Dehydrin-like protein expression profiles of maize seedlings during germination, low temperature stress, and genotype

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Dehydrin-like protein expression profiles of maize seedlings
during germination, low temperature stress, and genotype

by

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A thesis submitted to the graduate faculty
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This is to certify that the master's thesis of

Apsornsawan Arwatchanakarn

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
DEDICATION

I dedicate my thesis and my graduate studies to my mother, Channian Chinda, and to my daughter, Rutaiporn (Lin) Arwatchanakarn, who are at home in my native Thailand. Though we have been very distant you have never left my thoughts. Thank you for all your love, support, and understanding.
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ABSTRACT

Improving the cold tolerance of early season performance of maize is an important element of agronomic performance in many production areas. Dehydrin or Dehydrin-like proteins may be related to cold tolerance and their roles in chilling tolerance in many plant species have been a major focus of research. This research investigated the disappearance of dehydrin-like proteins in maize genotypes during seed germination and the re-induction of dehydrin-like proteins after the exposure of seedlings to low temperature stress. Inbred lines with putative differences in tolerance to low temperature stress were utilized. The genotypes analyzed were Co255 (cold tolerant), A619 (cold intermediate) and Mo17 (cold sensitive). To achieve these goals, the concentrations of total proteins and boiling soluble proteins of the three genotypes were analyzed in a split plot of a randomized complete block design. The protein profiles were evaluated by discontinuous SDS-PAGE. Dehydrin-like proteins were detected by immuno-blot assay of boiling soluble protein fractions. Dehydrin-like proteins did not disappear during seed germination while the 40kDa dehydrin-like protein of Mo17 disappeared after day 1 (imbibed embryos), the 22kDa dehydrin-like protein remained detectable throughout the course of this study in all genotypes. No re-induction of dehydrin-like proteins due to low temperature stress was found and their profiles were not affected by stress or genotype. This study might be the first to show; 1) dehydrin-like proteins are detectable in root maize 5-day-old seedlings and 6-day-old seedlings in 25°C and 2) dehydrin-like proteins profiles in 2 maize genotypes, A619 and Co255. The role of dehydrin-like proteins in cold tolerance and their re-induction following cold stress on a whole plant basis is no conclusively evaluated by this study, especially since only root tissues were used. Dehydrins or dehydrin-like protein expression in different tissues can vary. Because cold
tolerance is a complex trait, further investigations regarding the role of DHN in cold tolerance are warranted.
CHAPTER 1. INTRODUCTION

Lee and Lee (2003) have stated that “temperature is one of the most important environmental factors affecting plant development and crop productivity” Temperatures higher or lower than optimum can speed up or slow down numerous processes involved in germination, transpiration, photosynthesis, flowering and harvesting.

Growers usually like to use full season varieties under the assumption that this maximizes yield. Often this means planting early in the spring and harvesting prior to cold weather or rain at the end of the season (Ismail et al., 1997). Additionally, early planting can result in earlier canopy closure, earlier pollination, and having more of the grain filling period during long-light days (Mock and Pearce, 1975; Pendleton and Egli, 1969). Finally, earlier planting can result in earlier seed or grain maturity, drier grain at harvest, and reduce the risk of frost damage at the end of the season (Aidun et al., 1991). Taken together, these agronomic issues often lead to increasing the risk of exposing young seedlings to frost or low temperature stress, which may prevent or delay the mobilization of seed reserves in germinating seeds resulting in poor emergence and low seedling vigor (Harrington and Kihara, 1960; Pollock and Toole, 1966; Pollock et al., 1969; Hobbs and Obendorf, 1972; Ismail et al., 1997). The potential agronomic benefits of early planting and increased plant performance at low temperatures combined with the chilling sensitivity of some crops, have stimulated researchers to try to improve the cold tolerance of those crops.

In general, tropical and sub-tropical crops are damaged when they are exposed to temperature below 10°C (Xin and Browse, 2000). Unruh et al. (1996) and Madakadze et al. (2003) reported slow stand development and leaf damage in turf grass species under low temperature stress. Mungbean seedlings exposed to 4°C for 2 days in a growth chamber
showed chilling injury as defined by increased electrical conductivity (Chang et al., 2001). Lee (2001) reviewed the effect of low temperature on Korean rice. The critical temperature for germination appeared to be 10°C. Temperatures below this critical point resulted in poor and delayed germination.
CHAPTER 2. LITERATURE REVIEW

Maize (Zea mays L.): Cold tolerance and cold sensitivity

Maize is a tropical crop (Shaw, 1988) preferring temperatures ranging from 20-30 °C. (Kingston-Smith et al., 2000b). Even though it is considered a chilling sensitive species, it is an important crop in many areas of the world (Miedema, 1982). In fact, maize is widely grown in temperate regions where low temperature influences its performance and productivity (Fracheboud et al., 2002).

Several researchers have reported that numerous components of maize performance are affected by chilling stress including germination (Eagles and Brooking, 1981; Koscielniak, 1993; Hodges et al., 1995b), stand establishment, and seedling growth (Dubtz et al., 1962; Cal and Obendorf, 1972; Mock and McNeill, 1979; Aidun et al., 1991).

In previous studies, researchers have used different techniques to phenotypically distinguish between cold sensitive and cold tolerant maize genotypes. During the early growth phase “percent emergence, emergence index, and seedling dry weight” (Mock and McNeill, 1979), “speed of germination and median germination time” (Furter and Venter, 1990), and “percent germination, percent viability, and average time taken to germination” (Hodges et al., 1994) have been used. During the late growth phase, phenotypic differences such as, “shoot length, leaf viability, leaf color, dead plants” (Aidun et al., 1991) and shoot/root ratio (Richner et al., 1996) have been used.

Low temperatures reduce photosynthetic activity resulting in low dry matter accumulation (Miedema and Sinnaeve, 1980) and decreasing yield. Several researchers have studied the physiological and metabolic changes in photosynthesis of cold-sensitive species, such as maize, to determine if these traits were more accurate than phenotype in...
characterizing cold tolerant or sensitive genotypes. Massacci et al. (1995) and Hodges et al. (1997a) have shown that increased oxidative stress is an important mechanism by which photosynthesis is reduced at low temperatures. Recently, Lee et al. (2002) proposed using “Leaf Carbon Exchange Rate (CER), and rate of development (from planting to 7-leaf stage)” to characterize cold tolerant inbred lines. Studies of superoxide dismutase (Van Breusegem et al., 1999a, b) in maize leaf chloroplasts have shown that elevated levels of manganese superoxide dismutase (MnSOD) and iron superoxide dismutase (FeSOD) protect leaves from low temperature damage and may be indicators of cold tolerance in maize.

**Low temperature stress: Physiological and molecular changes in plants**

Numerous reports have been published illustrating that low temperature stress induces a number of alterations in the physiological and molecular processes of susceptible plants. Several investigators reported increases in oxidative stress during exposure to low temperatures as evidenced by elevated levels of antioxidants such as ε-tocopherol and glutathione (Leipner et al., 1999) as well as induction of several antioxidant enzymes. These enzymes include ascorbate peroxidase (ASPX), catalase (CAT), glutathione reductase and monodehydroascorbate reductase (MDHAR) (Anderson et al., 1995; Hodges et al., 1997b; Pastori et al., 2000). If the increased oxidative stress derived from the effect of low temperature on plants cannot be ameliorated by antioxidants and enzyme systems, damage to the plasma membrane resulting in losses of membrane function as evidenced by increased electrolyte leakage can occur (Crowe et al., 1992). Low-temperature induced changes in membrane permeability and losses in other critical membrane functions could substantially reduce cell/plant performance and eventually lead to cell death. The importance of these processes to plant performance has led some to hypothesize that membranes are the primary
site of cold damage (Levitt, 1972). Increasing the metabolic production of materials that protect the plant and plant membranes from stresses is probably one of the elements of plant acclimation to stress. Other metabolic responses that may support plant acclimation to stress include increased production of abscisic acid (ABA) (Chen et al., 1983), solutes like proline, betaine, polyols (Koster and Lynch, 1992; Kishitani et al., 1994) soluble sugars (Black et al., 1999), chloroplast protein (Schneider et al., 1995), unsaturated fatty acids (Diepenbrock and Stamp, 1982) and LEA (Late Embryogenesis Abundant) proteins (Close, 1997). Among LEA proteins, group 2 LEAs also known as dehydrins it has been suggested that they play a key role in protecting against membrane damage from stresses associated with dehydration or low temperature (Close, 1996).

**Dehydrin**

Dehydrin (DHN) or dehydrin-like protein (dlp) is the popular names for a group of proteins originally described by Dure et al. (1989) as group 2 LEAs because their levels increased dramatically in cotton embryos during the latter stages of seed development. Specifically, these proteins are grouped together based on size, amino acid composition, and other features. The term “dehydrin” was first proposed by Close et al. (1989) based on the putative role of these proteins in dehydration. The so-called “COR” (cold regulated) and “RAB” (response to ABA) protein groups also contain DHNs (Close, 1997). They can be found in Algae (Li et al., 1998), Cyanobacteria (Campbell and Close, 1997) and all plants (Close, 1997), including lower plants like Bryophyte (Mosses) and Pteridophyta (Ferns) (Campbell and Close, 1997; Mtwisha et al., 1998; Garay-Arroyo et al., 2000), higher plants like Coniferophyta (Gymnosperms) (Close et al., 1993; Reynolds and Bewley 1993; Bewley
et al., 1993; Hellwege et al., 1994; Jarvis et al., 1996; and Richard et al., 2000) and Angiospermophyta (Angiosperms) (Campbell and Close, 1997; Close, 1997).

**Characteristics of dehydrins in general**

DHNs are abundant in seeds during the late stages of embryogenesis (Dure, 1993). At this stage, the seed is maturing and has probably begun to dry. However, during germination and early seedling growth, the quantity of DHN decreases (Dure, 1997). Dehydrins can be re-induced by stressing seedlings (Close et al., 1989; Borovskii et al., 2002). Their molecular weights, as estimated from SDS-PAGE, range from 9 to 200 kDa (Oullet et al., 1993 and Takahashi et al., 1994). They comprise 0.1-5% of the total soluble protein in mature embryo and stressed seedlings (Close, 1997) and remain soluble at temperatures >95°C (Campbell and Close, 1997).

Members of the LEA protein family are generally hydrophilic, possess unique sequences specific to each family, usually contain a high percentage of glycine and lack of tryptophan and cysteine (Baker et al., 1988; Dure, 1993; Ingram and Bartels, 1996). Garay-Arroyo et al. (2000) indicated that LEA proteins are a subset of a broader group of proteins, which they called “hydrophilins.” This hypothesis was based on a screening of the Swiss Protein Database via an algorithm that selected members as a result of glycine content and hydrophilicity index. The hydrophilins, according to Garay-Arroyo et al. (2000), forms the basis for a hyperosmotic state in response to plant water deficit.

Although more work is required to determine the specific biochemical properties of DHNs, acknowledgement should be given to Dr. Close and his laboratory for expanding our understanding of DHNs. In 1996, Close proposed a nomenclature for DHNs based on a varied combination of segments that he called Y, S, K and Φ. The K-segments are composed
of a highly conserved 15-amino acid, Lys-rich consensus sequence (EKKGIMDKIKEKPLG), which occurs in each dehydrin studied so far (Close, 1997). Because it is a feature common to all DHNs studied to date and its sequence is highly conserved, the K-segment has been used to distinguish DHNs from the other LEA proteins. Thus, Close et al. (1993) produced antibodies against a synthetic K-consensus sequence. Currently, these antibodies are widely used to detect DHNs in plant tissues. The K-segments can be found at the C-terminus and may be repeated one to 11 times. For example, DHN1 of *Zea mays* has 2 K-segments (Close, 1997; Koag et al., 2003). Under certain conditions, the K-segments formed class A2 amphipathic α-helices and are believed to lead to binding of DHN to membrane phospholipids (Koag, 2003). Based on the potential phospholipid binding capacity of the K-segments, DHNs are believed to stabilize membranes during dehydration or cold stress (Ismail et al., 1999a; Buitink et al., 2000; Koag et al., 2003). The S, Y and Φ-segment are less conserved than K-segment (Close, 1996; 1997). The serine-rich (S) segment probably functions in protein phosphorylation processes (Vilardel et al., 1990; Alsheikh et al., 2003). The Y-segments are located at N-terminus. The function of the Y-segment is not known at this time. The φ-segments are rich in Proline, Alanine and polar amino acids like Glycine. The location and occurrence of the φ-segments varies among DHNs (Close, 1996). Low levels of Tryptophan are typical of DHNs (Close, 1997).

DHNs are believed to exist mainly as “random coils” in hydrated environments (Campbell and Close, 1997). Other authors describe this structural characteristic as “low secondary structure content” (Lisse et al., 1996), or simply “unfolded in a native state” (Close, 1996). Upon dehydration, however, DHNs are believed to fold in a more structured
fashion with the K-segments forming \( A_2 \) amphipathic \( \alpha \)-helical structures allowing DHNs to bind to lipid vesicles (Koag, 2003).

The genetic basis for dehydrin synthesis has been investigated. Of these investigations, the genetics of barley DHNs have received considerable attention. Choi and Close (1999) lists eleven genes (\( dhnl-11 \)) in barley that, together, encode \( YSK_2 \), \( Y_2SK_2 \), \( SK_3 \), and \( K_9 \) DHNs. Close (1997) lists six genes (\( dhnl-6 \)) that produce \( YSK_2 \), \( YSK_3 \), \( Y_2SK_2 \), \( SK_3 \) and \( K_9 \) DHNs, respectively in maize. Campbell and Close (1997) indicate that the “\( dhnl \) and \( rab17 \) products are nearly identical, differing in length by only one amimo acid, and encoding polypeptides of ca17-18 kDa”. The \( dhnl \) gene is located on chromosome 6L and \( dhn2 \) is located on chromosome 9S of maize (Campbell and Close, 1997). These genes encoded 2 peptides, DHN1 and DHN2, with molecular masses of ca 22 kDa (by SDS-PAGE analysis) (Close, 1996) and ca 40 kDa, (Asghar et al., 1994; Campbell et al., 1998), respectively. Campbell and Close (1997) reported that maize genome contains other \( dhn \) genes in addition to \( dhnl \) and \( dhn2 \).

The quantity and location of DHNs in cells varies depending on plant species, tissue, type of stress (Table 1), and stage of growth. Immunomicroscopy studies of DHNs indicated that they are predominately located in cytoplasm and nucleus of the cells (Egerton-Warburton et al., 1997).

The data from Table 1 indicated that DHNs might be found in the major plant organelles: cytoplasm, nucleus, plastid, chloroplast, mitochondria, protein and lipid bodies, vicinity of the plasma membrane and lumen of vacuoles. The sub-cellular location of DHNs has been used by researchers to develop hypotheses regarding the roles of plant DHNs.
Table 1. Subcellular localization of plant dehydrins

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Stress</th>
<th>Organelle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Craterostigma plantagineum</em></td>
<td>leaves</td>
<td>drought</td>
<td>cytoplasm</td>
<td>Schneider et al., 1993.</td>
</tr>
<tr>
<td>Maize</td>
<td>shoot and root apex</td>
<td>exogenous ABA</td>
<td>cytoplasm</td>
<td>Asghar et al., 1994.</td>
</tr>
<tr>
<td>Maize</td>
<td>embryos</td>
<td>dehydration</td>
<td>cytoplasm, nucleus, protein and lipid bodies</td>
<td>Asghar et al., 1994; Goday et al., 1994; Egerton-Warburton et al., 1997.</td>
</tr>
<tr>
<td>Maize</td>
<td>embryos</td>
<td>exogenous ABA</td>
<td>cytoplasm and nucleus, protein and lipid bodies</td>
<td>Goday et al., 1994; Egerton-Warburton et al., 1997.</td>
</tr>
<tr>
<td>Spinach</td>
<td>hypocotyls / cotyledons</td>
<td>cold and water deficit</td>
<td>cytoplasm, nucleus and endoplasmic reticulum</td>
<td>Nevin et al., 1993.</td>
</tr>
<tr>
<td>Wheat</td>
<td>crowns</td>
<td>cold</td>
<td>cytoplasm and nucleus</td>
<td>Houde et al., 1995; Sarhan et al., 1997.</td>
</tr>
<tr>
<td>Pea</td>
<td>root tip meristems</td>
<td>slow dehydration</td>
<td>cytoplasm and nucleus</td>
<td>Bracale et al., 1997.</td>
</tr>
<tr>
<td>Peach</td>
<td>bark</td>
<td>cold</td>
<td>Cytoplasm, nucleus and plastid</td>
<td>Wisniewski et al., 1999.</td>
</tr>
<tr>
<td>Birch</td>
<td>apices</td>
<td>cold</td>
<td>protein bodies and starch-rich amyloplasts</td>
<td>Rinne et al., 1999.</td>
</tr>
<tr>
<td>Celery</td>
<td>vascular</td>
<td>cold, drought and ABA</td>
<td>lumen of vacuoles</td>
<td>Heyen et al., 2002.</td>
</tr>
</tbody>
</table>

Possible role of plant dehydrins

DHNs are assumed to have multiple biological roles since they are found in diverse tissues and in response to diverse stresses. Dure (1993) proposed that they might stabilize proteins and membranes to protect the cell from dehydration damage. This stabilization theory seems to be the most popular as it has been investigated and supported by many researchers in monocot and dicot plant species (Close, 1997; Danyluk et al., 1998; Leobigildo, 2001; Borovskii et al., 2002; Koag et al., 2003; Soulages et al. 2003). In *in vitro* studies of woody plants, it has been hypothesized that DHNs may be involved in enhancing
enzyme activities of at least two enzymes during cold stress, \( \alpha \)-amylase (Rinne et al., 1999) and lactate dehydrogenase (Wisniewski et al., 1999). The DHNs in poikilohydric resurrection plants were hypothesized to work synergistically with sucrose in protecting against cell damage during dessication (Scott, 2000; Norwood et al., 2003). Nylander et al. (2001) found a dehydrin (RAB18) in stomatal guard cells of *Arabidopsis thaliana*, which led them to propose its role as “regulator of cell osmotic potential”. Supporting this idea, Kontunen-Soppela (2001) found a relationship between the concentration of 60-kDa-dehydrin and the osmotic potential in scots pine tissues. The last proposed role of DHN results from its potential calcium binding properties. Heyen et. al (2002) studied membrane – associated calcium binding protein from the vacuole membranes of celery (*Apium graveolens*). Based on biochemical characteristics and antigenicity, they indicated VCaB45 is a DHN. The calcium binding ability of VCaB45 was influenced by its phosphorylation state. Mediating the level of free calcium in the cell may be an important mechanism by which low-temperature stress can be ameliorated. This role was also supported by Alsheikh et al. (2003) in Arabidopsis. They reported that the ERD14 dehydrin in *Arabidopsis* showed calcium-binding properties which varied according to its phosphorylation state as possibly affected by casein kinase II activity.

**Relationships between dehydrin accumulation and stress tolerance in plants**

**The appearance of mRNA and dehydrin synthesis in seed during germination and seedling growth.**

During maturation drying, seed tissues lose water and the metabolic activities in the embryo decline. Those events obviously involve physiological and metabolic changes including mRNA and protein synthesis. Han et al. (1997) compared the levels of *lea* mRNAs
and the levels of storage proteins in mature seed. They found that the levels of *lea* mRNAs remained high in mature seeds, whereas the mRNA of storage proteins was undetectable. The regulation and expression of *lea* mRNAs differs among species, genotypes, and tissues. Galau et al. (1987) found that *lea* mRNAs in cotton (*Gossypium hirsutum*) are abundant just prior to desiccation and then disappear during the first day of germination (Hughes and Galau, 1987). In castor bean (*Ricinus communis* L.), *lea* mRNAs in endosperm are lost rapidly during germination, whereas dehydrin proteins are very stable and can be detected until 4 days after imbibition (Han et al., 1997). Close et al. (1989) reported that DHNs were not detectable in 40 mm long seedlings of maize but could be re-induced by 24-30 hours of dehydration. In pea cotyledons (*Pisum sativum*), DHNs accumulated to high levels at maturity, then declined after germination and became undetectable in 19 day-old the seedlings (Robertson and Chandler, 1992). The *RAB-17* maize mRNAs accumulate in embryo tissues and reach a maximum in the mature, dry embryo (Sanchez-Matinez et al., 1986). In 1988, Goday et al. studied the appearance of *RAB-17* maize mRNAs at various development stages. They reported that these mRNAs were not detected in young embryos, but they were detected after ABA treatment. They were abundant in mature embryos but undetectable in 2-day-old seedlings. However, Vilardell et al. (1990) found that *rab-17* mRNAs were undetectable in the first hours of imbibition, but RAB-17 proteins did not disappear until after 2 days of germination. The rapid disappearance of DHNs after germination has been reported by Mao et al. (1995) and Cordova-Tellez (2001). Cordova-Tellez (2001) showed that the ca 22 kDa and ca 40 kDa polypeptides were hardly detected after the shoot and root become visible (~2-3- day-old seedling). He also found that the ca 40 kDa disappeared earlier than the ca 22 kDa.
The induction of dehydrin proteins by drought and cold stress during seedling emergence

It is well established therefore, that DHNs are abundant in mature dry seeds and disappear upon germination. The timing of the disappearance of DHNs during germination depends on species, genotype, types of tissues and also types of dehydrin proteins. In general, LEA proteins levels are reduced over the course of germination (Dure, 1997). DHNs can be induced in vegetative plant tissues by abiotic stresses like cold, drought, salinity and ABA (Close, 1996; Bray, 1997; Sarhan et al., 1997; Whitsitt et al., 1997; Cellier et al., 1998; Danyluk et al., 1998; Thomashow, 1998; Ismail et al., 1999b; Borovskii et al., 2000, 2002; Tabaei-Aghdaei et al., 2000; Zhu et al., 2000; Nylander et al., 2001; Borovskii et al., 2002). These authors found that some DHNs were induced by specific stresses while others were induced by several different stresses. They also reported that, depending on the dehydrin, expression may or may not occur predominately in specific cells or tissues. For instance, dlp, 80kDa (P-80) in barley responded only to low temperature and accumulated mainly in vascular tissue and epidermis (Bravo et al., 1999). Similar results were reported for the induction of the LT130 DHN protein in Arabidopsis. Its quantity increased only in response to low temperature and tissue specific accumulation occurred in vascular tissue and anthers. Alternatively, LT129 and ERD14 DHN proteins responded to more than one stress (eg. low temperature, ABA and salinity) and were found in several different tissues (Nylander et al., 2001).

Dehydrin induction by drought stress

Robertson and Chandler (1992) studied the expression of pea dehydrin in different tissues during seedling dehydration. They reported that mRNA re-induction occurred after
one day of dehydration in both cotyledons and shoots and after two days in roots. The DHNs were detectable longer than the mRNAs. This means that mRNAs were degraded before DHNs. DHNs were detected in cotyledons and shoot in 6 and 10 days respectively after dehydration and were undetectable in root despite being exposed to the same environment as the other seedling tissues.

In sorghum (Sorghum bicolor [L.] Moench), DHN mRNA and DHN protein are more strongly induced in leaves and roots of mature plants by water deficit than in young seedlings (Wood and Goldsbrough, 1997). However, Chandler et al. (1993), working with seedlings and mature barley plants, found higher levels of dehydrin mRNAs in seedlings than in mature plants tissues.

A relationship between drought tolerance and dehydrin gene expression in wheat (Triticum durum) has also been reported. Labhilili et al. (1995) found a higher levels of dehydrin mRNA in drought-tolerant cultivars than in drought-sensitive cultivars. Further, drought tolerant cultivars induced the synthesis of dehydrin mRNA faster than the drought-sensitive cultivars. These results are similar to those reported for ‘aspen’ poplar trees. Pelah et al. (1997) reported that DHNs accumulate to a higher level in drought-tolerant Populus popularis than in less drought-tolerant Populus tomentosa.

**Dehydrin induction by cold stress**

As previously mentioned, cold or low temperature stress is one of the stresses involved in dehydrin induction in vegetative tissues. Welin et al. (1994) used northern blots to study dehydrin mRNA induction after they exposed leaves and stems of Arabidopsis thaliana to 4°C for 15 days. Following 2 h of cold treatment, they could detect mRNAs of dehydrins LT130 and LT140 and DHN/LEA/RAB proteins. The protein levels reached their
highest after 8 to 16 h, then declined gradually after 48 h and remained at that level throughout the entire treatment period. The dehydrin mRNAs were undetectable after 2 h of exposure to normal growth temperature, 22°C.

Stanca et al. (1995) monitored the accumulation of COR14 (cold-induced protein) after one week at 4, 6, 8, or 10°C in cold resistant and susceptible barley cultivars. They found higher levels of COR14 in the cold resistant cultivar in every temperature except 10°C. However, the cold susceptible cultivar synthesized COR14 only at 4°C. The COR14 protein is encoded by cDNA clone pt59 and its transcription pattern was also determined in related cereals. They concluded that the difference in COR14 levels between these cultivars was the result of differential gene expression in response to low temperatures. Clear evidence of a genetic basis for differential cold tolerance has been presented in cowpea (Vigna unguiculata). Ismail et al. (1997 and 1999a, b) reported a positive relationship between the presence of ca 35kDa dehydrin protein and cold tolerant cultivars in cowpea. They showed that the addition of a ca 35kDa dehydrin to cold intolerant cowpea lines improved the cold tolerance of those lines. This work included crossing the donor line to the cold intolerant lines and a backcrossing program to reduce the impact of “other genetic” differences. In two recent papers from Borovskii et al., 2000 2002, they investigated cold-induced proteins in the mitochondria of seedlings of rye and wheat at 4°C and maize at 10°C. They found that two dlpS, 63kDa and 52kDa in seedling mitochondria increased in response to low temperature stress. Higher accumulations of DHNs were found in winter rye (Secale cereale L.) than in winter wheat (Triticum aestivum). Exposure of 2-or 3-day-old maize seedlings to 10°C for 1 or 7 days increased dlp levels slightly more than 3-or 4-day-old maize seedlings at control
temperature, 27°C. They did not describe the single genotype used or compare its responses to those of any other maize genotypes. Therefore, their conclusions do not address whether exploitable variation in dlp expression in response to low temperature stress exists in maize and whether or not this variation was associated with cold tolerance or susceptibility.

This research investigated the changes of dehydrin-like proteins during germination and following cold stress. And to determine if inbred lines with putative differences in tolerance to low temperature stress respond to low temperature stress differently
CHAPTER 3. MATERIALS AND METHODS

Plant material

Three maize (*Zea mays* L.) inbreds were used in this study: a cold tolerant genotype, Co255 (Hodges et al., 1995a); a cold intermediate genotype, A619 (Hardacre and Eagles 1980); and cold sensitive genotype, Mo17 (Haldimann, 1998).

Seed planting and experiment set up

Seeds were surface-sterilized by soaking them in 1% (v/v) sodium hypochlorite for 1 min and then rinsing 3 times with sterile water, 1 min each time and blotted dry on paper towels before planting (McGee, 1988). The seeds were germinated in paper towels and placed upright in buckets (12 units in one bucket divided by a wire grid). The buckets were covered with black plastic bags and placed in dark growth chambers. Two growth chambers were used and controlled to be 25°C (control temperature) or 7°C (stress temperature).

The study was designed and conducted as a split plot of a randomized complete block design with time as the blocking factor. There were 5 main plots (germination times or days), 3 sub plots (genotypes) and 3 replications. Germination time or day treatments included 1-day-old imbibed seeds (D1), 3-day-old seedlings (D3), 5-day-old seedlings (D5), 6-day-old seedlings (D6) at 25°C and 6-day old seedlings with stress (D6S). The D6S treatment consisted of germinating seeds at 25°C for 5 days then exposing them to 7°C for 1 day. The combination of days and genotypes consisted of 15 treatments. Days and genotypes were randomized for the location inside the growth chamber and inside the buckets, respectively. The amount of proteins was compared and the appearance of DHNs was investigated.
Plant tissue and protein extraction

Proteins were extracted from freeze-dried, ground tissues of maize embryos and root. Embryos were excised from 1-day-old imbibed seeds and roots were separated from the seedlings from day3, day5 (control), day6 (control) and day6 stress using a sharp razor blade. The excised embryos and roots were put in the plastic bags and kept in -80°C for 24 h then freeze-dried for 72 h. The freeze-dried embryos were ground with a mortar and pestle (kept in refrigerator over night before use). Freeze dried root tissues were ground in a paint shaker (Red Devil Equipment, Brooklyn Park, MN, model no. 5400). The extraction buffer consisted of phosphate buffer (pH 7.5), 1mM EDTA and 1mM PMSF. The ratio of the dry mass of sample powder per volume of extraction buffer was 1:10. Proteins were extracted as described by the method of Cordova-Tellez (2001) except that the samples were shaken for an hour before centrifuging. The protein concentration was determined immediately following extraction with a Bio-Rad microassay procedure with bovine serum albumin (BSA) as the standard and using scanning spectrophotometer (Bio-Tek Instruments, Inc.). The supernatant and dry mass sample powder of both total proteins and boiling soluble proteins were kept in -20 °C when not in use.

SDS-PAGE

Both total proteins and boiling soluble proteins were separated and evaluated the protein profiles by electrophoresis on discontinuous SDS-PAGE (Laemmli, 1970) using the Mini-PROTEAN III Cell Electrophoresis Unit (Bio-Rad). The 15% polyacrylamide ready-made Tris-Glycine gels with 10 wells (Cambrex Bio Science Rockland, Inc.) were used in this study. Protein loading order in each gel was the same for individual genotype. For example, the first lane was molecular weight standards as molecular weight markers (Pre-
stained, Broad Range, Bio-Rad). The loading amount of the known molecular weight marker was 5µl (ca. 1.25µg). The other lanes were loaded in order of the protein samples extracted from D1, D3, D5, D6 and D6S with the same loading amount, 10 µg per lane. The electrophoresis was conducted in an 8 °C chamber at 200V for an hour. Two gels were run in the same unit for protein profile evaluation and for dehydrin detection. One gel was stained with Coomassie BlueR-250, destained, and photographed. The second gel (an unstained gel) was used for electroblottings.

**Transferring protein and Immuno-blot analysis**

Electroblottings were carried out using a Hoefer Transfer Electrophoresis Unit according to manufacturer’s recommendations (Amersham Pharmacia Biotech, California) for transferring protein from the gel to the membrane (Immobilon-P membrane, Millipore, MA). Transfers were conducted in an 8°C chamber at 35V. for 12 h. and the method of immuno-blot was the same as been described by Cordova-Tellez (2001) except that the primary antibodies were diluted in the ratio 1/3000. The completeness of the protein transfer was verified by staining the gel and the second membrane with Coomassie Blue. The first membrane was used to detect dlps. The primary antibodies were tested for effectiveness and sensitivity in both negative and positive controls to confirm that they reacted with the DHN consensus peptide. The negative control was made by using antigen-blocked serum (antibodies were blocked with the dehydrin consensus peptide) instead of primary antibodies (These antibodies were kindly provided by Dr. Timothy J. Close, University of California, Riverside). Because the assessment of DHN is based on the occurrence of this sequence, these proteins will be referred to as dehydrin-like proteins (dlps) in this thesis. The boiling soluble protein samples extracted from D1 (embryos) were used in both positive and negative
control of three maize genotypes, A619, Co255 and Mo17, because the DHNs are normally abundant during this stage. The membranes were air-dried and photographed as soon as possible.

**Statistical analyses**

Changes in the amount of protein from D1-D6 were analyzed by regression analysis, proc. GLM (SAS, 2000). The data comparing non-treated seedlings (D6) and treated seedling (D6S) was analyzed by contrast statements.
CHAPTER 4. RESULTS

Protein analyses of embryo and seedling root tissues within and among genotypes over time

The amount of total proteins (TPs), boiling soluble proteins (BSPs), and the percentage of BSPs in TPs from cold tolerant genotype Co255, cold intermediate genotype A619, and cold sensitive genotype Mo17, were not significantly different. However, the levels of these proteins changed significantly over time: TPs ($P < 0.001$), BSPs ($P < 0.01$), and in the percentage of BSPs in TPs ($P < 0.05$) (Table 2). The levels of TPs were highest at D1, 102.7 ug mg$^{-1}$ tissue, dropped sharply to 37.1 ug mg$^{-1}$ tissue by D3, decreased another 50% D3 to D5 but remained fairly constant on D5 in D6 and D6S (Table 3 and Fig. 1). Change in the levels of BSPs was similar to TPs until D3. In contrast to TPs, BSPs levels remained relatively constant in the D3, D5, D6, and D6S treatments (Table 3 and Fig. 2). The percentages of BSPs in TPs on D1 and D3 were similar but increased two fold by D5 (Table 3). While the percentages of BSPs in TPs increased from D3 to D5, the levels of TPs and BSPs decreased (Fig.1, 2 and 3). No significant interactions were found between days and genotypes for TPs, BSPs, or percentage of BSPs in TPs. There were no significant differences in TPs, BSPs, and percentage of BSPs in TPs (Table 2) between D6 and D6S.
Table 2. Mean squares of the level of total protein, boiling soluble protein, and the percentage of boiling soluble protein in total protein from maize embryos and seedling root tissues of 3 genotypes over time (D1, D3, D5, D6 and D6S).

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Total protein (TP)</th>
<th>Boiling soluble protein (BSP)</th>
<th>%BSP in TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Blk)</td>
<td>2</td>
<td>709.5*</td>
<td>58.2***</td>
<td>258.7NS</td>
</tr>
<tr>
<td>Day (D)</td>
<td>4</td>
<td>13064.1***</td>
<td>200.8***</td>
<td>665.8*</td>
</tr>
<tr>
<td>Blk x Day</td>
<td>8</td>
<td>309.6NS</td>
<td>14.7**</td>
<td>139.0NS</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>2</td>
<td>29.6NS</td>
<td>1.9NS</td>
<td>34.4NS</td>
</tr>
<tr>
<td>D x G</td>
<td>8</td>
<td>27.5NS</td>
<td>1.5NS</td>
<td>68.7NS</td>
</tr>
<tr>
<td>Blk x G</td>
<td>4</td>
<td>144.6NS</td>
<td>2.1NS</td>
<td>45.8NS</td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D6 x D6S</td>
<td>1</td>
<td>51.7NS</td>
<td>0.0NS</td>
<td>230.4NS</td>
</tr>
</tbody>
</table>

*, **, *** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

NS, not significant at the 0.05 level.

Table 3. Comparison of the average amount of total proteins (µg mg⁻¹ tissue), boiling soluble proteins (µg mg⁻¹ tissue), and the percentage (%) of boiling soluble protein in total protein from maize embryos and seedling root tissues of 3 genotypes over time (D1, D3, D5, D6 and D6S).

<table>
<thead>
<tr>
<th>Seed and seedling age</th>
<th>Total Protein(TP) µg mg⁻¹ tissue</th>
<th>Boiling Soluble Protein(BSP) µg mg⁻¹ tissue</th>
<th>% of BSP in TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day1(25°C)</td>
<td>102.7 a</td>
<td>14.5 a</td>
<td>13.8 b</td>
</tr>
<tr>
<td>Day3(25°C)</td>
<td>37.1 b</td>
<td>4.5 b</td>
<td>12.2 b</td>
</tr>
<tr>
<td>Day5(25°C)</td>
<td>15.5 c</td>
<td>3.9 b</td>
<td>28.4 a</td>
</tr>
<tr>
<td>Day6(25°C, control)</td>
<td>12.7 c</td>
<td>3.7 b</td>
<td>31.4 a</td>
</tr>
<tr>
<td>Day6stress((25°C, 5days and7°C,1day)</td>
<td>16.1c</td>
<td>3.7 b</td>
<td>24.2 a</td>
</tr>
<tr>
<td>LSD (α=0.05)</td>
<td>12.4</td>
<td>2.5</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Means within a column not followed by the same letter are not significantly different.

(P<0.05) according to Fisher’s Protected LSD.

LSD, Least significant difference.
Figure 1. The trend of total protein (µg mg^{-1} tissue) from day1 (D1) through day6 (D6) of the three average genotypes of maize embryos and maize seedling roots at 25°C. The change of those levels total proteins is shown by linear regression line from sigma plot program. The vertical bars represent the standard error of mean for each treatment with three replications and differences at $P<0.05$. 

\[ Y = -17.7833X + 108.7006 \]

$R^2 = 0.885$
Figure 2. The trend of boiling soluble protein (µg mg\textsuperscript{-1} tissue) from day 1 (D1) through day 6 (D6) of the three average genotypes of maize embryos and maize seedling roots at 25°C. The change of those levels boiling soluble proteins is shown by linear regression line from sigma plot program. The vertical bars represent the standard error of mean for each treatment with three replications and differences at $P<0.05$. 

$Y = -2.0927X + 17.394$

$R^2 = 0.766$
Figure 3. The trend of percentage of boiling soluble protein in total protein from day 1 (D1) through day 6 (D6) of the three average genotypes of maize embryos and maize seedling roots at 25°C. The change of those levels of percentage is shown by linear regression line from sigma plot program. The vertical bars represent the standard error of mean for each treatment with three replications and differences at $P<0.05$. 

$Y = 3.9913X + 6.504$

$R^2 = 0.811$
**Total protein and boiling soluble protein electrophoresis profiles of genotypes over time**

The protein profiles of maize genotypes with differences in cold tolerance were compared. On D1, the protein profiles of the 3 genotypes were similar in both TPs and BSPs in terms of the number and molecular mass of the bands. In the BSPs, however, there were bands in different positions between the 29.2 kDa and 21.1kDa (pointed arrow in plate 1B) migrated closely or slower than the others in these genotypes. About 15 protein bands in BSPs were found whereas there were about twice as many bands in the TPs fraction (Plate 1).

The protein profiles of these 3 maize genotypes were similar at D3, D5, D6 and D6S in both TPs and BSPs (Plate 2). The intensity of protein bands was consistent in D1 and D3 for TPs and in D1 for BSPs. The protein bands became diffuse after that. Comparing the protein profiles of interest in BSPs from non-stress treatments (D5 and D6, control) and cold-stressed treatments (DS6) of the 3 maize genotypes in individual gel (plate 2) or in the same gel (Plate 5A) resulted in no distinguishable differences in bands.
Plate 1. SDS-PAGE of extracted proteins from embryos (D1). Comparing the profiles of total protein from extraction buffer (A) and boiling soluble protein (B) from 3 maize genotypes, cold tolerant genotype Co255, cold intermediate genotype A619 and cold sensitive genotype Mo17. The first lane of each gel was pre-stained standards (STD) (Bio-Rad) with known molecular weight (MW) as markers (numbers in the left hand side). The next 3 lanes show the protein profiles of A619, Co255, and Mo17, respectively. Equal weight of protein samples, 10 µg from each genotype was loaded per lane in both total protein and boiling soluble protein. The arrow shows the location, which some bands of A619, Co255 and Mo17 migrated faster or slower than the others.
A. Total protein

B. Boiling soluble protein

Plate 2. SDS-PAGE of total protein from extraction buffer (A) and boiling soluble protein (B) from 3 maize genotypes, cold tolerant genotype Co255, cold intermediate genotype A619 and cold sensitive genotype Mo17. Individual gel was presented only one genotype. The protein samples were from each seedling age, day1 (D1), day3 (D3), day5 (D5), day6 (D6) and day6 stress (D6S). The first lane of each gel is pre-stained standards (STD) (Bio-Rad) with known molecular weight (MW) as markers (numbers in the left hand side). The next 5 lanes show the protein profiles of day1 (D1), day3 (D3), day5 (D5), day6 (D6), day6 stress (D6S) of each genotype. Equal weight of protein sample, 10 µg was loaded per lane.
Dehydrin-like protein (dlp) profiles in 3 maize genotypes

Immuno-blot analyses were conducted with the BSPs fraction. The dehydrin antibodies used in these experiments were tested for their effectiveness and the sensitivity to detect dlp. The plant tissue used was embryo, which is believed to have abundant dlp proteins. The positive control showed dlp bands in all 3 maize genotypes, A619, Co255 and Mo17 (Plate 3A) whereas no dlp bands were detected in the negative control (Plate 3B). The change in dlp profiles were investigated in each membrane for each genotype over time (Plate 4) or compared among the 3 maize genotypes in the same membrane in D1 (Plate 3A) and D5, D6 and D6S (Plate 5B). As we expected, the dlp profiles in embryos (D1) were the two pre-dominant bands, 22kDa and 40kDa found in genotype Mo17 and only 22kDa found in genotypes A619 and Co255. The fainter bands below the two dominant bands could be “either intact proteins or degradation protein products (Close et al., 1993)”. The 22kDa dlp was detected in every sampling (D1, D3, D5, D6, and D6S) and in all genotypes (Plate 4 and 5). It was abundant (the thickest bands) in embryos (D1) and started to decrease after D1. No evidence of its re-induction was found in 5-day-old seedlings exposed to low temperature stress of 7°C for 1 day (D6s) when compared to 5-day-old seedlings (D5) and 6-day-old seedlings (D6) at control temperature (25°C) in these 3 maize genotypes (Plate 5B). The 40kDa dlp in genotype Mo17 could not be detected after D1 (imbibed-embryo) (Plate 4 and 5).
Plate 3. Immuno-blots of dehydrin-like proteins (dlps) from embryos (D1) of 3 maize genotypes, cold tolerant genotype Co255, cold intermediate genotype A619 and cold sensitive genotype Mo17. Positive control (A) and negative control (B) were tested as described in materials and methods. The protein fractions from boiling soluble proteins were separated by SDS-PAGE. Equal weight of protein samples, 10 µg from each genotype was loaded per lane in both gels. The first lane was pre-stained standards (STD) (Bio-Rad) with known molecular weight (MW) as markers (numbers in the left hand side). Proteins were electroblotting to the membrane as described in materials and methods.
Plate 4. Comparing the protein profiles on SDS-PAGE (A) and immuno-blot (B) over time of 3 maize genotypes, cold tolerant genotype Co255, cold intermediate genotype A619 and cold sensitive genotype Mo17. Each gel and membrane was representative of each genotype. The first lane of each gel or membrane was pre-stained standards (STD) (Bio-Rad) with known molecular weight (MW) as markers (numbers in the left hand side). The protein samples were boiling soluble proteins from every seedling ages, day1 (D1), day3 (D3), day5 (D5), day6 (D6) and day6 stress (D6S). Equal weight of protein samples, 10 µg was loaded per lane.
Plate 5. Comparing the protein profiles on SDS-PAGE (A) and immunoblot (B) of 3 maize genotypes, cold tolerant genotype Co255, cold intermediate genotype A619 and cold sensitive genotype Mo17 in the same gel and membrane only 3 seedling ages, day5 (D5), day6 (D6) (non-treated cold treatment, control) and day6stress (D6S) (cold-treated treatment). The first lane of each gel or membrane was pre-stained standards (STD) (Bio-Rad) with known molecular weight (MW) as markers (numbers in the left hand side). The protein samples were boiling soluble and equal weight of protein sample 10 µg was loaded per lane.
CHAPTER 5. DISCUSSION AND CONCLUSION

Improving maize early season performance and reducing the risk of poor or failed stands are of widespread interest. The role of dehydrin in chilling tolerance in many plant species has been a major focus of research for many years. So far, the best evidence for the relationship between DHN and chilling tolerance related to the presence of a 35 kDa in cowpea (Ismail et al 1997 and 1999a,b). Borovskii et al. (2000) investigated cold-induced proteins in the mitochondria of maize, 3-day-old seedlings with exposure to 10°C for 1 day, which showed that dlp levels increased slightly. In a following study, they exposed 2-day-old seedlings to 10°C for 7 day and found dlp levels still increased slightly (Borovskii et al. 2002). The techniques used in this study produced information about dlps in both embryo and root of maize seedling genotypes with putative differences in cold tolerance from D1 through D6 and when 5-day-old seedlings (D5) were exposed to 7°C for 1 day.

Characterization of levels of dehydrin-like proteins during seed germination and seedling growth

The immuno-blot analysis in this study indicated the presence of two major DHNs, 22kDa and 40kDa, which are commonly found in maize embryo (Asghar et al., 1994; Close, 1996; Campbell et al., 1998; Cordova, 2001). The large molecular weight (40kDa) protein was detected only in the embryos (D1) of Mo17. The presence of both the 22kDa and 40kDa protein in Mo17 was also reported by Asghar et al. (1994) and Campbell et al. (1998). In contrast, in Co255 and A619, only the 22kDa protein was detected but could not detected in the genotypes Co255 or A619. Asghar et al. (1994) and Campbell et al. (1998) used only embryo tissue in their studies. In this study was also found the 22kDa protein in the root of maize seedlings up to D6 and D6S of the genotypes A619, Co255 and Mo17. The genotype
Mo17 showed the presence of 22kDa in D1 though D6 and D6S similar to the genotypes Co255 and A619. The 40kDa dlp in genotype Mo17 could not be detected in the other genotypes and disappeared after D1 (imbibed-embryo). Cordova (2001) reported that 40kDa was detected at low levels after the shoot and root become visible (~2-3-day-old seedling) and it disappeared earlier than the 22kDa dlp. Other researchers, Borovskii et al. (2000 and 2002) found large molecular weight dlp proteins, 52kDa and 63kDa in 3-4-day-old seedlings. However, our experiments did not detect these larger proteins. In this case, it might be because of the different protein extraction protocol and plant tissues used (mitochondria of seedling in Borovskii et al. and embryo and root tissue in this study). Lowton and Wilson (2003) showed that using different extraction buffer resulting in different protein fractions. Close et al. (1993) found bands between 18 and 24 kDa and larger bands at ca 40kDa and 60kDa from maize shoot during dehydration stress. Asghar et al. (1994) reported that DHNs accumulate differently in plant tissues and Nylander et al. (2001) reported that DHNs in Arabidosis thalina showed tissue-specific localization during low temperature stress.

The general characteristics of DHNs including boiling solubility (Campbell and Close, 1997), abundance in seeds during the later stages of embryogenesis (Dure, 1993) and decreased abundance during germination and early seedling growth (Dure, 1997) are supported by the results of the present study. The level of the abundant 22kDa dlp protein found in D1, started to decrease from D1 to D3 and from D3 to D5 and D6. In addition, the percentage of BSPs in TPs increased after 3 days of germination (Table 3 and Fig. 3). This increase in the percentage of BSPs in TPs might be the result of a slower rate of decrease in the BSPs relative to the TPs fraction.
The 22kDa dlp in maize genotypes in this study were present throughout the study from D1 until D6 and was not affected by stress (D6S) or genotypes. This result is not consistent with those from some previous studies. A number of researchers (Close, 1989; Vilardell et al., 1990, Plana, 1991; Mao et al., 1995; Cordova, 2001) have found that dehydrin mRNAs or dehydrin proteins in maize roots were not detected after 2 days of germination. Slower rates of disappearance of DHNs have been found in some dicotyledonous crops. Castor bean (*Ricinus communis* L.) DHNs can be detected until 4 days after imbibition (Han et al., 1997). Dehydrin in mature pea cotyledons (*Pisum sativum*) did not become undetectable until seedlings were 19-days-old (Robertson and Chandler, 1992). In this study, dlps were still present at the end of seedling age (D6 or D6S). Similar results were found in the studies of Borovskii et al. 2000 and 2002. They could detect dlps in maize seedlings after 4 days of germination. Our data might be the first findings that dlps were detectable in maize seedlings after 5 and 6 days of germination in control temperature.

**The re-induction of dlps after the exposure of seedlings to low temperature stress**

Previous studies have shown that DHNs or dlps can be induced in vegetative plant tissues by abiotic stresses like cold, drought, salinity and ABA (Close et al. 1989; Close, 1996; Bray, 1997; Sarhan et al., 1997; Whitsitt et al., 1997; Cellier et al., 1998; Danyluk et al., 1998; Thomashow, 1998; Bravo et al., 1999; Ismail et al., 1999b; Borovskii et al., 2000; Tabaei-Aghdaei et al., 2000; Zhu et al., 2000; Nylander et al., 2001; Borovskii et al., 2002). None of these studies have shown clear evidence that DHNs or dlps are re-induced by low temperature stress after their disappearance, similar to this study. Moreover, dlp appeared at D6 and at the stage when the seedling was exposed to low temperature stress (D6S) too. In comparing the amount of proteins, protein profiles and dlp profiles from control, non-cold-
treated treatment (D5 and D6) and cold-treated treatment (D6S) in this study, there were no differences in the amount of both TPs and BSPs between treatments. Taken together, no evidence of re-induction of dlp was found after the exposure of maize seedlings to 7°C for 1 day. There are at least two reasons to explain this result. Firstly, the dhnl gene-encoding the small molecular weight dlp of 22kDa in root might not respond to low temperature stress like the gene-encoding the larger molecular weight dlp of 63 and 52 kDa detected by Borovskii et al. (2002). This was supported by other reports from previous studies. Close et al. (1993) studied on dehydrin accumulation in different tissues of barley seedling and reported that some dehydrins are present in some tissues whether the tissues are stressed or non-stressed. The gene that encodes the 80kDa dlp, dhn5, in barley responded only to low temperature and this protein accumulated mainly in vascular tissue and epidermis (Bravo et al., 1999). A similar result was reported for the induction of the LT130 DHN protein, encoded by the gene LT130, in Arabidopsis. Its quantity increased at low temperature and accumulated in specific tissue like vascular tissue and anthers (Nylander et al., 2001). Kontunen-Soppela, 2001 reported that the DHNs from the needles of Scots pine seedlings showed no response to low temperature. Secondly, maize may not be a low temperature responsive species as reported in Danyluk et al. (1994). They found that Wcor410, an acidic dehydrin could be detected in gramineae species during abiotic stress but that only maize and rice showed no response to different low temperature regimes. However, there is no comparison of the relative intensity of dlp bands between non-treated cold treatment (D6) and cold-treated treatment (D6S) to support the result. Therefore, the comparison of relative intensities of protein bands might be of interest for further study.
Comparing the dlps response among inbred lines with putative differences in tolerance to low temperature stress

There were no significant differences in the amount of total proteins (TPs) and boiling soluble proteins (BSPs) among maize genotypes with putative differences in tolerance to low temperature stress (P<0.05). These three genotypes had very similar protein profile. Borovskii et al. (2000) also reported the similarities of TPs and BSPs profiles among cereal crops, wheat, rye and maize. Then, protein profiles might not be a good indicator to distinguish cold tolerant maize genotypes. The 22kDa dlp remained detectable throughout the course of this study in all genotypes while the 40kDa dlp of Mo17 disappeared after day 1 (imbiled embryos). The dlp profiles were not affected by stress or genotype. Kontunen-Soppela et al., 2000 also found no direct relationship of DHNs with freezing resistance or osmotic stress on the needles of Scots pine seedling (Pinus sylvestris). Thus, there is no evidence from the present study to support that dlps respond differently among inbred lines with putative differences in tolerance to low temperature stress. Additionally, cold tolerance in maize is a very complicated process involving not only dehydrin but also other mechanisms from physiological, biological, molecular and genetic factors and they may be synergistic as has been mentioned in the literature review.
Conclusion and further study

The 22kDa dlp of maize genotypes was present throughout the study in all genotypes whereas 40kDa dlp of Mo17 disappeared after day1. The level of boiling soluble proteins was abundant in day1 and reduced after day1. No re-induction of dlps due to low temperature stress was found. The absence of re-induction was consistent across genotypes.

The new findings are the presence of 22kDa dlp in 5-day-old seedlings and 6-day-old seedlings in maize genotypes and the protein profiles in 2 maize genotypes, A619 and Co255.

The present study does not provide conclusive proof regarding dlp re-induction due to low temperature, especially since the data is limited to root tissues. As previously mentioned, DHN or dlp expression in different tissues can vary. Even though cold tolerance is a complex trait, further investigations regarding the role of DHN in cold tolerance are warranted.
REFERENCES


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