Role of Abscisic Acid in the Induction of Desiccation Tolerance in Developing Seeds of Arabidopsis thaliana

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ABSTRACT

In contrast to wild-type seeds of Arabidopsis thaliana and to seeds deficient in (aba) or insensitive to (abi3) abscisic acid (ABA), maturing seeds of recombinant (abe,abi3) plants fail to desiccate, remain green, and lose viability upon drying. These double-mutant seeds acquire only low levels of the major storage proteins and are deficient in several low mol wt polypeptides, both soluble and bound, and some of which are heat stable. A major heat-stable glycoprotein of more than 100 kilodaltons behaves similarly; during seed development, it shows a decrease in size associated with the abi3 mutation. In seeds of the double mutant from 14 to 20 days after pollination, the low amounts of various maturation-specific proteins disappear and many higher mol wt proteins similar to those occurring during germination are induced, but no visible germination is apparent. It appears that in the abe,abi3 double mutant seed development is not completed and the program for seed germination is initiated prematurely in the absence of substances protective against dehydroxy. Seeds may be made desiccation tolerant by watering the plants with the ABA analog LAB 173711 or by imbibition of isolated immature seeds, 11 to 15 days after pollination, with ABA and sucrose. Whereas sucrose stimulates germination and may protect dehydroxylation-sensitive structures from desiccation damage, ABA inhibits precocious germination and is required to complete the program for seed maturation and the associated development of desiccation tolerance.

The significance of ABA for seed development has been probed by the analysis of ABA-deficient (aba) and -insensitive (abi) mutants of Arabidopsis thaliana. Thus, it was established that ABA is required for the induction of dormancy, which is instrumental in the prevention of precocious germination (16, 20). Seeds of the aba and abi mutants showed normal seed development and subsequent maturation and water loss. Similar observations were made during the analysis of the ABA-deficient sitiens mutant of tomato (11; S.P.C. Groot and C.M. Karssen, unpublished data). In both species, fertilization of the ABA-deficient mutant with wild-type pollen restricted ABA to the zygotic tissues of the developing seeds (11, 16) and inhibited precocious germination. Thus, it was proven that embryonic ABA plays the major role in seed development (17).

In wild-type seeds, ABA of maternal origin dominates quantitatively but has no significant function in development. Mutants of A. thaliana displaying reduced sensitivity to ABA as a result of a mutation at the abil or abil2 locus have a phenotype similar to that of the aba mutant and also exhibit wilting of vegetative tissues. However, mutants at the abi3 locus, although showing reduced sensitivity to ABA in the inhibition of germination, do not exhibit increased wilting (21) and are only slightly less responsive to ABA with respect to effects on seedling growth (9). When ABA deficiency and insensitivity are combined into aba,abil or aba,abit2 mutant plants, wilting is much more serious, but no additional phenotypic effects are seen (9). In contrast, the recombinant of the abi3 mutation with aba exhibits aberrant seed development: the seeds fail to desiccate at the normal time, remain green until ripe, and lose viability upon drying (19). Isolation of developing seeds and incubation in water lead to the rapid protrusion of the radicle. When kept at high RH, these seeds can survive and give rise to new plantlets. Under ambient conditions, however, radicle elongation soon stops and seedlings do not emerge. These observations prove that the ability of seeds to desiccate and survive depends on the perception of very low concentrations of ABA.

It was shown previously that recombinant aba,abi3 seeds did not form the late abundant 2S and 12S storage proteins (21). Likewise, other proteins necessary for the completion of seed development might not be synthesized in this double mutant. Apart from the deposition of storage proteins, ABA has been shown to induce mRNAs and proteins associated with desiccation tolerance in isolated embryos of wheat (26), barley (2), and maize (3). Moreover, ABA-induced proteins are associated with protection to drought stress in leaves (8, 28). Thus, proteins of the double mutant at different stages of seed development were analyzed to determine whether the reduced ABA content and action are associated with the absence of specific proteins that may play a role in desiccation tolerance.

MATERIALS AND METHODS

Collection of Developing Seeds

Wild-type Arabidopsis thaliana (Landsberg erecta) and homozygous aba (A26) and abi3 (CIV) mutants were the same as described previously (20, 21). Homozygous aba,abi3 double mutants were selected from crosses within a population heterozygous for ABA deficiency (19). Individual seeds were placed in pots in a mixture of nutrient-rich soil, perlite, and sand (3:1:1) and grown in a greenhouse at high RH.

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some experiments, 2.5 mL of a 10 μM solution of the ABA analog LAB 173711 (15) per plant was applied to the soil three times a week from flowering onward.

Seeds were collected 8, 14, and 20 dap, unless indicated otherwise. Staging of the developing seeds was performed by tagging individual flowers at the day of anthesis. Unripe seeds were collected by cutting the siliques open with a needle. For germination experiments, seeds were placed in Petri dishes on filter paper soaked with solutions prepared in sterile distilled water. Germination was scored as the percentage of seeds from which a radicle had protruded. For protein analyses, harvested seeds were frozen immediately in liquid N2 and stored at −80°C.

Collection of Germinating Seeds

For reference purposes, fully developed wild-type seeds at different stages of germination were analyzed. Seeds were sown in Petri dishes on water-saturated filter paper, allowed to germinate, dried for 2 h at room temperature, and stored at −20°C. Visibly germinated developing seeds harvested at 14 dap were similarly collected.

Testing for Desiccation Tolerance

Seeds at different stages of development were incubated for 2 to 4 d in darkness at 24°C in various solutions made up in sterile distilled water. After the seeds were washed with sterile distilled water, they were placed in Petri dishes on dry filter paper and air dried for 1 to 4 d under the same conditions. After addition of water or 0.1 mM GA4/7, germination was tested under continuous white fluorescent light (Philips TL 57) at 9 W·m−2 and 24°C. In one type of experiment, double-mutant seeds, after drying, were slowly rehumidified to 25% water content by placing them in a water-saturated atmosphere (13). After further equilibration for 30 min at 24°C, germination was tested as described before.

Protein Extraction

Proteins of seeds at 8, 14, and 20 dap were extracted according to the method of Heath et al. (12) with minor modifications. Approximately 40 mg of seeds was homogenized at 2°C in a 1.5-mL Eppendorf tube, using a metal rod (Potter, IKA-RW15). To remove pigments, seeds were extracted three times with 500 μL of 80% acetone, and the remainder was collected each time by centrifugation for 2 min. The resulting pellet was dried under vacuum and extracted by further homogenization for 5 min in 500 μL of buffer containing 63 μM Tris-HCl, 0.5 M NaCl, 1% (w/v) PVP, 0.1% (w/v) ascorbic acid, and 0.1% (v/v) 2-mercaptoethanol (pH 7.8). The homogenate was centrifuged for 5 min. The resulting supernatant, containing the soluble proteins, was thoroughly mixed with an equal volume of phenol-mix (100 g of distilled phenol, 0.1 g of 8-hydroxyquinoline, and 25 mL of chloroform, equilibrated with 30 mL of extraction buffer) (18) and centrifuged for 2 min. To minimize losses, the buffer phase was reextracted with 0.5 volume of phenol mix, whereas to the phenol phase 0.5 volume extraction buffer was added, and the procedure was repeated. Both phenol phases were combined, 5 volumes of 10% TCA in 100% acetone were added, and the proteins were precipitated overnight at −20°C. After centrifugation, the pellet was washed four times with 80% acetone and dried in vacuum. The dried pellet was dissolved either in SDS sample buffer (10 mM Tris-HCl, 1 mM EDTA [sodium salt], 10% glycerol, 2% SDS, and 5% 2-mercaptoethanol [pH 8.0], containing a trace of bromophenol blue) or in IEF sample buffer (9.5 M urea, 5% (v/v) 2-mercaptoethanol, 2% Nonidet P-40, and 2% Pharmalytes 5–8 or 3–10) for protein analysis.

The pellet originating from the homogenization in protein extraction buffer and containing the bound proteins was washed twice with the extraction buffer and resuspended in SDS sample buffer. After centrifugation, the supernatant was boiled for 5 min and centrifuged again. The resulting supernatant was used directly for SDS-PAGE or treated further for IEF. For IEF, samples were precipitated with TCA-acetone as described above. The precipitated proteins were dissolved in IEF sample buffer. All samples were stored at −20°C until analysis.

Heat-stable proteins were obtained by heating the homogenates in extraction buffer at 100°C for 10 min. After centrifugation, the supernatant was treated with phenol mix as described above.

Table I. Relative Contents of Phenol-Soluble Proteins Recovered from Seeds (20 dap) of Wild-Type, aba, ab3, and aba,ab3 Arabidopsis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100</td>
</tr>
<tr>
<td>aba3</td>
<td>71–89</td>
</tr>
<tr>
<td>aba</td>
<td>87–114</td>
</tr>
<tr>
<td>aba,ab3</td>
<td>33–69</td>
</tr>
</tbody>
</table>

Abbreviations: dap, days after pollination; IEF, isoelectric focusing; pl, isoelectric point; 2D-PAGE, two-dimensional PAGE.
Figure 2. 2D-PAGE patterns of phenol-soluble proteins from (A–C) wild-type and (D–F) aba,abi3 double-mutant seeds at 8 (A and D), 14 (B and E), and 20 (C and F) dap. The positions of spots referred to in the text that are either absent or present at reduced levels in the double mutant are within the areas marked or indicated by arrows.
The amounts of protein present in the samples were determined according to the method of Bradford (4).

Electrophoretic Analyses

Samples in SDS sample buffer were separated on a 12.5% (w/v) polyacrylamide gel slab according to the method of Laemmli (22). 2D-PAGE was performed according to the method of O'Farrell (25) with minor modifications, as described by Van Telgen and van Loon (29). In the first dimension, IEF extended from either pH 5 to 8 or 3 to 10, and a 12.5% polyacrylamide-SDS gel slab was used in the second dimension. Reproducibility was optimized, gels were stained with Coomassie blue or silver, and patterns were analyzed as described in detail by Klerk and van Loon (18). The 2S and 12S storage protein polypeptides were identified as described earlier (19). Staining for glycoproteins with periodic acid-silver was essentially as described by Dubray and Bezard (6).

RESULTS

Analysis of Soluble Proteins

Phenol-soluble proteins were analyzed from developing seeds of wild-type, aba and abi3 single mutants, and the aba,abi3 double mutant at 8, 14, and 20 dap. Only slight quantitative differences were apparent between the four genotypes at the earliest stage. As evidenced from Coomassie blue-stained gels, by day 14 storage protein polypeptides had accumulated in wild-type, aba, and abi3 seeds, and other low mol wt proteins in the range of 15 to 20 kD had become more conspicuous (Fig. 1). These changes had become more pronounced by day 20. In contrast, in the aba,abi3 double mutant, the accumulation of the storage polypeptides, as well as of the specific minor proteins, was strongly reduced and ceased prematurely. Small amounts of the storage polypeptides were present by day 14, and their amounts were not increased by day 20. Additional bands at 22 and 25 kD, seen by day 20, had slightly different mobilities from the storage polypeptides present in the wild-type and single mutants. At day 20, the pattern of the double mutant further differed in that several bands present at 8 dap seemed still recognizable (Fig. 1). Although similar amounts of protein were applied to the gel shown in Figure 1, the relative complexity of the pattern of the double mutant was not primarily due to the low amount of storage protein present, as evidenced by analyzing patterns resulting from application of a range of protein concentrations (data not shown). In addition, the total amount of phenol-soluble proteins recovered from the aba,abi3 double mutant was about half that from the wild-type or the aba mutant. Proteins in the abi3 mutant were reduced by about 20% (Table 1).

Changes in the protein patterns were further analyzed by 2D-PAGE. A complex pattern of proteins with molecular masses to >100 kD and pl values ranging from 5 to 8 was revealed. Virtually no additional spots were apparent when IEF was extended from pH 3 to 10 (cf. Fig. 4A). Because of the silver stain used, storage protein polypeptides were less intense but stood out as a result of their golden yellow color. As shown in Figure 2 (A–C), the wild-type polypeptides with molecular masses particularly in the range of 20 to 35 kD increased substantially in intensity during seed development, whereas several spots in the range of 35 to 60 kD were reduced in intensity or seemed to be lost. The relevant spots are depicted schematically in Figure 3.

Similar changes were seen during development of the seeds of the aba and the abi3 mutants (data not shown). However, in the double mutant, hardly any increase occurred in the pattern in the 20 to 35 kD region, but the pattern in the 35
to 70 kD range became substantially more complex (Figs. 2, D–F, and 3). Because by 20 dap double-mutant seeds have acquired the ability to immediately start germination when sufficient moisture is present within the siliques, it was envisioned that additional spots present in the latter region might be related to germination. Indeed, compared with the pattern of wild-type seeds 20 dap (Fig. 4B), the pattern of double-mutant seeds (Fig. 4A) strongly resembled that of precociously germinated wild-type seeds at the same stage of development (Fig. 4C) or after-ripened wild-type seeds 2 d after germination (Fig. 4D). Thus, instead of accumulating maturation-specific proteins, the double mutant seems to prepare for germination, even though no visible germination occurs in the absence of added water.

**Analysis of Bound Proteins, Heat-Stable Proteins, and Glycoproteins**

The deficiency in several soluble low mol wt proteins in seeds of the double mutant, may be related to desiccation intolerance. However, additional proteins might be lacking that remain bound to sensitive structures and could physically protect those from the damaging effect of water loss. Bound proteins were solubilized from the residue remaining after extraction of the soluble proteins. As shown in Figure 5A, several protein bands, notably those at 19, 22, 30, and 33 kD, increased during development of wild-type seeds. As revealed by 2D-PAGE, several of these bands comprised multiple polypeptides with varying pI values (Fig. 6). These proteins were less abundant in the aba and abi3 mutants and did not accumulate in the double mutant (Figs. 5A and 6). Instead, increases in proteins at 18, 29, and 50 kD were noticeable in the latter.

Because some ABA-induced proteins have been reported to be heat stable (9, 14, 27), soluble protein extracts were boiled, and samples enriched in heat-stable proteins were analyzed. Whereas in 8-d samples many polypeptides withstood boiling, fewer bands remained at later stages of de-
opment (Fig. 5B). By 20 dap, wild-type seeds had accumulated heat-stable proteins of 29, 33, 60, 65, and >100 kD. The extent of accumulation was similar for the abi3 mutant but less so in the aba mutant. With the exception of the >100 kD protein, these proteins did not appear in the double mutant (Fig. 5B).

The >100 kD polypeptide formed a major band among the accumulating heat-stable proteins. However, its size seemed to vary. Whereas its apparent mol wt was identical and did not change during seed development in the wild-type and the aba mutant, its size was slightly reduced when it first appeared in the abi3 and double mutant and further reduced from 14 to 20 dap in these seeds. Staining for glycoproteins (Fig. 5C) revealed that this polypeptide is the major glycoprotein present in developing Arabidopsis seeds. These observations indicate that the abi3 mutation influences the processing of a glycoprotein of >100 kD. Moreover, this glycoprotein is almost completely lost from double-mutant seeds from 14 to 20 dap, concomitantly with the accumulation of germination-specific polypeptides.

**Induction of Desiccation Tolerance in Double-Mutant Seeds**

Throughout development, seeds of the aba,abi3 double mutant readily germinated in water (Fig. 7) but lost viability when dried for 2 d at 24°C in the dark (0% germination; not shown). As a result of leakiness of the abi3 mutation (19), germinability and desiccation tolerance were acquired by application of the ABA analog LAB 173711 to the plants (Fig. 7). Such treatment largely restored the protein pattern of the seeds to that of the wild type: storage proteins accumulated, low mol wt proteins became more conspicuous, and the complexity of the pattern in the higher mol wt region was reduced (Fig. 8). Thus, LAB 173711 treatment of the plants at the time of seed development restored both the protein pattern and desiccation tolerance.

To determine whether the desiccation intolerance of double-mutant seeds was due to imbibitional damage during rehydration, seeds harvested 20 dap and left to dry for 2 d were slowlyrehumidified in moist conditions before testing germination. No germination occurred when incubation took place at room temperature, but up to 15% of the seeds germinated afterrehumidification for 6 h at 4°C. Thus, only a small percentage of the seeds were rescued, indicating that impairment of factors before water uptake must be significant. To allow possible repair mechanisms to operate more fully, the rate of germination was slowed by application of ABA and/or osmotic treatments to freshly harvested seeds before drying. ABA was ineffective in inducing dormancy in double-mutant seeds 20 dap. However, seeds 11 to 15 dap, which had acquired full germinability in water (Fig. 7), showed responsiveness to ABA and were exploited to determine whether the substances could protect them from dehydration damage. Seeds were incubated for 2 to 4 d in the various solutions, left to dry for 1 to 4 d at 24°C, and reimbibed in germination-stimulating GA₄/7.

In the first experiment, shown in Table II, ABA by itself did not protect against desiccation. Osmotic treatments with 3% sucrose or glucose were ineffective because they stimulated germination of the seeds during the pretreatment. Higher

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**Figure 5.** SDS-PAGE patterns of bound proteins (residual proteins solubilized in SDS) (A), heat-stable proteins (soluble proteins heated for 10 min at 100°C) (B), and glycoproteins (C) from developing seeds of wild type (wt), abi3 mutant, aba mutant, and aba,abi3 double mutant (dm) at 8, 14, and 20 dap. The positions of bands referred to in the text are indicated by arrows.
concentrations of the sugars did prevent germination but induced vitrification and loss of viability due to their osmotic effects. Osmotic pretreatment with PEG or mannitol allowed dehydration of some of the seeds, leading to 10 and 22%, respectively, of viable plantlets. These values were not increased in the presence of ABA. In contrast, pretreatment with ABA in the presence of 3% glucose and, even more so, sucrose maintained viability of a large percentage of the seeds. In subsequent experiments with seeds from different batches of plants and harvested at different times of the year, viability after desiccation was retained to different extents as a result of the pretreatments. Notably, pretreatment of the seeds with ABA alone often proved highly effective, allowing up to 80 and 100% survival at concentrations of 10 and 100 μM, respectively. Thus, developing seeds isolated from untreated plants can acquire desiccation tolerance by treatment with ABA, or ABA and sugars, before drying. Upon germination, the resulting plantlets developed normally into fully fertile seedlings.

**DISCUSSION**

Development of desiccation tolerance in *Arabidopsis* seeds occurs when only minute amounts of ABA are perceived, because only a combination of ABA deficiency and insensitivity in seeds of the aba,abi3 double mutant caused a reduced water loss and the loss of viability upon drying. In contrast, all single mutants developed normally; they only showed reduced dormancy and inhibitory effects of applied ABA on germination and seedling growth, indicating that these responses are less sensitive to the hormone. Thus, biochemical differences found between the double mutant, on the one hand, and the wild-type and single mutants, on the other hand, are likely to be associated with the lack of desiccation tolerance in the former.

In spite of many investigations (8, 23, 28), the mechanism of desiccation tolerance has not been elucidated. Dehydration increases the transition temperature of the phospholipids in membranes, resulting in the formation of gel phase domains (5). Upon rehydration, leakage of cell constituents results before the membranes regain their liquid crystalline state. For pollen, this detrimental damage can be alleviated by prior equilibration in moist air. Under such conditions, repair mechanisms are thought to operate, and less rapid intrusion of water occurs during subsequent imbibition (5). Equilibration in moist air was hardly effective in maintaining viability in double-mutant seeds. Apparently, the damage occurring during desiccation was no longer reversible.

Proteins may physically protect membranes from the dele-
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Figure 8. 2D-PAGE patterns of phenol-soluble proteins from seeds of the double mutant 20 dap (A), double mutant 20 dap from plants watered repeatedly with LAB 173711 (B), and abi3 mutant 20 dap (C). Note the increase in the spots in the marked area and indicated by arrows as a result of treatment of the double mutant with LAB 173711 (B) to the level present in the abi3 mutant (C).

Figure 8. 2D-PAGE patterns of phenol-soluble proteins from seeds...masked area and indicated by arrows as a result of treatment of the double mutant with LAB 173711 (B) to the level present in the abi3 mutant (C).

Figure 8. 2D-PAGE patterns of phenol-soluble proteins from seeds of the double mutant 20 dap (A), double mutant 20 dap from plants watered repeatedly with LAB 173711 (B), and abi3 mutant 20 dap (C). Note the increase in the spots in the marked area and indicated by arrows as a result of treatment of the double mutant with LAB 173711 (B) to the level present in the abi3 mutant (C).

Terious effects of dehydration. Such a mechanism is thought to operate in the resurrection plant Craterostigma plantagineum, in which desiccation-related proteins are abundantly expressed in dried leaves and in ABA-treated dried callus (1). The only proteins that appear to be present in sufficient quantities to cover and physically protect membranes in Arabidopsis seeds are the storage polypeptides. These accumulated to high levels in wild-type, aba, and abi3 mutants. Only small amounts of the 125 polypeptides appeared in the aba,abi3 mutant, and these did not increase during the later stage of seed development. However, it appears unlikely that, in addition to functioning as storage reserves, these proteins can be generally protective to membranes, because they are supposedly localized exclusively in protein bodies.

Double-mutant seeds were also deficient in other low mol wt polypeptides of lower abundance. These polypeptides may be similar to Lea proteins induced by ABA in cultured embryos (8, 10) and resemble polypeptides induced in vegetative tissues by environmental stresses such as drought (26, 28). The function of these proteins is unknown, but they could be involved in the production of protective substances. Although the majority of these proteins were soluble, bound proteins displaying similar mol wts and pI values were also present, suggesting that they could be associated with membranes and play a role in stabilizing sensitive structures. A fraction enriched in such proteins was obtained by boiling the extracts, indicating that some of these proteins must have a rigid conformation. Particularly intriguing is the occurrence of a >100 kD heat-stable glycoprotein, accumulating in wild-type and single mutants but disappearing prematurely from double-mutant seeds. During seed development, a reduction in its size occurred in mutants containing the abi3 allele, suggesting that this locus controls a posttranslational modification, involving partial proteolysis and/or deglycosylation.

Table II. Acquisition of Desiccation Tolerance by Developing Seeds of the aba,abi3 Double Mutant

Developing seeds were harvested 12 dap, pretreated for 2 d in the various solutions, and dried for 2 d at 24°C. For germination, seeds were imbibed in 0.1 mM GA4/7.

<table>
<thead>
<tr>
<th>Seed Pretreatment</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>10 μM ABA</td>
<td>0</td>
</tr>
<tr>
<td>100 μM ABA</td>
<td>86 c</td>
</tr>
<tr>
<td>3% sucrose</td>
<td></td>
</tr>
<tr>
<td>3% glucose</td>
<td></td>
</tr>
<tr>
<td>10 μM ABA + 3% sucrose</td>
<td>75</td>
</tr>
<tr>
<td>100 μM ABA + 3% sucrose</td>
<td>93 c</td>
</tr>
<tr>
<td>10 μM ABA + 3% glucose</td>
<td>40</td>
</tr>
<tr>
<td>30% PEG (-1.1 MPa)</td>
<td>10</td>
</tr>
<tr>
<td>12.5% mannitol (-1.1 MPa)</td>
<td>22</td>
</tr>
<tr>
<td>10 μM ABA + 30% PEG</td>
<td>8</td>
</tr>
<tr>
<td>10 μM ABA + 12.5% mannitol</td>
<td>25</td>
</tr>
</tbody>
</table>

* Means from 7 experiments; different letters indicate statistically significant differences (P < 0.01) according to Wilcoxon’s signed-rank test. ** On glucose and sucrose, germination occurred already during pretreatment; germinated seeds lost viability when dried.
During seed development in the double mutant, the pattern of high mol wt proteins became considerably more complex. In addition to proteins characteristic of seed development, it comprised polypeptides occurring during normal germination, suggesting that in the virtual absence of perceived ABA, seed development is not completed and the program for seed germination is initiated prematurely in the absence of sufficient protective substances. Although the double mutant is considerably less responsive to ABA and applied ABA did not prevent radicle protrusion of freshly harvested seeds 20 dap, when transferred immediately into solution, ABA did prevent precocious germination when applied to immature seeds 11 to 15 dap. This behavior thus marks a stage at which developing seeds are most sensitive to ABA. Treatment of plants with ABA or LAB 173711 throughout the sensitive stage largely counteracted the effects of the mutations (19) and allowed the program for seed maturation to be completed. Moreover, immature seeds at this stage became desiccation tolerant when incubated in a high concentration of ABA or lower concentrations of ABA and sugars, particularly sucrose. Whereas sucrose stimulated germination, ABA seemed to be required for the development of desiccation tolerance. Simple sugars such as sucrose can also protect membranes by replacing bound water molecules during dehydration (5). Thus, the effect of the sucrose may be ascribed to its functioning as a readily accessible reserve, as well as a protective substance. Recently, high concentrations of sucrose were reported in naturally developing rape seeds and wheat embryos and considered to confer protection against desiccation damage (7, 24). Because in the Arabidopsis double mutant high concentrations of ABA eliminated the need for added sucrose, it is possible that one of the actions of ABA is the stimulation of the formation or release of protective sugars in the isolated immature seed. The requirement for sucrose in some experiments, but not in others, could be explained by varying contents of endogenous sugars present at the time of harvest in different batches of seeds. The possible relationship among ABA, proteins, and sugar metabolism is currently being further explored.

ACKNOWLEDGMENTS

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LITERATURE CITED


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