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
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Agronomy
Interdepartmental Major: Plant Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

Post the Major Department
Signature was redacted for privacy.

For Interdepartmental Major
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1990
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INTRODUCTION

Significance and objectives  Seed corn ears are normally harvested at seed moisture contents as high as 400 g H$_2$O kg$^{-1}$ fresh weight and then mechanically dried to 110-120 g H$_2$O 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 H₂O 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 H₂O kg⁻¹ hydration is different than that above
290 g H₂O 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). Moreover, 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 H₂O 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 H₂O 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.
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 H₂O 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 et al., 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.
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$_2$O kg$^{-1}$ fw (oven method). Physiological maturity for the inbreds used in these studies occurs at approximately 350 to 380 g H$_2$O 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$_2$O 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$_2$O 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-µm 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-NH₂ 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 H₂O kg⁻¹ fw decreased during preconditioning, but not in embryos harvested at 390 to 400 g H₂O 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 (R²=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 $\text{H}_2\text{O}$ kg$^{-1}$ fw for A632 at $H_1=535$, at $H_2=454$, and at $H_3=393$ and for B73 at $H_1=547$, at $H_2=442$, and at $H_3=399$. 
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$_2$O kg$^{-1}$ fw at H$_1$=535, at H$_2$=454, and at H$_3$=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 H₂O kg⁻¹ fw at H₁=547, at H₂=442g, and at H₃=399.
<table>
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<th>Soluble sugar content</th>
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<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>Suc</td>
<td>Raf</td>
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<td>g H₂O kg⁻¹ fw h</td>
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Total sugar content including unidentified sugars.

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 H₂O kg⁻¹ fw; B: harvest moisture 393 g H₂O 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 H₂O kg⁻¹ fw; B: harvest moisture, 399 g H₂O 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

<table>
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<th>Sugar composition (%)</th>
<th>Germination (%)</th>
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<th>Conductivity (µamp)</th>
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<td>Warm</td>
<td>Cold</td>
<td></td>
</tr>
<tr>
<td>A632 x (H99xH95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.78</td>
<td>-0.92**</td>
<td>0.85*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.67*</td>
<td>0.71*</td>
<td>-0.81*</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.79*</td>
<td>0.91**</td>
<td>-0.78*</td>
</tr>
<tr>
<td>Raf/Suc</td>
<td>0.83**</td>
<td>0.95**</td>
<td>-0.80*</td>
</tr>
<tr>
<td>B73 x (H99xH95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.68**</td>
<td>-0.79**</td>
<td>0.41*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.27</td>
<td>0.31*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.88**</td>
<td>0.92**</td>
<td>-0.78**</td>
</tr>
<tr>
<td>Raf/Suc</td>
<td>0.89**</td>
<td>0.91**</td>
<td>-0.78**</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.01.
DISCUSSION

Seed corn when harvested above 400 g H₂O 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 H₂O 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 H₂O 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 H₂O 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 H₂O 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 NaHCO₃ 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 NaHCO₃ 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 (5µm) 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 µm) 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 preconditioning times. Results were analyzed using a linear regression ($r^2 = 0.82$ and $0.64$ for conductivity and sugar leakage, respectively)
Table 3. Changes in membrane phospholipids of maturing corn embryos during preconditioning

<table>
<thead>
<tr>
<th>Preconditioning Time (hours)</th>
<th>Phospholipids (μg/g. dw)</th>
<th>PI</th>
<th>PS</th>
<th>PE</th>
<th>PC</th>
<th>PC/PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>207.1</td>
<td>13.7</td>
<td>70.8</td>
<td>259.8</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>220.3</td>
<td>45.8</td>
<td>88.8</td>
<td>296.6</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>189.7</td>
<td>50.1</td>
<td>80.6</td>
<td>309.1</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>186.9</td>
<td>37.4</td>
<td>62.3</td>
<td>371.9</td>
<td>5.97</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>177.1</td>
<td>41.4</td>
<td>92.1</td>
<td>483.4</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>161.2</td>
<td>33.2</td>
<td>104.6</td>
<td>839.9</td>
<td>8.02</td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>63.9</td>
<td>22.3</td>
<td>35.2</td>
<td>89.3</td>
<td>1.05</td>
<td></td>
</tr>
</tbody>
</table>

*PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine.
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

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

*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 counterparts 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
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 H₂O 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 H₂O 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 (5μm) 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 K₂HPO₄. 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 β-mercaptoethanol. 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.
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

<table>
<thead>
<tr>
<th>Preconditioning time (h)</th>
<th>Germination (%)</th>
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<tbody>
<tr>
<td></td>
<td>3 days*</td>
<td>5 days</td>
<td>7 days</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>22</td>
<td>38</td>
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<tr>
<td>6</td>
<td>17</td>
<td>60</td>
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<td>52</td>
<td>54</td>
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<td>24</td>
<td>28</td>
<td>54</td>
<td>56</td>
</tr>
<tr>
<td>36</td>
<td>23</td>
<td>57</td>
<td>62</td>
</tr>
</tbody>
</table>

*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 et al., 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$_2$O 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.
Preconditioning at 35°C for 6-48 hours prior to drying

High moisture seed corn (intolerant to high temperature drying)

- Induction of high temperature drying tolerance in terms of membrane stabilization
  - Phospholipids composition and phase transition
  - Carbohydrate stabilizing membrane
  - ABA and Dehydration Induced Protein
  - High seed vigor, Low conductivity and sugar leakage

- 45°C or 50°C drying
  - Reduce seed quality due to membrane involved drying damage
  - Low seed vigor, High conductivity and sugar leakage

Fig. 11. A summary of the effects of preconditioning on membrane stabilization and high-temperature desiccation tolerance during seed corn drying
LITERATURE CITED


ACKNOWLEDGMENTS

I wish to express my deep appreciation and gratitude to Dr. Joseph S. Burris, my major professor, for his direction, patience and encouragement.

My gratitude to Drs. Cecil R. Stewart, Irvin C. Anderson, Russell E. Mullen, Ethan Hack and Eve Wurtele for serving on my graduate committee and for reviewing this dissertation. Their helpful suggestions are deeply appreciated.

My thanks to Dr. Pamela J. White, Department of Food Nutrition, for the use of her DSC. Also my thanks to Dr. Verlyn Fick, Dr. Stephen Malone, Randy Madden, Helene Lawrence, and all members of the Seed Science Center for their help and friendship during my graduate study at Iowa State University.

I am deeply grateful to Gustafson Inc. for their scholarship to support my graduate study at Iowa State University. Special thanks go to Dr. K. W. Rushing, Dr. R. P. Knake and S. C. Shen of Gustafson Inc.

Finally, I am greatly indebted to my wife, Lisha, and my daughter, Jiabei, for their understanding and support during this endeavor.
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.