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Development of Desiccation Tolerance during Embryogenesis in Rice (*Oryza sativa*) and Wild Rice (*Zizania palustris*)[1]

Dehydrin Expression, Abscisic Acid Content, and Sucrose Accumulation

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The ability of seeds to withstand desiccation develops during embryogenesis and differs considerably among species. Paddy rice (*Oryza sativa* L.) grains readily survive dehydration to as low as 2% water content, whereas North American wild rice (*Zizania palustris* var *interior* [Fasset] Dore) grains are not tolerant of water contents below 6% and are sensitive to drying and imbibition conditions. During embryogenesis, dehydrin proteins, abscisic acid (ABA), and saccharides are synthesized, and all have been implicated in the development of desiccation tolerance. We examined the accumulation patterns of dehydrin protein, ABA, and soluble saccharides (sucrose and oligosaccharides) of rice embryos and wild rice axes in relation to the development of desiccation tolerance during embryogenesis. Dehydrin protein was detected immunologically with an antibody raised against a conserved dehydrin amino acid sequence. Both rice and wild rice embryos accumulated a 21-kD dehydrin protein during development, and an immunologically related 38-kD protein accumulated similarly in rice. Dehydrin protein synthesis was detected before desiccation tolerance had developed in both rice embryos and wild rice axes. However, the major accumulation of dehydrin occurred after most seeds of both species had become desiccation tolerant. ABA accumulated in wild rice axes to about twice the amount present in rice embryos. There were no obvious relationships between ABA and the temporal expression patterns of dehydrin protein in either rice or wild rice. Wild rice axes accumulated about twice as much sucrose as rice embryos. Oligosaccharides were present at only about one-tenth of the maximum sucrose concentrations in both rice and wild rice. We conclude that the desiccation sensitivity displayed by wild rice grains is not due to an inability to synthesize dehydrin proteins, ABA, or soluble carbohydrates.

Most seeds enter a period of desiccation and physiological quiescence during the last phase of development. However, there are considerable differences among species in their ability to survive desiccation. Orthodox seeds such as paddy rice (*Oryza sativa* L.) caryopses readily tolerate desiccation to <5% water content, and their longevity in storage increases as temperature and water content decrease (Roberts, 1973). In contrast, recalcitrant seeds exhibit varying degrees of desiccation tolerance and storage longevity. North American wild rice (*Zizania palustris* var *interior* [Fasset] Dore) seeds, for example, are tolerant of desiccation only under restricted dehydration and rehydration conditions. Wild rice is an aquatic grass whose seeds (caryopses) normally abscise at a relatively high moisture content, fall into the water, remain dormant through the winter, and germinate the following spring. Wild rice seeds were thought to be intolerant of dehydration <30% moisture content (Probert and Brierly, 1989; Probert and Longley, 1989), but the seeds can survive dehydration to as low as 6% moisture content if dehydration and rehydration occur at 15 to 25°C and imbibitional damage is avoided (Kovach and Bradford, 1992). Wild rice seeds survive desiccation only under specific dehydration and rehydration conditions and may be classified as minimally recalcitrant (Farrant et al., 1988).

The cellular mechanisms allowing orthodox seeds to tolerate extreme dehydration remain obscure. Recently, interest has focused on a class of LEA proteins that are expressed in seeds during development and in other tissues in response to water stress or ABA (Skriver and Mundy, 1990). Based on conservation of amino acid sequences across species, extreme hydrophilicity, heat stability, and induction by dehydration, a subset of LEA proteins, called "dehydrin" (Close et al., 1989) or RAB proteins (Mundy and Chua, 1988), has been hypothesized to play a role in desiccation tolerance (Dure et al., 1989). Robertson and Chandler (1992) described dehydrin expression during pea (*Pisum sativum* L.) seed development but did not correlate dehydrin synthesis with desiccation tolerance. We recently showed that mature wild rice embryonic axes contained "dehydrin-like" proteins (Bradford and Chandler, 1992). Other proteins correlated with the development of desiccation tolerance during embryo maturation have been reported in angiosperms (Bartels et al., 1988; Blackman et al., 1991) and gymnosperms (Leal and Misra, 1993). However, the presence of "maturation proteins" alone was not sufficient to confer desiccation tolerance on excised germinating soybean axes (Blackman et al., 1992).

ABA is also known to induce the expression of a number of genes in developing seeds, including dehydrins (reviewed by Kermode, 1990; Skriver and Mundy, 1990; Hughes and Galau, 1991; Williams and Tsang, 1991). A role for ABA in

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Abbreviations: DAA, days after anthesis; LEA, late embryogenesis abundant; RAB, responsive to ABA.

seed development has been demonstrated in *Arabidopsis* by Koornneef et al. (1989). They isolated ABA-deficient (*aba*) and ABA-insensitive (*abi3*) seeds and found that recombinant *aba/abi3* mutant seeds displayed reduced water loss, incomplete testa and embryo development, and intolerance to desiccation. Meurs et al. (1992) found that the *aba/abi3* mutant failed to accumulate storage proteins during development. Application of an ABA analog restored protein accumulation and desiccation tolerance in the double-mutant seeds, indicating that some ABA is required for seed maturation and desiccation tolerance. During embryo development, *abi3* mutants synthesized a protein homologous to an ABA- and desiccation-inducible protein from *Craterostigma*, whereas the desiccation-intolerant double mutants did not (Ooms et al., 1993).

Accumulation of disaccharides and oligosaccharides has also been implicated in desiccation tolerance by preserving membrane and protein integrity during dehydration (Crowe et al., 1992). The disaccharides are thought to prevent membrane fusion during desiccation by interacting with polar head groups of phospholipids and functional groups of proteins. Trehalose has been shown to be a particularly effective protectant in anhydrobiotic organisms such as yeast, nematodes, fungi, and bacteria, whereas in seeds and pollen, Suc appears to play the same role (Crowe et al., 1992). Many orthodox seeds accumulate high amounts of Suc and oligosaccharides during maturation (Amuti and Pollard, 1977), and sugar accumulation has been correlated with an increase in desiccation tolerance (Leprince et al., 1990; Blackman et al., 1992). Oligosaccharides have been proposed to inhibit Suc crystallization, a situation that is favored by slow drying (Koster and Leopold, 1988), and may be involved in desiccation tolerance by promoting glass formation at water contents <10% (Bruni and Leopold, 1991). Desiccation tolerance of *Arabidopsis aba/abi3* double-mutant seeds increased when they were cultured on media containing both ABA and Suc (Meurs et al., 1992). On the other hand, wild-type *Arabidopsis* seeds accumulated relatively little soluble sugar during development compared to desiccation-intolerant *abi3* genotypes (Ooms et al., 1993). Sugar accumulation in the mutant genotypes occurred late in embryogenesis, however, after desiccation tolerance had already developed in wild-type seeds.

To determine whether dehydrins, ABA, or saccharides are critical for the development of desiccation tolerance during embryogenesis, the temporal sequence of their expression or accumulation patterns can be compared to the time of attainment of desiccation tolerance. We report here the development of germinability and desiccation tolerance during rice and wild rice seed maturation in relation to the expression of dehydrin protein, ABA content, and saccharide accumulation.

MATERIALS AND METHODS

Plant Materials and Culture

Rice (cv M201) and wild rice (cv NC1) plants were grown outdoors in large tanks containing soil submerged under 10 cm of water. Panicles were tagged on the day of female anthesis, and seeds were harvested 11 to 41 DAA. Embryonic axes of wild rice and embryos of rice (32 of each) were excised aseptically and either cultured directly on agar containing quarter-strength Murashige and Skoog basal medium with 3% Suc (w/v) and 0.75% agar (w/v) at 20°C under fluorescent light or dehydrated over saturated $MgCl_2$ (33% RH) at 25°C for 48 h to 7.3 and 9.3% water content (fresh weight basis) for rice and wild rice, respectively. After dehydration, the axes or embryos were rehydrated in 100% RH for 24 h before culturing. Continued growth and greening of the embryonic axes was scored as completion of germination.

Intact wild rice seeds were dried to water contents of 7 to 9%, rehydrated in water for 1 month at 15°C, and then stored in water at 2.5°C for 5 months to break dormancy before germination was tested as described previously (Kovach and Bradford, 1992). Intact rice seeds were dried at room temperature by forced air to 5% water content, dehulled, and germinated in Petri dishes on blotter paper saturated with distilled water. In separate experiments, mature wild rice seeds (three replications of 25–50 seeds) were slowly dried over saturated $NaNO_2$, $MgCl_2$, or $ZnCl_2$ to water contents of 4 to 15%, and their viability was determined by tetrazolium tests or germination after stratification (Kovach and Bradford, 1992).

Similar experiments were conducted on plants grown in 1990 and 1991 with the exception of measurement of ABA content, which was determined for embryos from the 1991 harvest only. The overall patterns of development, dehydrin expression, and saccharide accumulation between the two years were similar. Data presented are from the 1991 experiments unless otherwise noted.

Protein Labeling, Extraction, and Electrophoresis

For labeling of newly synthesized proteins, 12 to 15 excised axes or embryos were incubated for 2 h in 2 volumes (w/v) of distilled water containing 4 mCi of [^{35}S]Met (ICN Biomedicals, Inc.; 1100 Ci mmol^{-1} specific activity) g^{-1} fresh weight at room temperature, rinsed with 200 mL of double-distilled water, and stored in liquid nitrogen. The axes and embryos were ground in 10 volumes (w/v) of extraction buffer (20 mM Tes, 0.5 M NaCl [pH 8.0]) and centrifuged to remove insoluble material. Aliquots were removed from the supernatant and heated in a 100°C water bath for 10 min and then cooled on ice. The samples were centrifuged, and the resulting supernatant contained the heat-soluble protein fraction. Protein content was determined by the bicinchoninic acid method (Smith et al., 1985) using BSA as a standard. Incorporation of labeled Met into protein was determined by scintillation counting after precipitation with cold 10% TCA.

The supernatant was combined with an equal volume of SDS buffer, and discontinuous gel electrophoresis was run on 12% acrylamide gels (Laemmli, 1970). Each lane was loaded with equal counts (150,000 cpm, before hot water bath treatment) of heat-soluble or total protein. Only the heat-soluble protein gels are shown.

Western Blots

Proteins for western blots were extracted as described above. Aliquots containing 8 μg of total protein were electrophoresed in 12% acrylamide SDS gels and electroblotted to

nitrocellulose membranes, and the bands containing dehydrin protein were made visible using antiserum raised against a conserved dehydrin/RAB amino acid sequence (kindly provided by Dr. T.J. Close, University of California, Riverside; Close et al., 1993a).

ABA Analysis

ABA was extracted from three replicates of 10 axes or embryos essentially as described previously (Bradford and Chandler, 1992). Extracted ABA was measured by an indirect ELISA (Walker-Simmons, 1987) using monoclonal antibody to ABA (Idetek Inc., San Bruno, CA) and (±)ABA as a standard. Because the monoclonal antibody is specific to (−)ABA, standard curve concentrations were multiplied by 0.5. To test for sample loss and false positives, a known quantity of (±)ABA in distilled water was taken through the extraction process and partially purified using Sep-pak C_{18} reverse-phase chromatography (Waters Associates Inc., Milford, MA) (Tahara et al., 1991). The average recovery of six extractions was 87% (±8%). To test for nonspecific interference in extracts (Rodbard, 1974; Pengelly, 1985), representative samples from the beginning, middle, and end of the harvest period were diluted with extraction buffer, and the amount of ABA expected was checked against the amount of ABA measured. The plot of expected versus measured had a slope that was not significantly different from unity and an intercept that passed through the origin (undiluted sample), demonstrating that nonspecific interference was not evident at any point during seed development (data not presented). As another test for nonspecific interference, 20-μL aliquots from representative samples were added to a standard curve. This resulted in a curve parallel to the standard curve and offset by the expected amount, again indicating that there was no interference within the linear range of binding (data not shown).

Suc and Oligosaccharide Extraction and Analysis

Rice embryos (50–100) and wild rice axes (25) were excised from freshly harvested seeds throughout development. The embryos or axes were then lyophilized and stored in liquid nitrogen until the sugars were extracted. The axes or embryos were pulverized in liquid nitrogen and then rehydrated with boiling water (HPLC grade) for 10 min. The samples were centrifuged, and the supernatant was collected and filtered through a 0.45-μm nylon filter. Suc and oligosaccharides were analyzed by HPLC using an Aminex carbohydrate 87C column and an HPX-42A column (Bio-Rad, Hercules, CA), respectively. There was a linear response of the refractive index to oligosaccharides up to degree of polymerization 6. Consequently, total oligosaccharide concentrations were based on peak areas using Glc as a standard.

RESULTS

Sensitivity of Wild Rice Embryos to Dehydration

Mature wild rice seeds could be dried to embryo water contents of 9 to 10% without affecting viability (Fig. 1). Survival declined sharply at water contents of <7%, and no embryos survived dehydration of <5% water content (Fig. 1). Viability determined by tetrazolium tests gave values slightly higher than germination tests for a given water content, but both methods showed the same sensitivity to water content (Fig. 1). Rice grains, on the other hand, can survive dehydration to water contents as low as 2% if rehydration occurs slowly to avoid imbibitional damage (Ellis et al., 1989). Several methods of slowly increasing the water content of wild rice grains did not improve survival, making it unlikely that the reduced viability at low water contents can be attributed to imbibitional damage. Thus, despite their conditional tolerance of dehydration, wild rice seeds cannot be considered fully desiccation tolerant. In subsequent tests of desiccation tolerance, embryos were not dried to <7% water content.

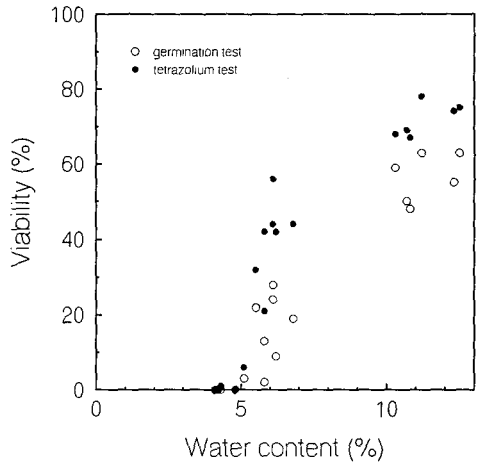

Figure 1. Viability of wild rice seeds as a function of embryonic axis water content. Viability was determined by tetrazolium tests (●) or by germination tests (○) after cold stratification to break dormancy as described by Kovach and Bradford (1992).

Germinability and Desiccation Tolerance of Rice and Wild Rice Seeds during Development

Cultured rice embryos were capable of growth as soon as they could be excised, and by 23 DAA germination was 100% (Fig. 2A). The percentage of excised embryos that were desiccation tolerant increased more gradually, reaching 100% by 31 DAA (Fig. 2A). The ability of intact seeds to withstand desiccation was essentially the same as that of excised embryos (Fig. 2A). The point when all embryos were desiccation tolerant coincided with the completion of embryo dry weight accumulation (Fig. 2B). The water content of rice embryos was approximately 46% at 39 DAA (Fig. 2B), remained relatively constant until 53 DAA, then declined to 34% by 65 DAA, and was 8% by 72 DAA.

Similarly, most cultured wild rice embryonic axes were capable of growth virtually as soon as they were large enough to be located and excised from the seed (11–14 DAA) (Fig. 3A). However, most excised axes did not survive dehydration until 25 DAA, approximately 2 weeks later. Seeds that were dried intact became desiccation tolerant 6 to 8 d earlier than excised axes (Fig. 3A), possibly because the slower dehydra-

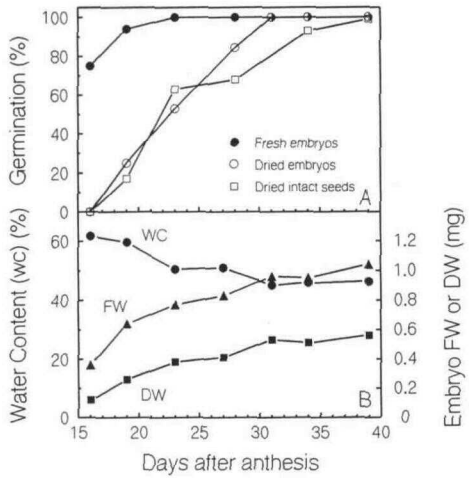

Figure 2. A, Development of germinability and desiccation tolerance of intact rice seeds (□) and fresh (●) and dried (○) excised embryos. B, Fresh weight (FW, ▲), dry weight (DW, ■), and water content (WC, fresh weight basis; ●) of rice embryos during development.

tion of the axes within the seed allowed development to continue. The initial water content of wild rice axes was 70% and then remained at 50% between 18 and 36 DAA before decreasing to 10% by 41 DAA (Fig 3B). Most axes became tolerant of desiccation after the initial decline in water content, coinciding approximately with maximum axis dry weight accumulation (Fig. 3B). Germination of both fresh and dried excised axes declined later during development (Fig. 3A), possibly due to embryo dormancy, because the excised axes did not deteriorate for a period of several months

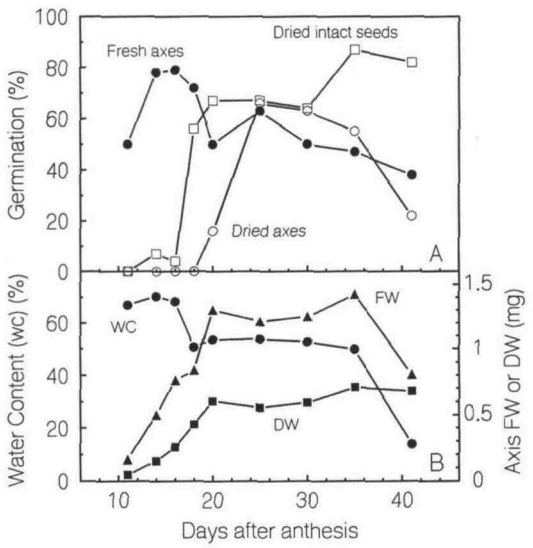

Figure 3. A, Development of germinability and desiccation tolerance of intact wild rice seeds (□) and fresh (●) and dried (○) excised embryonic axes. B, Fresh weight (FW, ▲), dry weight (DW, ■), and water content (WC, fresh weight basis; ●) of wild rice embryonic axes during development.

but simply failed to grow under culture conditions. In contrast, germination of intact seeds after drying, rehydration, and stratification remained high throughout seed maturation.

Expression of Dehydrin Protein in Rice Embryos and Wild Rice Axes during Development

Labeling indicated that a 21-kD protein was being synthesized in developing rice embryos by 15 DAA (Fig. 4A). Accumulation during development of a dehydrin protein of the same apparent molecular mass as the labeled protein band was confirmed on protein (western) blots using antiserum to a conserved dehydrin amino acid sequence (Fig. 4B). Additionally, another protein of about 38 kD was recognized by the dehydrin antibody, and its appearance coincided with that of the 21-kD dehydrin protein (Fig. 4B). Neither band was visible using preimmune serum. The initial appearance of dehydrin proteins occurred just as the percentage of desiccation-tolerant embryos began to increase (Fig. 4A). Dehydrin protein accumulation closely paralleled the increase in embryo dry weight and the increasing percentage of desiccation-tolerant embryos and continued after maximum embryo dry weight and desiccation tolerance had been attained (Figs. 2 and 4B).

During wild rice embryo development, labeling of a newly synthesized, heat-stable 21-kD protein was evident by 18 DAA (Fig. 5A). Western analysis revealed the accumulation of a 21-kD dehydrin protein, the initial appearance of which preceded the attainment of desiccation tolerance in developing axes (Fig. 5B). Based on these criteria and the results reported by Bradford and Chandler (1992), we conclude that the 21-kD protein is a dehydrin protein. Although the max-

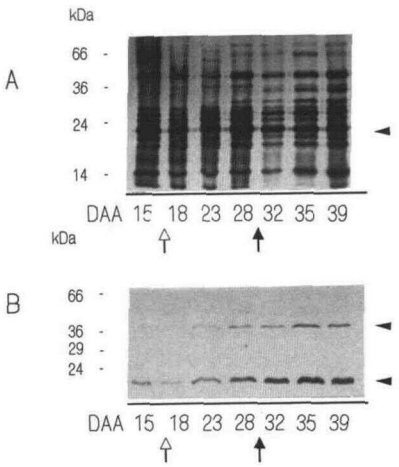

Figure 4. Expression of dehydrin protein in developing rice embryos. A, Incorporation of [^{35}S]Met into a heat-soluble, 21-kD protein band (arrow, right ordinate). B, Staining on protein (western) blots of 21- and 38-kD protein bands (arrows, right ordinate) using antiserum prepared against a conserved dehydrin oligopeptide sequence. The open arrows perpendicular to the abscissa indicate the beginning of the period during which the percentage of desiccation-tolerant axes began to increase; the closed arrows indicate when the percentage of desiccation-tolerant embryos reached its maximum.

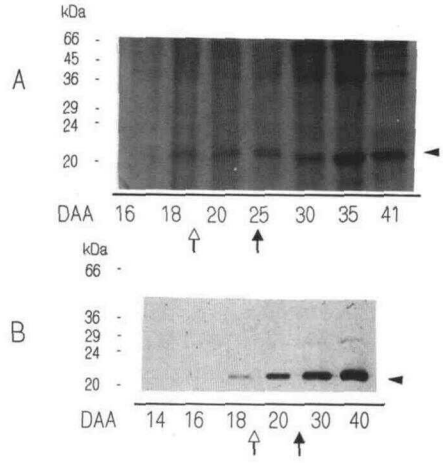

Figure 5. Expression of dehydrin protein in developing wild rice embryonic axes. A, Incorporation of [^{35}S]Met into a newly synthesized heat-soluble, 21-kD protein band (arrow, right ordinate). B, Staining on protein (western) blots of a 21-kD protein band (arrow, right ordinate) using antiserum prepared against a conserved dehydrin oligopeptide sequence. The open and closed arrows perpendicular to the abscissa indicate the period during which desiccation tolerance developed.

imum percentage of axes was tolerant of desiccation by 25 DAA, dehydrin protein continued to accumulate in increasing amounts after that date. A second faint protein band of approximately 27 kD also appeared in samples 30 and 40 DAA (Fig. 5B).

ABA Content during Development of Rice and Wild Rice Seeds

ABA content of rice embryos increased markedly between 21 and 28 DAA and then declined between 28 and 32 DAA (Fig. 6A). About 50% of the embryos had already attained desiccation tolerance before the sharp increase in ABA content occurred (Figs. 2A and 6A). Calculations of the molar concentration of endogenous ABA were based on the water content of the axes during development. The calculated concentration of ABA ranged from 0.06 μM at 15 DAA to 0.92 μM at 28 DAA (Fig. 6B). The peak in ABA concentration occurred at 28 DAA, whereas dehydrin synthesis occurred as early as 15 DAA and persisted after the ABA concentration had decreased to near the initial one (Figs. 4A and 6A).

ABA accumulation in wild rice axes increased by about 3-fold between 14 and 18 DAA and then declined sharply between 18 and 20 DAA (Fig. 6A). The increase in ABA content preceded the onset of desiccation tolerance, and the percentage of desiccation-tolerant seeds increased as ABA content decreased (Figs. 3A and 6A). When converted into molar values, the pattern of ABA accumulation increased from a low of 0.08 μM at 11 DAA to a peak of 3.5 μM at 18 DAA, followed by a plateau of 1.4 to 1.8 μM (Fig. 6B). The maximum ABA content of 4.8 μg g^{-1} dry weight in wild rice was about twice that found in rice. Dehydrin protein was first detectable on western blots in appreciable amounts concomitantly with peak ABA content (Fig. 5B). However, dehydrin synthesis and accumulation continued after ABA content decreased (Fig. 5).

Suc and Oligosaccharide Accumulation during Development of Rice and Wild Rice Seeds

Data for Suc and oligosaccharide accumulation in rice embryos were pooled for the 1990 and 1991 harvests. Suc accumulation in developing rice embryos reached about 30 mg g^{-1} dry weight at 35 DAA (Fig. 7). Suc accumulation paralleled the increasing percentage of desiccation-tolerant excised embryos and intact seeds during development (Figs. 2A and 7). Total oligosaccharides never exceeded 4 mg g^{-1} dry weight (Fig. 7).

Suc content of wild rice axes was about twice that of rice embryos, reaching 69 mg g^{-1} dry weight at 36 DAA (Fig. 7). Suc continued to accumulate after the greatest number of wild rice axes were desiccation tolerant, and maximum Suc content coincided with maximum germination of dried intact wild rice seeds (Figs. 3A and 7). The sharp increase in Suc content did not begin until the axes had reached about 87% of their final dry weight (Figs. 3B and 7). Oligosaccharide contents were comparatively low, ranging from 2.5 mg g^{-1} dry weight at 18 DAA to 6.8 mg g^{-1} dry weight at maturity (Fig. 7). The identities of the oligosaccharides were not resolved, but raffinose is the major oligosaccharide present in whole wild rice grains (Becker and Lorenz, 1981).

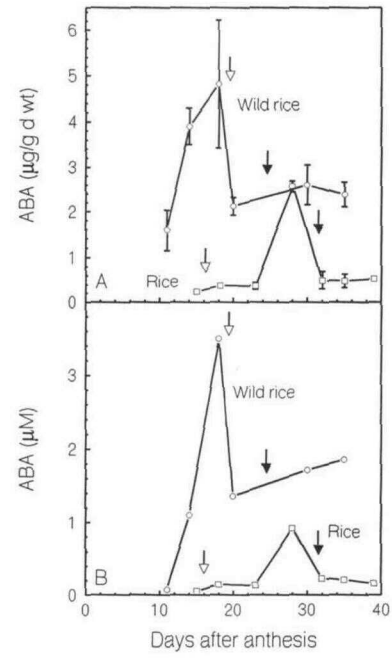

Figure 6. ABA content during development of rice and wild rice seeds expressed on a dry weight basis (A) or converted to molar concentrations based on tissue water content (B). The data represent the means of two or three replicates of 10 wild rice embryonic axes (○) or rice embryos (□). The open and closed arrows indicate the period during which desiccation tolerance developed. Error bars in A indicate ±SE.

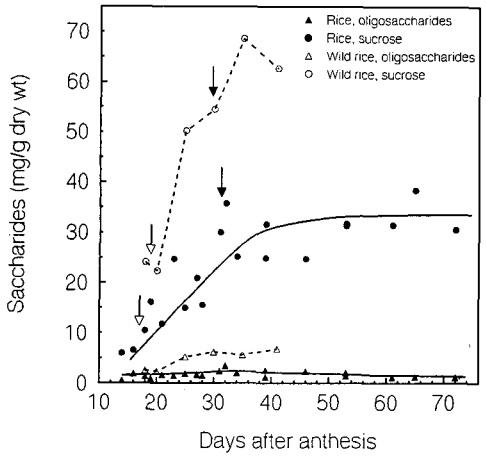

Figure 7. Suc (circles) and oligosaccharide (triangles) accumulation during development of rice embryos (solid symbols) and wild rice axes (open symbols). Data for rice were pooled from 1990 and 1991 harvests. The open and closed arrows indicate the period during which desiccation tolerance developed.

DISCUSSION

Seeds exhibit a range of tolerances to dehydration, from completely intolerant to survival of water contents as low as 2% (Roberts, 1973; Farrant et al., 1988; Ellis et al., 1989). Wild rice seeds, for example, survive desiccation to about 7% water content (Fig. 1) but only when dehydrated and rehydrated at 15 to 25°C and when rapid imbibition is avoided (Kovach and Bradford, 1992).

Because of their extreme hydrophilicity, predicted structure, and induction by dehydration and ABA, it has been suggested that dehydrins protect intracellular components against dehydration injury (Dure et al., 1989). In some seeds (e.g. cotton), group II LEA proteins (sharing amino acid sequences with dehydrin or RAB proteins) accumulate only late in embryogenesis after abscission of the ovule from the funiculus (Hughes and Galau, 1991). However, in rice (Fig. 4), wild rice (Fig. 5), and pea (Robertson and Chandler, 1992), synthesis of dehydrins begins early in embryogenesis, and the proteins accumulate in parallel with the increase in embryo dry weight. Dehydrin protein synthesis was detected before desiccation tolerance was attained by any wild rice axes (Fig. 5). Similarly, at 15 DAA in rice embryos, a small amount of dehydrin was present, and few embryos were tolerant of desiccation (Fig. 4). As more embryos became tolerant, more dehydrin was detected.

Desiccation tolerance, as measured in this study, is a discrete or quantal response, i.e. an embryo or axis was either tolerant and survived the dehydration regime or was intolerant and did not. Therefore, we cannot interpret the development of desiccation tolerance (Figs. 2 and 3) as a reflection of increasing tolerance of a given seed but only as an increase in the percentage of seeds in the population that was tolerant at a given time. Dehydrin proteins were present and accumulating when an increasing number of seeds were becoming desiccation tolerant (Figs. 4 and 5). The accumulation of dehydrin protein during this period could be due to the initiation of synthesis in an increasing number of embryos or axes, or to a quantitative increase in expression per embryo, or both. Distinguishing between these alternatives would require assaying on a single embryo/axis basis. The absence of maturation protein accumulation from highly recalcitrant seeds (Farrant et al., 1992) or ABA deficient/insensitive seeds (Meurs et al., 1992) suggests that a minimal amount of such protein is required for desiccation tolerance. However, maximum dehydrin synthesis in rice and wild rice embryos occurred after all seeds were tolerant of desiccation, indicating that the late accumulation of dehydrin proteins during embryogenesis was not required for survival of desiccation, but may serve other roles in seed quality or longevity (Galau et al., 1991; Ellis et al., 1993).

The significance of the accumulation of an additional dehydrin (38 kD) in rice and wild rice (27 kD) during embryogenesis is not known, but multiple bands have often been observed (Close et al., 1989, 1993a, 1993b; Robertson and Chandler, 1992). In several species, multiple genes have been identified that code for distinct dehydrin proteins (Close et al., 1993b). This possibility is supported by the observation of two mRNA transcripts (approximately 0.8 and 2.4 kb) from wild rice axes (data not shown) and seedlings (Bradford and Chandler, 1992) that hybridized with a *rab16a* cDNA probe (kindly provided by Dr. N.-H. Chua, Rockefeller University, New York) under low-stringency conditions. An additional possibility is that, in rice, the higher molecular mass band is a dimeric form of the protein. The native form of dehydrins in maize is thought to be dimeric, and structural evidence is consistent with the formation of dimers by hydrophobic interactions between conserved α-helical regions (Close et al., 1993b). At higher concentrations of dehydrin protein all dimers might not dissociate in the SDS-reducing buffer. It is also possible that the dehydrin proteins synthesized by wild rice axes are functionally impaired compared to those in rice embryos. Testing this hypothesis, however, awaits identification of dehydrin function in vivo.

ABA contents measured in these experiments are similar to those reported previously for wild rice grains (Albrecht et al., 1979). However, we found somewhat lower ABA contents in wild rice axes at maturity (2.2 $\mu g\ g^{-1}$ dry weight) than did Bradford and Chandler (1992) in axes after extended cold storage in water (7.2 $\mu g\ g^{-1}$ dry weight). The ABA contents of rice embryos in our study agree closely with that reported previously (Bradford and Chandler, 1992), but the maximum ABA content during development was 3.3 times greater than that found by Aldridge and Probert (1993). ABA has been implicated in the development of desiccation tolerance because peak contents of ABA during seed development often coincide with the attainment of desiccation tolerance, and the expression of many *LEA* genes is stimulated by ABA (Hughes and Galau, 1989; Bochicchio et al., 1991; Williams and Tsang, 1991). Although the timing of expression of LEA proteins is often coincident with high ABA content, not all *LEA* genes respond to ABA (Hughes and Galau, 1989, 1991), and other recalcitrant seeds have been shown to contain high amounts of ABA (Pence, 1991). An mRNA hybridizing to *rab16a* was detected at the earliest sampling time in both rice embryos and wild rice axes (data not shown), when ABA contents were still low (Fig. 6). High concentrations of ABA

were not necessary for the initiation or continued synthesis and accumulation of dehydrin protein or mRNA. There was no obvious correlation between ABA and the expression of dehydrin mRNA and protein during development. Sensitivity to ABA also changes as seeds mature (Walker-Simmons, 1987; Welbaum et al., 1990; Trewavas, 1991; Xu and Bewley, 1991); therefore, ABA content alone may not accurately reflect ABA action. Nonetheless, sensitivity to extreme desiccation in wild rice seeds was not due to an inability to accumulate ABA during development, because concentrations were more than twice those found in rice embryos (Fig. 6).

The percentage of desiccation-tolerant seeds increased as the average Suc content increased during maturation in both wild rice and rice. Suc accumulated in wild rice axes to about twice that found in mature rice embryos (Fig. 7). Yet, wild rice seeds were still sensitive to dehydration conditions and were unable to survive very low water contents (Fig. 1). Their sensitivity to desiccation must not be due to a lack of Suc or the oligosaccharides measured in our studies. We did not identify the specific oligosaccharides; therefore, it remains possible that a deficiency in a particular oligosaccharide contributes to wild rice desiccation sensitivity.

Despite the accumulation of dehydrin proteins, and more than twice the amount of ABA and Suc as in rice embryos, wild rice seeds are not tolerant of dehydration to moisture contents <7% and are sensitive to drying and imbibition conditions. The temperature dependencies of dehydration and imbibitional damage in wild rice seeds (Kovach and Bradford, 1992) suggest that membrane phase transitions are involved, as in imbibitional injury of dry pollen and yeast (Crowe et al., 1992). Additional studies with more extremely recalcitrant seeds would be useful in determining whether dehydrin proteins, ABA, and saccharide accumulation are essential components of desiccation tolerance (Farrant et al., 1993).

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