-tolerant stage, however,

resumes protein synthesis at almost the control level.
Recently, stress induced proteins, such as heat shock
proteins, were shown to be related to stress tolerance in
plants (Cooper and Ho, 1983; Cooper et al., 1984; Heuss-
LaRose et al., 1987; Sachs and Ho, 1986). These proteins may
confer thermal protection as they are generally located in
the nucleus, mitochondria, and ribosomes, indicating the
mobilization of major cellular organelles in order to cope
with the stress. Other seed developmental proteins such as
"Lea" proteins in cotton (Baker et al., 1988) and embryo
storage proteins in maize (Kriz and Schwartz, 1986) may also
be related to stress tolerance. A possible role of some Lea
proteins in the desiccation tolerance through membrane
stabilization has been suggested based on their amino acid
sequences (Baker et al., 1988). Abscisic acid (ABA), a
hormone known to inhibit the germination of mature embryos,
is also thought to have a role in regulation of protein
accumulation associated with precocious germination and other
stress conditions during seed development. The ABA
regulation of protein synthesis may be at both the
transcriptional and translational level and may be related to
some environmental stress tolerance during seed development
(Eisenberg and Mascarenhas, 1985).

The preconditioning system was used to study the ABA
levels and specific proteins accumulation as related to the

induction of high-temperature desiccation tolerance during
post-harvest drying of seed corn. Whether some specific
proteins may be accumulated, and their relationship with ABA
during the induction of desiccation tolerance will be
studied.

## MATERIALS AND METHODS

**Plant material**  The hybrid corn seed was produced at Iowa State University, Ames, IA.  The inbred line B73 was used as seed parent, and H99xH95 was the common pollen parent.  Seeds were harvested at moisture contents of 500 to 450 g $H_2O$ $kg^{-1}$ fw (oven method).

**Induction of high-temperature desiccation tolerance**  Samples were preconditioned at 35°C, relative humidity (RH) 22% for 0, 6, 12, 24, 36, and 48 h as described in Parts I and II, to induce high-temperature desiccation tolerance before transfer to 50°C, RH 17% for the completion of the drying process down to 120 g $H_2O$ $kg^{-1}$ fw moisture content.

**Determination of seed germinability**  Eight replications of 10 seeds with different times of preconditioning were surface sterilized with 1% hypochlorite, and cultured in sterilized agar medium.  Seed germination was counted each day after seed began started germination.

**ABA determination**  Eight replications of 40 embryos were ground with 15 mL of 80% methanol using a polytron homogenizer (Roberts and Hooley, 1988).  The extracts were adjusted to pH 8, and held in the dark overnight at 23°C after which the methanol was evaporated at 35°C under vacuum. The mixture was then centrifuged at 27,000 g for 10 min, the supernatant was saved as a water soluble fraction and the pH was adjusted to 9.0.  The water soluble fraction was

extracted with ether 3 times and the aqueous phase was saved and adjusted pH to 3. The aqueous phase was then extracted with ether 3 additional times, and the organic phase which contained ABA and some PA, DPA (Dorffing and Tietz, 1983) was saved. The organic phase was evaporated to dryness under nitrogen at 35°C and the extracts were resuspended in methanol, separated and determined by high performance liquid chromatography (HPLC) with a UV detector at 190 nm (Shimadzu Corporation, Kyoto, Japan) (Ciha et al., 1977). A Chromogobond MC-18 (5$\mu$m) column (Supelco Inc. Bellefonte, PA) (Guinn et al, 1986) was used with a mobile phase of methanol and flow rate of 2 mL min$^{-1}$.

**Protein extraction and SDS PAGE** All procedures were conducted at 0 to 5°C except as noted. Twenty corn embryos (endosperm removed) were homogenized in 5 mL grinding buffer containing 50 mM $K_2HPO_4$. The slurry was centrifuged at 20,000 g for 15 min. The supernatant was saved as a crude extract and diluted 1:5 with SDS-PAGE sample buffer containing 62.5 mM Tris, 1.25% SDS, 1.25% of glycerol and 0.125% of ß-mercaptomethanol. SDS-PAGE electrophoresis was performed according to the method of Laemmli (1970). Slab gels were 1 mm thick and consisted of a 4.5 and 12% (w/v) resolving and separation gels, respectively. Samples were heated at 100°C for 2 min prior to loading into each lane. Gels were stained with Coomassie blue R-250 after

electrophoresis at 3 mamp (resolving gel) and 30 mamp (separation gel) constant current for 16 h.

electrophoresis at 3 mamp (resolving gel) and 30 mamp (separation gel) constant current for 16 h.

## RESULTS

Preconditioning the ears of high moisture corn prior to drying induced desiccation tolerance. Seed vigor as indicated by cold test germination increased by 20% following 24 hours of preconditioning prior to drying at 50°C. The conductivity and the sugar leachate from dried seed when soaked in water were significantly decreased in the seed with 6 hours or more of preconditioning (Part I, Figs. 1, 2). Thus, the preconditioning model system allowed the study of induced desiccation tolerance during seed corn drying, changes in abscisic acid (ABA) levels and protein synthesis patterns during different times of preconditioning.

Preliminary results showed that ABA content (separated by HPLC only after partial purification without calculation of the recovery) remained at a constant and high level through 24 hours of preconditioning and then dropped dramatically after 36 hours as the moisture content in the embryos began to decrease (Fig. 9). A small (8%) and linear loss in seed moisture occurred during the 36-hour preconditioning period indicating no direct correlation between moisture loss and the changes in ABA content. The determination of the seed germinability (Table 5) shows an improvement in seed germinability and a decrease in germination inhibitors during preconditioning treatment, although ABA level remained relatively high within 24 hours

of preconditioning.

SDS polyacrylamide gel electrophoresis showed a new protein band (68K) appearing after 6 hours of preconditioning, and increasing in quantity with increasing preconditioning time. The 68k polypeptide reached its highest concentration after 36 hours (Fig. 10) when the ABA content began to decrease, which shows no direct relationship between 68k protein and ABA levels.
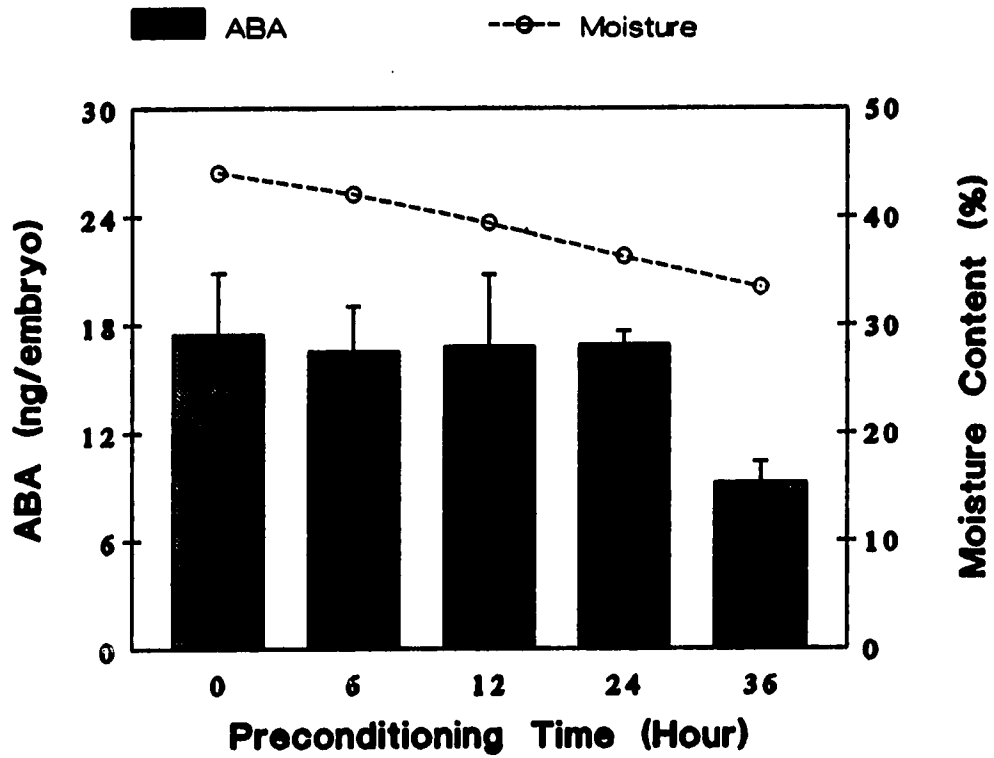
Fig. 9.  Endogenous ABA level in maturing maize embryo during
         preconditioning.  Results are the means and standard
         errors of eight assays

Table 5. Seed germinability following preconditioning
in maturing seed

| Preconditioning | Germination (%) | | |
|---|---|---|---|
| time (h) | 3 days[a] | 5 days | 7 days |
| 0 | 12 | 22 | 38 |
| 6 | 17 | 60 | 61 |
| 12 | 19 | 52 | 54 |
| 24 | 28 | 54 | 56 |
| 36 | 23 | 57 | 62 |

[a]Days after planting on the medium.

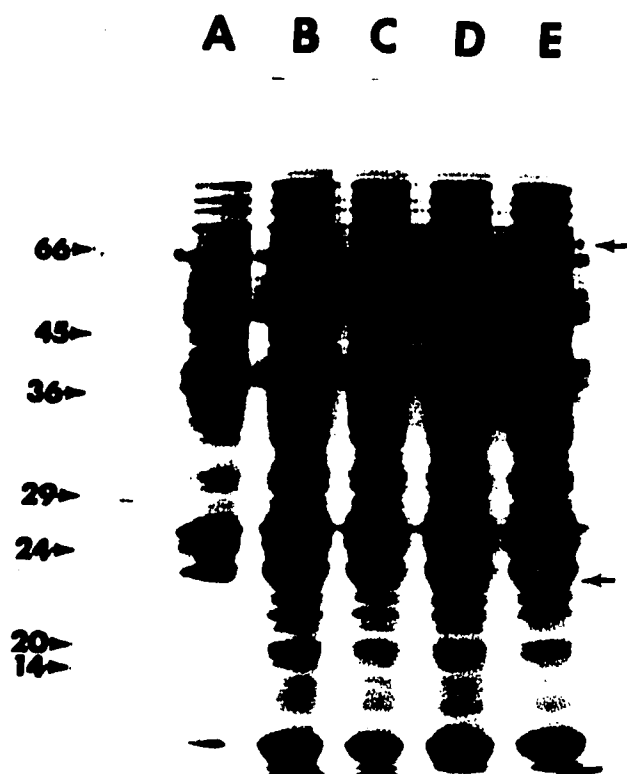Fig. 10.  SDS-PAGE (10%) of soluble proteins in maturing
maize embryo showing temporal changes in protein
synthesis during preconditioning.  A, B, C, D, and
E represent 0, 6, 12, 24, 36 hours of
preconditioning, respectively

## DISCUSSION

Induction of environmental stress tolerance depends upon the properties of the membrane (Parts I and II of this dissertation) and/or the soluble enzymes outside of the membranes (Hale and Orcutt, 1987). It is generally believed, however, that there may be several mechanisms of plant acclimation to environmental stress. These may include the induction of protein synthesis and/or altered protein function. Sachs and Ho (1986) concluded that in all of the environmental stress conditions studied, alteration in gene expression resulted in the induction of new proteins and repression of at least some normally expressed proteins. They believed that these stress-induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with the stress condition. In our studies (Parts I and II) of preconditioning induction of high-temperature desiccation tolerance, membrane seems to be a sensitive site of high-temperature desiccation damage. Using the same preconditioning system, two polypeptides (68kd and 21 kd in SDS PAGE) appeared after 6 hours of preconditioning and increased in quantity with increasing the preconditioning time as seeds become tolerant to high-temperature desiccation (Fig. 10). Meanwhile, ABA, a plant growth regulator widely reported to improve tolerance to stress, was found to remain at a relatively high level through 24 hours of

preconditioning (Fig. 9), then decreased rapidly during the late stage of seed development (Bewley and Black, 1985). A seed germinability test (Table 5), however, shows an improvement in seed germinability after 6 hours of preconditioning, although the ABA levels were still relatively high at this time.

The roles of 68k desiccation-induced protein is not clear. It may be possible that these proteins, as has been suggested for Lea proteins D-11 and D-113 (Baker et al. 1988), function in the "solvation" of cytosolic structure to protect the membranes, or have other metabolic functions related to high temperature desiccation tolerance.

ABA effects on protein synthesis are widely reported (Eisenberg and Mascarenhas, 1985; Goldbach and Goldbach, 1977; Ho, 1983; Mohapatra et al., 1988). The phytohormone ABA has been implicated in plant responses to several environmental stresses (Dale and Campbell, 1981; Quatrano, 1986) and has, therefore, been proposed as a common mediator for plant responses to stress. ABA may also affect gene expression and protein synthesis during seed development as related to stress tolerance. During seed development the addition of ABA at physiological concentration to developing embryos (soybean) prevents their precocious germination and encourages the continuation of developmental processes

(Kermode at el., 1989). The ABA inhibited precocious germination but enhanced: a) the accumulation of storage proteins (Bray and Beachy, 1984; Eisenberg and Mascarenhas, 1985), b) development of enzyme activities in later embryogenesis (Choinski et al., 1981), and c) production of certain mRNA and protein fractions characteristic of late embryogenesis (Lea proteins) (Galau and Dure, 1986). Water deficits cause an increase in ABA in a number of species of higher plants. Gomez et al. (1988) described an experiment to isolate ABA-induced genomic clones and its cDNA in maize embryo and found that its mRNA accumulates in epidermal cells where it is also induced by water stress. They suggested that the increase in ABA levels in the embryo before desiccation is part of the program ensuring the survival of embryos during seed development. Therefore, we believe that the high ABA level prior to 24 hours of preconditioning (though not necessary to inhibit seed germination) and the newly-induced proteins may relate to the transition from desiccation intolerance to tolerance during post-harvest drying of high moisture seed corn. The results indicate that the 35°C preconditioning may require a reduced drying rate, and result in maintenance of a high ABA level, and induction of protein synthesis required for coping with the high temperature stress. The biological function of these proteins, however, is not clear. It seems that the

desiccation-induced proteins found during preconditioning are synthesized as maturing seed transfers from desiccation sensitive to desiccation tolerance state, i.e., from developmental mode to germination mode (Kermode et al, 1989), whereas, ABA regulation of other proteins found in developing embryos such as Lea proteins (Baker et al., 1988; Galau and Dure, 1986) and embryo storage proteins (Eisenberg and Mascarenhas, 1985; Kriz and Schwartz, 1986) may maintain embryo metabolism in a developmental, i.e., largely anabolic, mode and prevent precocious germination which may not be related to desiccation tolerance. Hence, the molecular biology of ABA in induction of high-temperature desiccation tolerance, and the relationship between desiccation induced proteins and membrane stabilization requires further study.

The strategy of such a study would begin with the purification of the desiccation-induced proteins (DIP), preparation of antibodies, and determination of amino acid sequences via automated Edman degradation of the N terminus or of internal peptide fragments (Sussman and Harper, 1989). The antibodies could be used to isolate genes by screening expression libraries. DNA oligonucleotide probes could be generated, based on the amino acid sequences, and used to screen the DNA libraries. The possible function of these proteins, then, may be clarified after such studies.

## GENERAL SUMMARY

Seed corn (*Zea mays* L.) ears harvested at seed moisture contents greater than 400 g $H_2O$ $kg^{-1}$ fresh weight (fw) are sensitive to high-temperature (>45°C) drying, but tolerant to low-temperature drying. Preconditioning the ears of the high-moisture seed corn prior to the high-temperature drying induced the desiccation tolerance. These results suggest that membranes are a sensitive site of high-temperature desiccation damage and that membrane stabilization is important in the induction of desiccation tolerance. Studies of soluble sugar effects on desiccation tolerance indicated that the percentage composition of sucrose and a larger oligosaccharide, raffinose, increased significantly during preconditioning. Soluble-sugar compositional relationships, especially the ratio of raffinose to sucrose, may play an important role in preserving membrane function as indicated by membrane integrity. Membrane phospholipid composition and thermal properties in preconditioned seed were studied to relate high-temperature desiccation tolerance to membrane stabilization. Alterations in phospholipid molecular species (increase PC/PE ratio), and changes in fatty acid composition to a more saturated composition during preconditioning and maturation could be common mechanisms in membrane stabilization under high-temperature desiccation. Thermal properties of membrane fractions also correlated well with

these biochemical changes. Further studies of the plant growth regulator (ABA) and protein synthesis during preconditioning indicated that high levels of ABA through 24 hours of preconditioning and newly-induced proteins may be related to the transfer from desiccation intolerance to desiccation tolerance during post-harvest drying of high-moisture seed corn. The relationships between ABA, protein synthesis and membrane stabilization during high-temperature desiccation are uncertain. A proposed model of the preconditioning process during membrane stabilization and high-temperature desiccation tolerance is summarized in Fig. 11. These relationships, however, require further study.
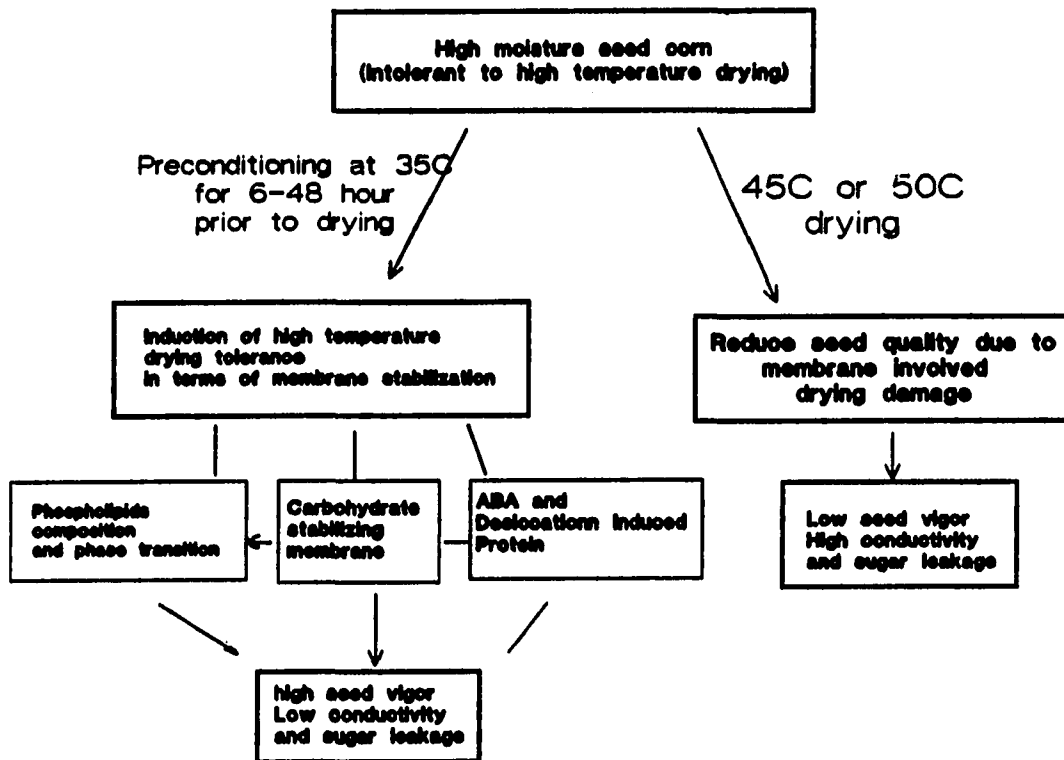
Fig. 11. A summary of the effects of preconditioning on
membrane stabilization and high-temperature
desiccation tolerance during seed corn drying

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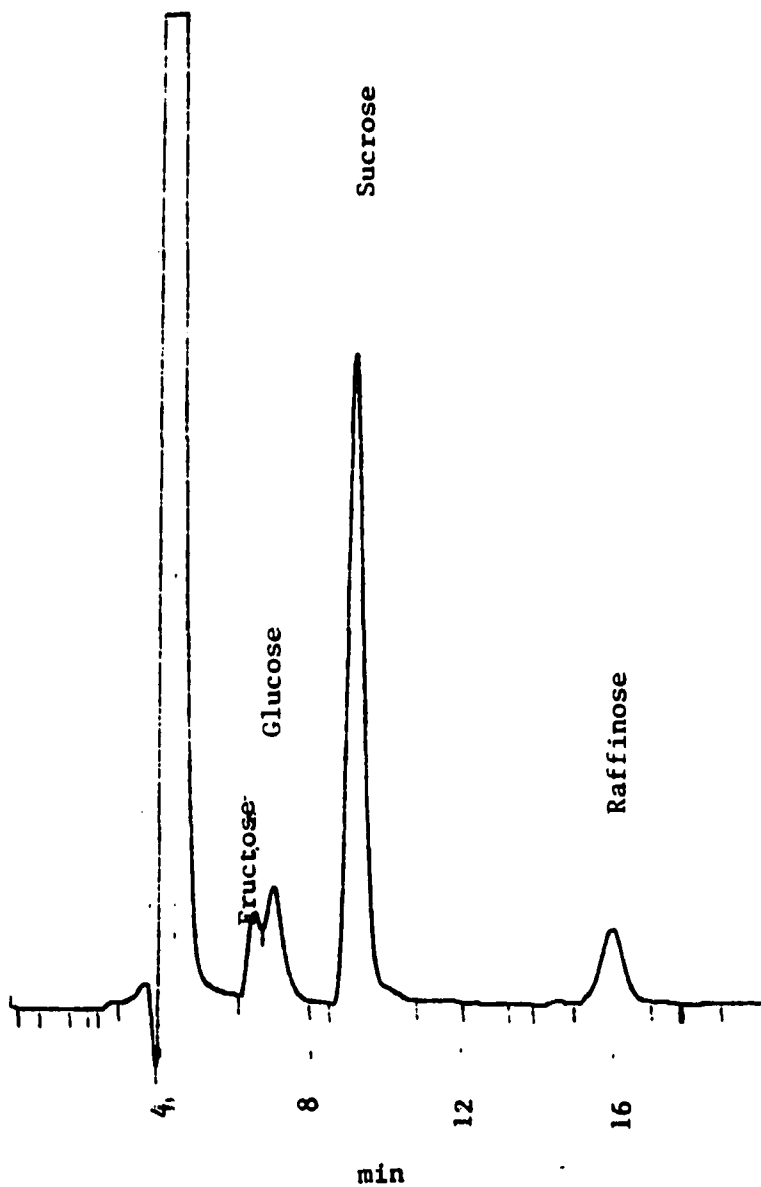
# APPENDIX

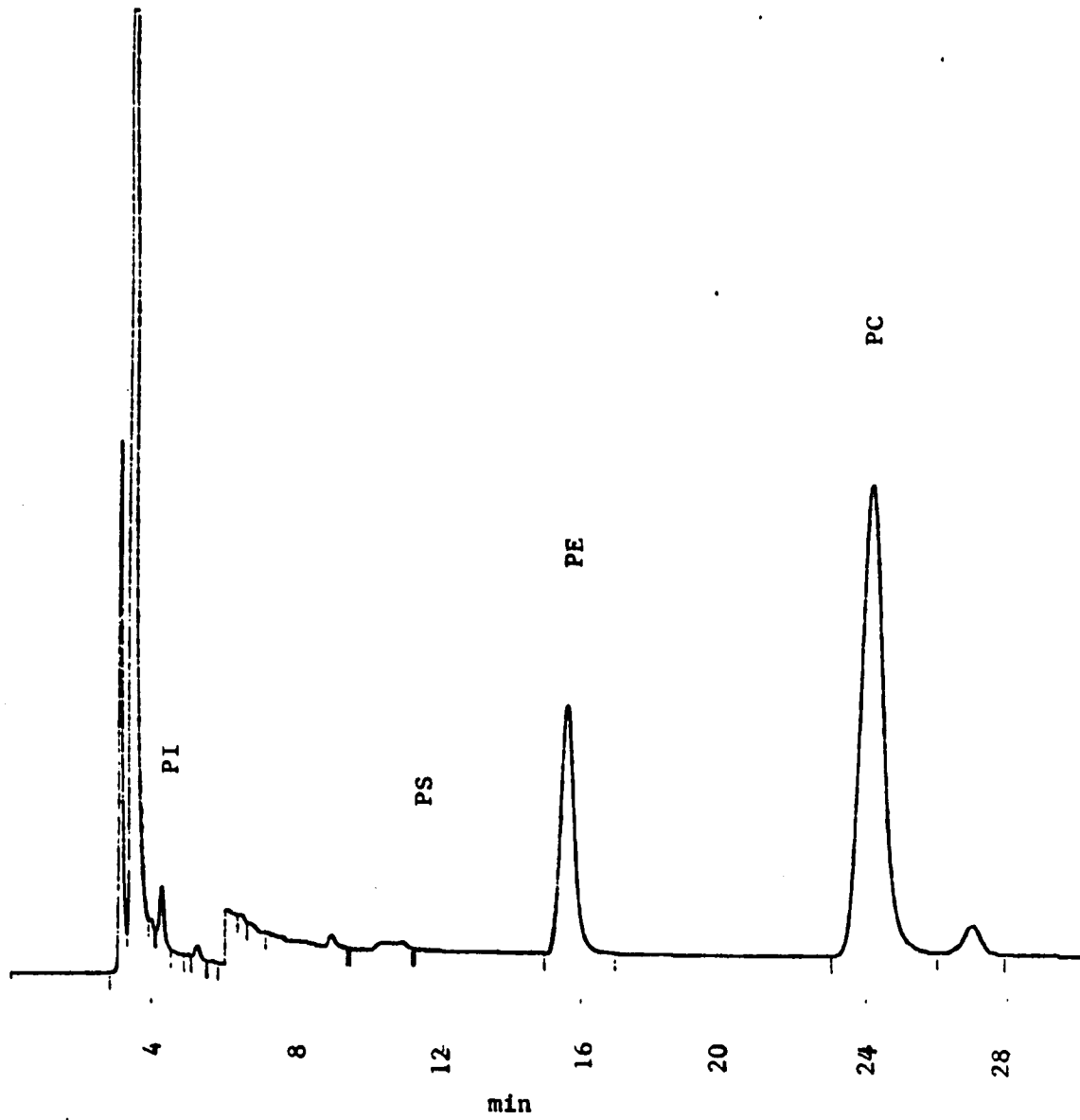Figure A-1.   HPLC-chromatogram of soluble sugars from maturing maize embryos

Figure A-2.  HPLC-chromatogram of phospholipids from membrane
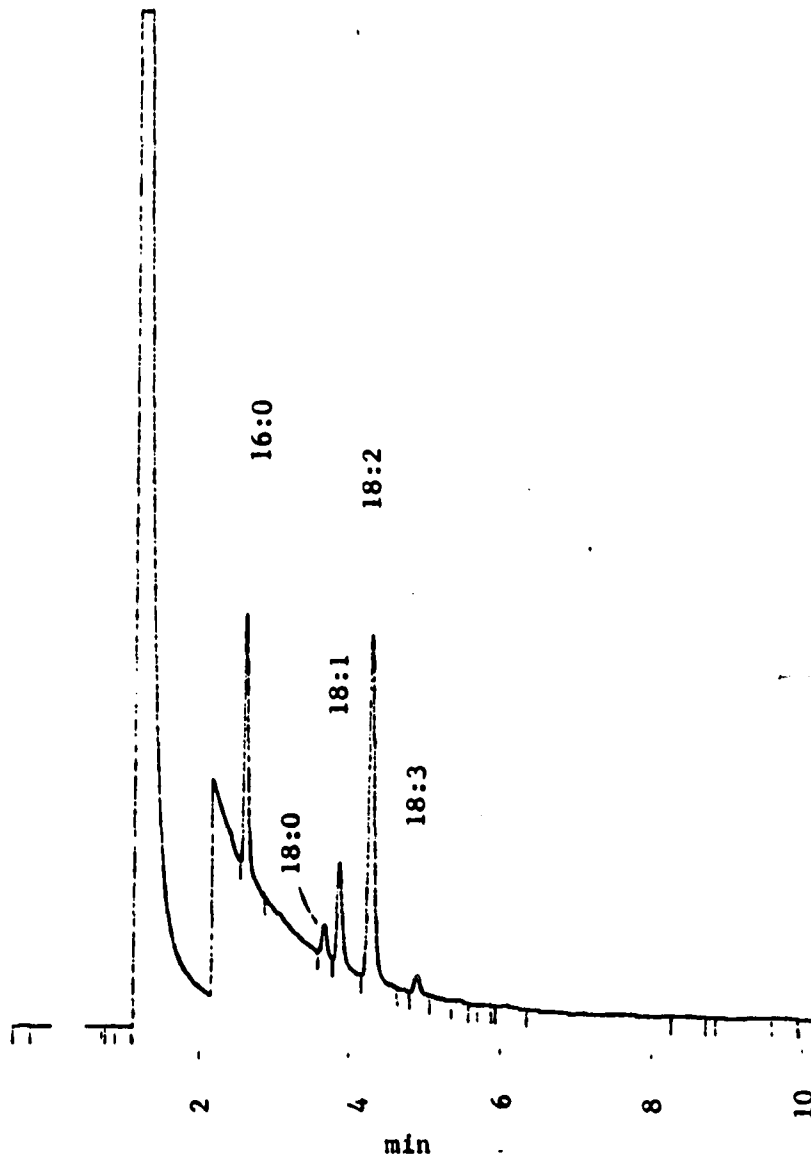fraction of maturing maize embryos

Figure A-3.  GC-chromatogram of fatty acid from membrane
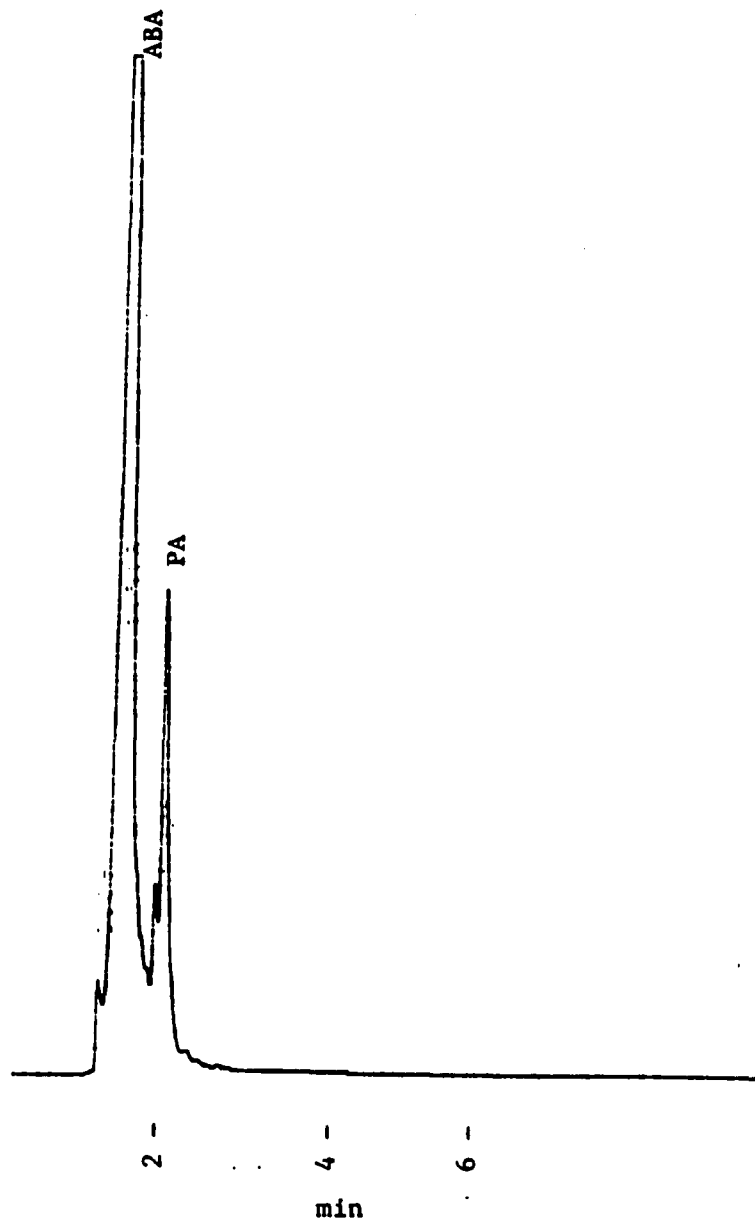phospholipid fraction of maturing maize embryos

Figure A-4.  HPLC-chromatogram of ABA from maturing maize
embryos.  ABA samples were partially purified by
ether partition.