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Group 3 Late Embryogenesis Abundant Proteins in Desiccation-Tolerant Seedlings of Wheat (*Triticum aestivum* L.)[1]

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Dormant seeds and young seedlings of wheat (*Triticum aestivum* L.) tolerate desiccation. A transcript expressed in this desiccation-tolerant tissue has been cloned and sequenced (J. Curry, C.F. Morris, M.K. Walker-Simmons [1991] Plant Mol Biol 16: 1073-1076). This wheat cDNA clone encodes a protein that is homologous to other group 3 late embryogenesis abundant (LEA) proteins. In this report, we describe the production of polyclonal antibodies to the protein product of the cDNA clone and assess group 3 LEA protein levels in desiccation-tolerant tissue. The group 3 LEA antibodies detected four major proteins in wheat with molecular masses from 27 to 30.5 kD. The genes for these proteins mapped to wheat chromosomes 1A, 1B, and 1D. The group 3 LEA proteins were present in mature seed embryos and were maintained when growth-arrested, dormant seeds were hydrated for 111 h. However, in germinating seeds the group 3 LEA proteins declined and were no longer detectable by 111 h. We severely dehydrated seedlings (more than 90% water loss) to assess group 3 LEA transcript and protein accumulation in tissues of these desiccation-tolerant plants. In response to dehydration, abscisic acid (ABA) levels increased dramatically and group 3 LEA mRNAs were induced in root, shoot, and scutellar tissue. However, group 3 LEA proteins were detected only in shoot and scutellar tissue and not in root tissue. Treatment of nonstressed seedlings with 20 μM ABA resulted in low levels of group 3 LEA proteins in the roots, whereas higher levels were found in the shoot and scutellar tissue. When dehydrated seedlings were rehydrated, shoot and scutellar tissue resumed growth. The roots did not resume growth and subsequently died. New roots developed later from the scutellar tissue. Thus, in severely dehydrated wheat seedlings, the accumulation of high levels of group 3 LEA proteins is correlated with tissue dehydration tolerance.

Dormant seeds and young seedlings of wheat (*Triticum aestivum* L.) are desiccation tolerant. Dormant seeds can be dried and rehydrated with little loss of viability. When moisture conditions are sporadic, this ensures that seeds will survive to germinate later under more favorable environmental conditions. Seedlings also are desiccation tolerant because they can survive losses of over 95% moisture (Blum et al., 1980). Although some root death may occur after desiccation, seedlings can resume growth after rehydration. This desiccation tolerance ends when germinating seedlings reach the first leaf stage. At this stage, plants can be killed by drought stress because they can no longer survive large moisture losses. The biochemical causes for this drastic decrease in desiccation tolerance with seedling development are not understood.

The high desiccation tolerance of dormant wheat seeds and young seedlings provides a useful system to examine desiccation tolerance mechanisms. As part of our research efforts to elucidate the mechanism of dehydration stress responses, we have cloned mRNAs that are expressed in hydrated, dormant seeds (Curry et al., 1991; Morris et al., 1991). All of these cDNA clones represent transcripts that are induced by dehydration or ABA. When ABA is applied to dormant seed embryos, germination is blocked and all the transcript levels are enhanced. When seedlings are dehydrated, endogenous ABA increases, followed by induction of the transcripts.

The cDNA clones represent transcripts that are found in desiccation-tolerant wheat tissue and comprise six gene families (Curry et al., 1991; Morris et al., 1991; Anderberg and Walker-Simmons, 1992; Curry and Walker-Simmons, 1993). One clone has sequences homologous to protein kinases (Anderberg and Walker-Simmons, 1992), whereas the other sequenced clones are homologous to LEA proteins. LEA proteins accumulate in late embryogenesis and often in dehydrated seedlings. These proteins are hydrophilic and have been proposed to be desiccation protectants (Chandler et al., 1988; Close et al., 1989; Dure et al., 1989; Lane, 1991). ABA-inducible proteins, including the LEA proteins, remain soluble even after boiling, suggesting that the proteins have a high affinity for water (Close et al., 1989; Ried and Walker-Simmons, 1990; Thomann et al., 1992). Such proteins may help maintain a minimal water content during dehydration and in the process stabilize cytoplasmic structures (Lane, 1991).

One of the wheat cDNA clones that we have identified has sequence homology to the group 3 LEA proteins (Dure et al., 1989; Curry et al., 1991). The deduced amino acid sequence of the wheat group 3 LEA cDNA contains a repeating 11-amino acid tract. This tract is repeated 10 times and is characteristic of group 3 LEA proteins (Curry et al.,

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Abbreviation: LEA, late embryogenesis abundant.

1991). The deduced amino acid sequence of the wheat group 3 LEA indicates that the protein is a highly polar, basic protein.

The group 3 LEA genes have been reported in several plant species. The barley group 3 LEA protein HVA1 (Hong et al., 1988) is over 95% homologous at the amino acid level to the wheat group 3 LEA protein (Curry et al., 1991). HVA1 contains nine repeats of a homologous amino acid tract. Group 3 LEA proteins with similar repeating amino acid tracts have also been reported for cotton (Baker et al., 1988), carrot (Choi et al., 1987; Dure et al., 1989), and rapeseed (Harada et al., 1989). The presence of group 3 LEA mRNAs in these plant species indicates that a common mechanism for desiccation protection may be present.

If the group 3 LEA proteins function as desiccation protectants, then it is important to demonstrate group 3 LEA protein accumulation in plant tissue that is resilient to dehydration stress. It is well established that dehydration causes an increase in LEA mRNAs, including the group 3 transcripts (Chandler et al., 1988; Mundy and Chua, 1988; Close et al., 1989; Curry et al., 1991; Hetherington and Quatrano, 1991; Hong et al., 1992; King et al., 1992). However, the presence of LEA transcripts does not always result in the accumulation of the protein product. For example, in ABA-treated wheat seedlings, the Em transcript was induced, but the Em protein could not be detected with antibodies (Hetherington and Quatrano, 1991). The accumulation of group 3 LEA protein has been measured in dehydrated barley seedlings, although no comparison with desiccation tolerance was included (Hong et al., 1992).

For this study of dehydrated wheat, we were particularly interested to determine if group 3 LEA protein accumulates in the scutellar tissue, i.e. scutellum and axis. Survival of this tissue is essential for desiccation tolerance because it contains the meristematic nodes of leaves and roots. For example, when seedlings are dried, roots can be stressed so severely that they do not survive, but upon rehydration the plants resume growth with new roots developing near the scutellar node.

Here, we report the production of antibodies to the group 3 LEA cDNA protein product. We have used these antibodies to map the chromosomal location of the corresponding genes in wheat, to compare group 3 LEA protein levels in hydrated dormant seeds with those in germinating seeds, and to determine if group 3 LEA proteins accumulate in desiccated seedlings that are capable of surviving drying.

MATERIALS AND METHODS

Plant Materials

Seed stocks of winter wheat (*Triticum aestivum* L. cv Brevor) were obtained from field plots harvested at Spillman Agronomy Farm near Pullman, WA, and then after-ripened at room temperature to eliminate dormancy. Dormant seed stocks were obtained from plants grown in the greenhouse (22°C day/12°C night, with a supplemental photoperiod of 16 h). Seeds were harvested at physiological maturity and stored at −20°C.

To compare protein and mRNA levels between dormant and germinating seeds, whole seeds from each seedlot were placed on water-saturated blotting paper in 100-mm Petri dishes and incubated at 20°C and 100% humidity with a daily light period of 16 h. At various times, the embryo (scutellum plus axis) or scutellar region (scutellum plus axis in a germinating seed) was excised from the endosperm, frozen in liquid N_2, and stored at −70°C prior to protein extraction. Embryonic axes were isolated from dry seeds (Morris et al., 1991). To compare protein, mRNA, and ABA levels in seedlings, nondormant seeds were allowed to imbibe water for 4 to 5 d in a high-humidity chamber. At this developmental stage, 2 to 5 mm of the first leaf extended beyond the tip of the coleoptile. Roots and shoots were detached from the plant, and the scutellar region was excised from the endosperm, frozen in liquid N_2, and stored at −70°C prior to analysis.

Dehydration or ABA Treatment of Seedlings

For dehydration studies, seedlings were placed in 100-mm Petri dishes (15 seedlings/plate) containing blotting paper and 10 mL of water. This amount of water was sufficient to saturate the blotting paper and provide several milliliters excess. The uncovered Petri dishes were incubated under ambient conditions (20–22°C, 25–30% humidity) for up to 48 h. The water evaporated after 24 h, causing the roots to be exposed directly to air. Relative water content of the root, shoot, and scutellar tissues was determined by comparing the amount of water in dehydrated tissue with that in unstressed tissue. For ABA treatment, seedlings were transferred to Petri dishes containing blotting paper and 10 mL of either a water control containing 0.4% (v/v) ethanol or 20 μM (±)ABA (Sigma Chemical Co.[2]) in 0.4% ethanol. After incubating the Petri dishes for 48 h in a high-humidity environment, the root, shoot, and scutellar tissues were detached from the seedlings, washed three times with 1% (w/v) Suc, blotted to remove excess moisture, and stored at −70°C.

ABA Analysis

Frozen tissue samples were lyophilized prior to ABA extraction. The samples were subsequently pulverized in microcentrifuge tubes using a stainless steel pestle and transferred to 15-mL polypropylene tubes using 2 to 3 mL of extracting methanol (Walker-Simmons, 1987). Additional extracting methanol was added to a final volume of 5 mL, and the mixtures were stirred with magnetic stirbars for 48 h at 4°C in the dark. ABA in the extracting methanol was quantified by immunoassay using a monoclonal antibody for ABA (Walker-Simmons, 1987).

Antibody Production

Polyclonal antibodies to a wheat group 3 LEA polypeptide were produced using the gene fusion vector pRIT2T (Pharmacia LKB Biotechnology). The wheat group 3 LEA cDNA

[2] Mention of a specific product name by the U.S. Department of Agriculture does not constitute an endorsement or imply a recommendation over other suitable products.

was subcloned into this vector from pMA2005 (Curry et al., 1991). The resulting 55-kD protein was isolated according to the manufacturer's instructions and consisted of approximately equal molecular masses of truncated protein A linked to the wheat polypeptide. For the initial injection, a 250-μL PBS solution containing 100 μg of the fusion protein was injected into the lumen of a wiffle-type golf ball previously implanted in a New Zealand white rabbit (Ried et al., 1992). At 1-month intervals, 100 μg of the fusion protein was injected into the golf ball in either a PBS solution or bound to nitrocellulose in a PBS suspension. After four boosts, the fluid was removed from the ball and used directly in immunoblot analyses.

Protein Extraction and Analysis

Lyophilized tissue was pulverized in a microcentrifuge tube with a stainless steel pestle. Extraction buffer, pH 6.8 (15 mM Tris-HCl, 20 μM leupeptin, 1% [v/v] β-mercaptoethanol), was added (10 μL/mg dry weight) to the tube containing the tissue and vortexed for 1 min. Additional extraction buffer was added (5 μL/mg dry weight) and the tube was vortexed again, then centrifuged for 15 min at 4°C, 13,000 rpm in a microcentrifuge. The supernatant was removed, incubated at 70°C for 10 min, and then centrifuged again for 15 min at 4°C. This second supernatant, identified as the heat-soluble fraction, was stored at −70°C.

For immunodetection by western blotting, equal volumes of heat-soluble proteins (corresponding to equal amounts of lyophilized tissue) were electrophoresed through a 12.5% (w/v) polyacrylamide minigel, transferred to a PVDF membrane (Millipore Corp. or Bio-Rad Laboratories), and probed with the antibody as previously described (Ried et al., 1992) except that the membrane was developed in a solution of 100 mM Tris-HCl (pH 8.8), 100 mM NaCl, 5 mM $MgCl_2$, 0.15 mg/mL 5-bromo-4-chloro-3-indolyl phosphate, and 0.3 mg/mL nitro blue tetrazolium.

RNA Extraction and Analysis

Total RNA was extracted from tissue stored at −70°C using an acid guanidine thiocyanate procedure (Chomczynski and Sacchi, 1987). Five micrograms of RNA were denatured and electrophoresed through 1.2% (w/v) agarose gels containing formaldehyde (2.7%, v/v). RNA was transferred to nylon membranes (Zeta Probe, Bio-Rad) using alkaline conditions and hybridized with ^{32}P-labeled DNA probes synthesized by the random hexanucleotide-priming method (Morris et al., 1991). Filters were prehybridized for 15 min and hybridized for 16 h at 65°C in 1% (w/v) BSA, 1 mM EDTA, 0.5 M NaH_2PO_4, 7% (w/v) SDS, 0.1% (w/v) $Na_4P_2O_7$. Following hybridization, the filters were washed essentially as described in the Bio-Rad protocol and quantified using a radioanalytic imaging system (Ambis, San Diego, CA).

Rehydration of Dehydrated Seedlings

Seedlings dehydrated for 48 h were rehydrated by adding water to the blotting paper. Seedlings were incubated for 7 d at 20°C under high-humidity conditions, then transplanted to soil and placed in a greenhouse (22°C day/12°C night, supplemental photoperiod of 16 h).

RESULTS

Antibodies to a Group 3 LEA Fusion Protein Recognize Four Wheat Proteins

A group 3 LEA protein, encoded by a cDNA clone, was used to produce antibodies. In embryos of wheat grains that had been allowed to imbibe, these antibodies detected four proteins ranging from 27 to 30.5 kD (see Fig. 1), even though the antibodies were produced to one purified gene fusion product. The corresponding cDNA clone hybridized to a single 1.0-kb mRNA band (Curry et al., 1991).

Because bread wheat is a hexaploid, most genes are expected to be present in six copies (two per genome). The loci in each genome can code for unique protein products as exemplified by wheat storage proteins (Payne et al., 1980). We examined the genetic basis for the four group 3 LEA proteins using wheat aneuploid lines (cv Chinese Spring). These lines are each deficient for a chromosome pair and can be used to map genes to specific chromosomes. All the proteins mapped to the group 1 chromosomes. The most intensely staining protein band (30 kD) and a minor band (30.5 kD) mapped to chromosome 1A (Fig. 1). The other major cross-reacting polypeptides (27 and 29 kD) map to chromosomes 1B and 1D, respectively. The data from Southern analysis using the cDNA further support this interpretation (S.S. Jones and M.K. Walker-Simmons, unpublished data).

The structural similarity of the multiple bands was established using affinity-purified antibodies. The polyclonal antibodies that bound to the 27-kD band were eluted and used in subsequent western analyses. These 27-kD-binding antibodies cross-reacted with the 29-, 30-, and the 30.5-kD proteins, as well as the 27-kD protein (data not shown). These results indicate that the four proteins contain similar amino acid sequences.

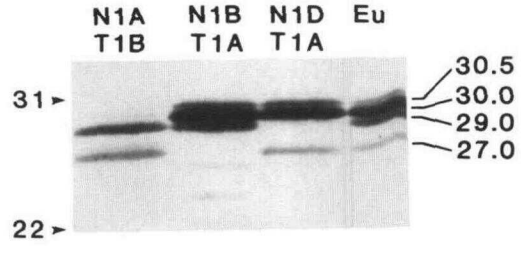

Figure 1. Chromosomal mapping of group 3 LEA proteins. Heat-soluble proteins were extracted from the embryo of aneuploid seeds derived from the wheat cultivar Chinese Spring and analyzed by western blotting using antibody produced against the wheat group 3 LEA protein. Genotype designations: N1A/T1B, nullisomic 1A, tetrasomic 1B; N1B/T1A, nullisomic 1B, tetrasomic 1A; N1D/T1A, nullisomic 1D, tetrasomic 1A; Eu, euploid. The sizes of the polypeptide bands (kD) are listed on the right, and the positions of the molecular mass markers (in kD) are listed on the left.

Levels of Group 3 LEA Proteins in Germinating and Dormant Seed Embryos

Group 3 LEA transcript levels decrease significantly by 24 h postimbibition in germinating wheat embryos (Morris et al., 1991). To determine the fate of the corresponding protein, we visually compared levels using western analyses. Protein levels were assessed in embryos dissected from nondormant and dormant seeds at varying times after imbibition (Fig. 2). By 16 h postimbibition, group 3 LEA proteins were detected in approximately equal amounts in both the germinating and dormant seeds. After 16 h, levels declined in the germinating seeds, and after 111 h, none of the proteins was detectable in the germinating seeds. In contrast, the levels of group 3 LEA proteins in dormant seeds remained constant through 111 h, the length of the experiment.

ABA, Group 3 LEA mRNA, and Protein Levels Increase in Response to Seedling Dehydration

The effects of severe dehydration of wheat seedlings on ABA concentration and group 3 LEA gene expression were determined. Three tissues (root, shoot, and scutellar) were analyzed. For the drying experiments, the seedlings were removed from a high-humidity environment and placed on water-saturated filter paper under low RH. Use of the wet filter paper ensured that the roots dried gradually. The relative water content (based on the amount of water per dry weight in unstressed tissues) decreased slowly during the first 12 h in shoot and scutellar tissues, but more rapidly in root tissues (Fig. 3A). By 24 h, the relative water content in roots had decreased to 4.3%, much lower than either shoot (45.4%) or scutellar (30.5%) tissues. By 48 h, the water content in the roots had dropped to 0.2%, whereas shoot and scutellar tissues were 8.9 and 2.7%, respectively.

ABA levels increased dramatically in response to the severe dehydration, with over a 10-fold increase occurring in all tissues between 12 and 24 h (Fig. 3B). By 48 h, the ABA level decreased in shoot and scutellar tissue, which probably indicates that ABA degradation had occurred. The data presented in Figure 3 are from a single experiment but are representative of multiple experiments.

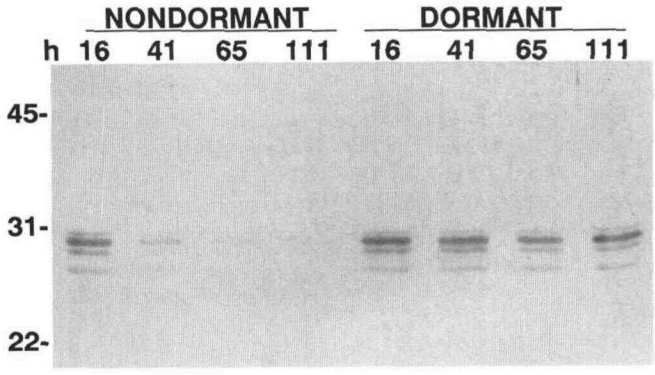

Figure 2. Expression of group 3 LEA proteins in nondormant and dormant seeds that had been allowed to imbibe. Heat-soluble proteins were extracted from embryos and scutellar tissues isolated from whole seeds allowed to imbibe for various times. Proteins were subjected to western blot analysis with antibody produced against the group 3 LEA fusion protein. Protein from the equivalent dry weight of embryos or scutellar tissue was loaded in each lane. The time of imbibition in hours is listed above each lane, and the positions of molecular mass markers are listed on the left in kD.

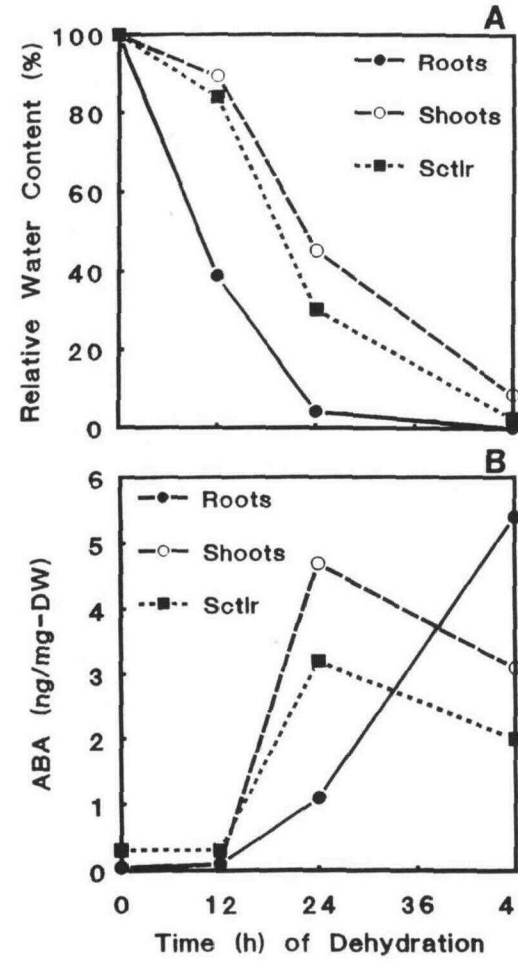

Figure 3. Water content and ABA levels in root, shoot, and scutellar (Sctlr) tissues of seedlings subjected to severe dehydration. Relative water content was determined by comparing the amount of water at various dehydration times with control tissue at time 0. The amount of ABA in each tissue was quantified by indirect ELISA using a monoclonal antibody specific to (+)ABA (Walker-Simmons, 1987).

Group 3 LEA mRNA was induced by 24 h of dehydration and reached high levels by 48 h in all tissues (Fig. 4B). Group 3 LEA proteins, however, accumulated only in shoots and scutellar regions, and were never detected in roots (Fig. 4A). To enhance the detection of group 3 LEA proteins in the roots, an 8-fold greater amount of sample was used for these experiments than those depicted in Figure 2. Even with this higher amount of sample, group 3 LEA proteins were not detected in dehydrated roots. In shoots, trace amounts of group 3 LEA proteins were present at 0 h, but by 48 h the levels had increased dramatically. In the scutellar tissue, a substantial level of group 3 LEA proteins was present at 0 h, and that amount increased with dehydration. Using the

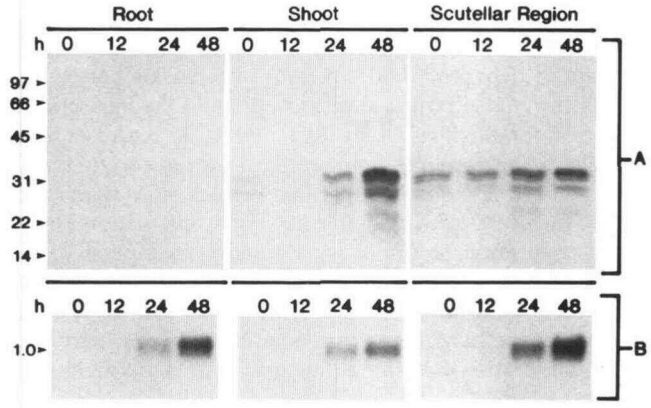

Figure 4. Expression of group 3 LEA proteins (A) and mRNAs (B) in dehydrated seedlings. A, Heat-soluble proteins were extracted from root, shoot, and scutellar tissues from dehydrating seedlings at various times and subjected to western analysis using antibody produced against the group 3 LEA fusion protein. The positions of the molecular mass markers are listed in kD. B, Total RNA was isolated from the tissues dehydrated for the same times as in A, fractionated by electrophoresis, transferred to nylon membranes, and hybridized with ^{32}P-labeled probes synthesized from a randomly primed cDNA insert of a group 3 LEA mRNA. The size of the transcript is shown at the left in kb.

higher amount of sample, several cross-reacting bands of about 22 kD were detected in the 24- and 48-h shoot and scutellar samples.

Dried Seedlings Resume Growth upon Rehydration

The seedlings that had been dehydrated for 48 h were resupplied with water and incubated at 20°C in a humid environment to promote growth. After 3 d, new roots began to grow from the scutellar region. The original roots, which were present during dehydration, turned brown and did not resume growth. In all plants the basal part of the first leaf became rehydrated and continued to expand, but in some plants the upper portion of the leaf remained dry and did not grow. After 7 d of rehydration, the seedlings were planted in soil and placed in the greenhouse. By 1 month, all plants had produced a true leaf, and the roots continued to proliferate (Table I).

Table I. *Growth of roots and plants from rehydrated seedlings*

Thirty seedlings (4 or 5 d old) were severely dehydrated for 48 h and subsequently rehydrated. After 7 d, the seedlings were planted in soil and monitored for 30 d. Root survival was determined by periodically uprooting plants and looking for growth of the radicle and seminal roots. Whole plant survival was determined by observing the production of new leaves. During the course of the experiments, all 30 plants produced new roots.

Treatment	Percent
Dried plants rehydrated	100
Plants that resumed growth	100
Roots that resumed growth	0

Table II. *Endogenous ABA levels after ABA treatment or dehydration*

Two-day-old seedlings were supplied with water with or without 20 μM ABA or the seedlings were dehydrated for 48 h. ABA was measured by immunoassay (Walker-Simmons, 1987).

Treatment	ABA Level		
	Root	Shoot	Scutellar
	pg/mg dry wt		
Water	515	50	115
ABA	23,800	620	9,820
Dehydrated	6,130	2,005	2,655

Expression of Group 3 LEA Transcripts and Proteins in Response to ABA

The rapid drying of the roots during the severe dehydration could have prevented group 3 LEA protein accumulation, even though ABA and group 3 LEA transcripts were induced. To address this, we determined the effects of ABA application in the absence of tissue dehydration on group 3 LEA gene expression in roots and the other seedling tissues. Seedlings (110 h) were placed in 20 μM ABA or a control water solution for 48 h. At the same time, another set of seedlings was dehydrated. ABA levels increased in the root, shoot, and scutellar tissues of both the ABA-treated and dehydrated seedlings compared with the water control seedlings (Table II). Seedling roots placed directly in ABA had high ABA levels, as would be expected.

Trace amounts of the group 3 LEA cross-reacting proteins were detected in the ABA-treated seedlings but not in the dehydrated root tissue (Fig. 5A). For shoots, the level of protein present in ABA-treated seedlings was much less than in dehydrated seedlings, whereas in scutellar tissue, approximately the same amounts of group 3 LEA proteins were present in ABA-treated and dehydrated seedlings.

Group 3 LEA mRNA levels were also compared in the ABA-treated and dehydrated seedlings (Fig. 5B). Transcripts were detected in all tissues whether the seedlings were treated with ABA or dehydration. Compared with the dehydrated seedlings, the ABA-treated seedlings contained 25% less message in the roots, and the shoots and scutellar tissues contained less than 10% of the message level.

DISCUSSION

Using antibodies produced to a group 3 LEA protein, we have shown that these LEA proteins accumulate in desiccation-tolerant seed and seedling tissues of wheat. These antibodies have also been used to identify group 3 LEA proteins (24 and 29 kD) in maize (Thomann et al., 1992). Our results (Fig. 2) demonstrate that the group 3 LEA proteins are maintained when dormant seeds are hydrated. Dormant seeds can be subjected to repeated wetting and drying, and the continued presence of LEA proteins may contribute to the desiccation tolerance in these seeds.

In this report, we have examined the desiccation tolerance of wheat seedlings before the development of the first seedling leaf. At this early developmental stage seedlings can

resume growth, even after moisture losses of over 95%. Survival after severe drying is aided by the presence of endosperm reserves and possibly by desiccation protectants such as the group 3 LEA proteins. With the emergence of the first leaf, tolerance to dehydration is lost and presumably the seedling can now nourish itself through photosynthesis and an ever-increasing root system.

Early experiments with wheat seedlings subjected to severe dehydration (Milthorpe, 1950) demonstrated that roots are not capable of resuming growth upon rehydration. Our experiments with dehydrated wheat seedlings showed the same effects on roots and revealed that group 3 LEA proteins were not detectable in the desiccation-intolerant root tissue. We plan in future experiments to extend our measurements of group 3 LEA proteins to older seedlings with less desiccation tolerance. It is interesting to note that a comparison of group 3 LEA protein accumulation in 3- and 7-d-old barley seedlings showed that the older seedlings accumulated one-tenth the group 3 protein levels of the younger seedlings (Hong et al., 1992). No assessment of desiccation tolerance was made in the barley study, but it is possible that the older seedlings had lost desiccation tolerance.

Differences in ABA levels did not account for differences in group 3 LEA protein levels or desiccation tolerance among root, shoot, and scutellar tissues (Table II). Upon desiccation, high levels of ABA and group 3 LEA mRNA accumulated in the roots, but no corresponding protein could be detected. ABA application to nonstressed seedlings increased root ABA levels markedly and induced the group 3 LEA transcript. However, even these high ABA levels did not cause significant accumulation of group 3 LEA proteins in the roots.

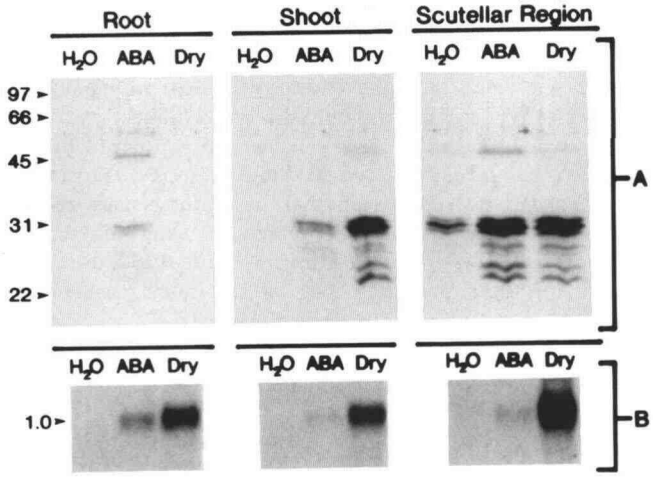

Figure 5. Expression of group 3 LEA proteins (A) and group 3 LEA mRNAs (B) in seedlings treated with ABA or dehydration for 48 h. A, Heat-soluble proteins were extracted from root, shoot, and scutellar tissues from seedlings and subjected to western blotting using the antibody against the group 3 LEA fusion protein. The positions of the molecular mass markers (in kD) are shown at the left. B, Total RNA was isolated from these tissues, fractionated by electrophoresis, transferred to nylon membranes, and hybridized with ^{32}P-labeled probes synthesized from a randomly primed cDNA insert of a group 3 LEA mRNA. The size of the transcript is shown at the left in kb.

The presence of LEA proteins is not always sufficient to confer desiccation tolerance. Wild rice seeds that are less tolerant of dehydration at low temperatures compared with paddy rice are still capable of accumulating the LEA protein dehydrin (Bradford and Chandler, 1992). Decreases in heat-soluble, embryo maturation proteins were associated with the loss of desiccation in soybean seedlings (Blackman et al., 1991). In contrast, heat-soluble protein accumulation in maturing soybean seeds, under experimentally manipulated conditions, did not always confer desiccation tolerance. Whether group 3 LEA protein accumulation contributes to desiccation tolerance, particularly in wheat, or is merely correlated with desiccation tolerance awaits further experimental determination.

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