474

rnal of Experimental Botany, Vol. 43, No. 251, pp. 747–757, June 1992

Imbibitional Damage and Desiccation Tolerance of Wild Rice (*Zizania palustris*) Seeds

AVID A. KOVACH and KENT J. BRADFORD[1]

epartment of Vegetable Crops, University of California, Davis, CA 95616–8631, USA

eceived 12 September 1991; Accepted 20 December 1991

ABSTRACT

eeds (caryopses) of North American wild rice (*Zizania palustris* var. *interior*), a temperate aquatic grass, have been reported to e viability when desiccated (i.e. to be 'recalcitrant' or 'homoiohydrous'). They are also deeply dormant at maturity and require s much as six months of stratification to break dormancy. It has been suggested that wild rice seed may have been misclassified s recalcitrant in some experiments due to the presence of dormancy. We report here that wild rice seeds can survive desiccation s seed and embryonic axis moisture contents as low as 6–8% (fresh weight basis). However, maximum survival of desiccation to ese low moisture contents is possible only if dehydration occurs at temperatures ≥25 °C and a slow imbibition period (3 weeks inimum) is allowed at temperatures between 10 and 25 °C prior to stratification or dormancy-breaking treatments. The eduction in survival of dehydration at temperatures <25 °C appears to occur only when embryonic axis moisture contents are educed below about 8%. We also show that various techniques to assess viability of dry dormant seeds (moisture contents 30%), such as tetrazolium tests or scarifying the pericarp above the embryo, can cause a reproducible loss of viability due to mbibitional damage that has previously been interpreted as intolerance of desiccation. Most reports of the desiccation intolerance wild rice seeds can be explained on the basis of the temperatures of dehydration or rehydration, failure to break dormancy, or mbibitional injury during viability testing. The successful dehydration and rehydration of wild rice seeds has important practical mplications for seed storage and germplasm preservation. In addition, the temperature dependence of desiccation tolerance in ild rice seeds represents a novel relationship between seed viability, temperature, and moisture content.

ey words: wild rice, *Zizania palustris* var. *interior*, seed, germination, dormancy, moisture content, desiccation tolerance, ecalcitrant, temperature.

INTRODUCTION

North American wild rice (Poaceae, *Zizania palustris* var. *nterior*) is native to shallow lakes and streams of the orthern United States and southern Canada, and was a taple food of native Americans in that area (Aiken, Lee, Punter, and Stewart, 1988). Currently, wild rice is harested from native lake populations and is grown commerially in paddies in Minnesota and California. The paddies reseed naturally under Minnesota conditions, but, in California, planting seed must be stored over winter and replanted the following spring. Under natural conditions, the seeds (caryopses) abscise at a relatively high moisture content (MC≥ 30%, fresh weight basis) and fall into the water, sinking to the bottom and remaining dormant until the following spring. A number of early studies concluded that wild rice seeds must be stored submerged at near-freezing temperatures to maintain viability (Brown and Scofield, 1903; Duvel, 1906; Muenscher, 1936; Simpson, 1966). However, this conclusion is complicated by the presence of a deep dormancy at maturity that requires up to 6 months of hydrated cold storage (stratification) before all viable intact seeds will germinate (Atkins, Thomas, and Stewart, 1987; Cardwell, Oelke, and Elliot, 1978; Kovach and Bradford, 1992; Simpson, 1966). Total viability of unstratified or partially stratified seeds must be assessed by methods such as the tetrazolium test (Grabe, 1970) or by scraping or slitting the pericarp over the embryo (perhaps with the addition of gibberellin or fusicoccin) to break dormancy (Cardwell *et al.* 1978; Woods and Gutek, 1974; our unpublished results). It has been noted that in a number of studies of the desiccation tolerance of wild rice seeds, the dried seeds had not been stratified; failure to germinate after imbibition might, therefore, have been due to the maintenance of dormancy rather than to loss of viability during storage (Ellis, Hong, and Roberts,

[1] To whom correspondence should be addressed.

1985). Simpson (1966) also noted that the seed coat of wild rice is highly impermeable to water, requiring scarification or long periods of submersion to hydrate fully before germination is possible. Consequently, the germination test period in some studies may not have been extended long enough to assess viability accurately. The apparent 'recalcitrant' behaviour in storage (Roberts, 1973) might, therefore, be due to dormancy or delayed imbibition rather than to intolerance of desiccation. On the other hand, Probert and coworkers (Probert and Brierley, 1989; Probert and Longley, 1989) found that viability of both stratified and dormant seeds declined when their embryonic MC declined below 45%, with 50% of the embryos failing to survive dehydration to 30% MC (≈20% seed MC). Since they used techniques to break dormancy and overcome seed coat impermeability, they concluded that wild rice seeds are truly recalcitrant.

There are, however, some conflicting data on the desiccation tolerance of wild rice seeds. For example, Fyles (1920) reported that freshly harvested wild rice seeds could be stored in air at ambient temperatures for up to 25 d and still retain approximately 50% viability. Simpson (1966) found 77% viability in wild rice seeds air-dried at room temperature for 14 d, although viability was lost after 90 d under these conditions. Unfortunately, seed MC were not reported in these studies, so the extent of dehydration attained is unknown. Oelke and Stanwood (1988), however, found that wild rice seeds dried to 11·5% MC at 22 °C and then stored at −2 or 2 °C for 6 months were still highly viable after a further 6 months of stratification in cold water. A subsequent study (Oelke, McClellan, and Leif, 1990) found that ≈50% of wild rice seeds survived when dehydrated under ambient conditions to 9% MC, stored for up to 9 months at 3 °C, and imbibed and stratified at 3 °C for 3 months. Thus, survival of wild rice seeds to relatively low MC may occur under some conditions. It would be extremely valuable for germplasm preservation and breeding programmes and for storage of commercial seed for planting to determine whether conditions do exist to circumvent the apparent recalcitrance of wild rice seeds and preserve viability at low moisture contents.

The previous studies of wild rice seed dormancy and storage have established some general principles, but have also left a number of inconsistencies and gaps in our knowledge. There is evidence that the embryos are unable to survive desiccation to very low MC, at least under some conditions, but a moderate level of dehydration below full imbibition may be tolerated. The seeds do not germinate immediately upon maturation, as do many recalcitrant seeds, but rather have an extended dormancy period. In their native temperate habitat, the seeds survive near-freezing or subzero temperatures while overwintering, and in fact require long-term stratification to break dormancy. These properties taken together would classify wild rice seeds as being 'minimally recalcitrant' according to a recent scheme (Farrant, Pammenter, and Berjak, 1988). Wild rice may, therefore, provide a good model system in which to study the mechanisms of desiccation tolerance in plant tissues, since seed development in wild rice is similar to that of 'orthodox' (desiccation tolerant) grass seeds until the dehydration phase of development (LaRue and Avery, 1938). Most studies of seed desiccation tolerance have compared tolerant orthodox seeds to either highly recalcitrant seeds, which are quite different taxonomically, structurally, and physiologically, or to orthodox seeds that have germinated to the point where they have become desiccation intolerant. While these approaches are useful, understanding the limitations to survival of dehydration in wild rice seeds should provide clues to the minimal requirements for desiccation tolerance in the absence of major differences in structural or developmental patterns.

The objective of the current study was to investigate the desiccation tolerance of wild rice seeds in relation to moisture content and temperature. A wide range of conditions and approaches were tested in an attempt to reconcile the conflicting reports on recalcitrance of wild rice seed. We report here that wild rice seeds are tolerant of desiccation, but only under restricted dehydration and rehydration conditions. These results have significant implications for storage of wild rice seeds for agricultural or germplasm conservation purposes, as well as for our understanding of the basis of desiccation tolerance.

MATERIALS AND METHODS

Plant material and dehydration/rehydration conditions

For the first experiment on dehydration at 5 °C, wild rice (*Zizania palustris* var. *interior* cv. NC1) seeds (caryopses) grown in the field were collected directly from a mechanical harvester. The seeds were returned to the laboratory on ice, cleaned of debris using an air column cleaner, and placed on stainless steel screens in constant relative humidity (RH) chambers at 5 °C within 3 h after harvest. Relative humidities of approximately 93, 81, 78, 65, 58, 46, 37, 29, 17, and 12% were maintained using saturated salt solutions (Greenspan, 1976). Air was circulated continuously within the sealed 6 dm^3 chambers with small electric fans. Oxygen, CO_2, temperature, and RH within the chambers were measured weekly; O_2 and CO_2 were measured on a gas chromatograph with a dual thermistor detector, and temperature and RH were measured with a Vaisala (Helsinki, Finland) HMI 31 temperature/humidity probe. The levels of O_2 and CO_2 remained within 18 to 21% and 0 to 1·5%, respectively. Samples of seed were removed from the chambers weekly for the first month, then monthly thereafter, for MC determinations and viability tests.

A similar experiment was subsequently conducted with hand-harvested fresh seeds utilizing only four RH levels (12, 33, 54, and 75%), but dehydrating at 5, 10, 15, 20, 25, and 30 °C. Saturated salts within sealed chambers were used to control the RH, but the air was not circulated. Due to the large number of samples involved, whole-seed samples were collected periodically and stored sealed at −25 °C for 1 to 3 months before dissection to determine embryonic axis MC. After 1 to 4 months

of dehydration, depending upon the temperature and RH, the seeds from each temperature and RH condition were imbibed for 1 month in water at 5, 10, 15, 20, 25, and 30 °C. All seeds were then transferred to water at 2·5 °C for 5 months of stratification before germination was tested as described below. After 21 d in the germination tests, the pericarps of any ungerminated seeds were slit to break dormancy and the seeds were incubated a further 21 d. The total germination of both intact and slit seeds after 42 d incubation is reported; the maximum additional percentage germination after slitting was 3%.

Germination and viability tests

Seed viability was determined by tetrazolium (TZ) tests by cutting 50 seeds longitudinally through the embryo, soaking in 0·15% (w/v) 2,3,5-triphenyltetrazolium chloride solution for 20 to 24 h at 20 °C in the dark, and scoring according to intensity and location of staining using criteria similar to those for other grains (Grabe, 1970). Germination tests were conducted for 21 d with seeds submerged under 3·0 cm of deionized water at 20 °C under fluorescent light. Seedlings were scored as germinated when they had a normal green shoot and seminal roots had emerged. Seeds showing protrusion of the epiblast and swelling of the coleoptile and mesocotyl (Aiken *et al.*, 1988) were not scored as germinated unless further growth occurred. The relationship between seed MC and viability during dehydration at 5 °C was analysed by the PROC PROBIT procedure of the SAS statistical package (SAS Institute, Inc., Cary, North Carolina). The initial response rate (total per cent viable seeds) was set at 90% (i.e. OPTC = 0·1), equal to the initial viability of the freshly harvested seed. Means for the dehydration/rehydration temperature experiment are based on three replicates of a minimum of 100 seeds per replicate. The arcsin-transformed germination percentages in the dehydration/rehydration temperature experiment were analysed by analysis of variance as a factorial design of dehydration RH, dehydration temperature, and rehydration temperature.

Moisture content determinations

Whole seed (including the lemma and palea) MC were determined by oven drying at 130 °C for 6 h. This method was found in comparison tests to agree with the 2-step method, where ground seed is oven-dried at 130 °C for 1 h (International Seed Testing Association, 1985). Excised embryonic axis MC were determined in some experiments by oven drying at 130 °C for 1 h. Moisture contents were also determined with a coulometric Karl Fischer titrator (CKFT) using a Mitsubishi model CA-06 automatic titrator and a model VA-06 vaporizer (Cosa Instruments, Norwood, New Jersey, US). A single embryonic axis or seed segment (consisting of that portion of the seed between 5 to 8 mm from the radicle end, and containing both embryonic and endospermic tissue) was weighed on a Cahn microbalance, heated at 155 °C in a stream of dry nitrogen in the vaporizer, and the evaporated water was quantitatively titrated in the Karl Fischer reaction. Segments rather than intact seeds were used to prevent overloading the titrator. Means for embryonic axes and intact seed segments measured by CKFT are based on five replicate axes or segments. No significant differences were found between MC determinations by the oven method and the CKFT method. Unless otherwise indicated, MC values are on a fresh weight basis.

RESULTS

Viability versus MC: dehydration at 5 °C

The consensus from previous literature indicated that near-freezing temperatures were necessary to preserve wild rice seed viability. Accordingly, wild rice seeds harvested at 33% MC were either placed in water or in constant RH chambers at 5 °C (Fig. 1A). Widely differing rates of dehydration were achieved, with up to 16 weeks being required to achieve equilibrium MC at the lower humidities (no further change in MC occurred up to 24 weeks of storage; data not shown). As the seeds were dormant, viability was assessed by the TZ test at intervals during storage (Fig. 1B). Loss of viability occurred within 4 weeks at RH < 79%, and even at 93% RH most seeds had apparently died after 16 weeks of storage (MC = 25%).

The relationship between seed viability and MC was constructed using all individual data points from Fig. 1. Regardless of the rate of dehydration, seed viability as a function of MC was described by a normal distribution with a mean of 20·9% and a standard deviation of 5·1% MC (Fig. 2). According to probit analysis, a 10% loss in viability from the initial level would be expected at a seed MC of 27·4%. The relationship in Fig. 2 is confounded somewhat by the duration of storage after achieving a given MC, as viability was gradually lost at constant MC with extended time (e.g. 93% RH in Fig. 1). Thus, the apparent critical MC for long-term survival under these conditions according to the TZ test is probably slightly above the values predicted from the probit curve of Fig. 2.

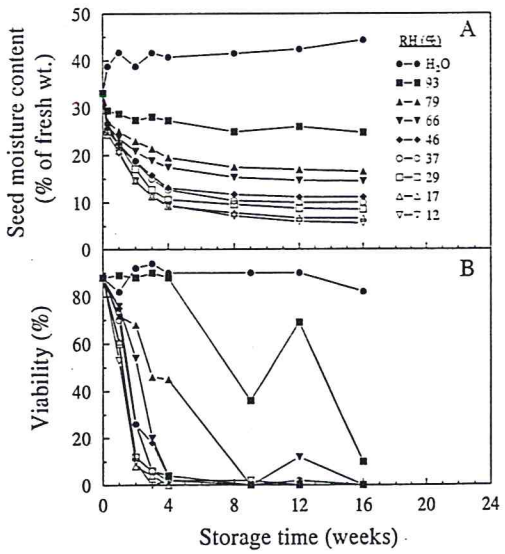


FIG. 1. (A) Dehydration time-courses of intact wild rice seeds maintained at 5 °C at the indicated relative humidities (RH). The seeds for the curve designated H_2O were submerged in water continuously. Whole seed MC was determined by the oven method. (B) Viability (TZ test) of wild rice seeds during dehydration at a range of RH, corresponding to the curves in panel (A).

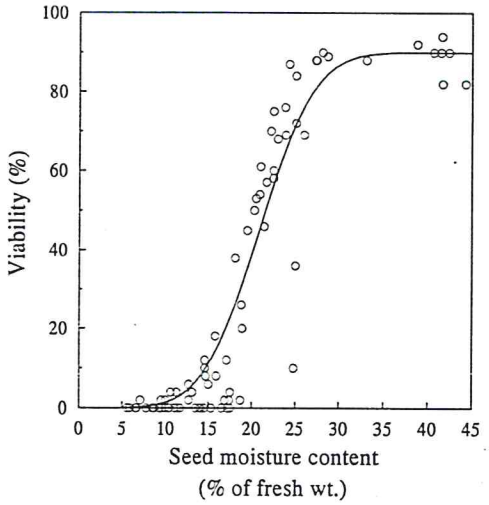


FIG. 2. The relationship between wild rice seed MC and viability based on the TZ test. The symbols indicate the MC and viability values determined at each sampling time for all RH levels in Fig. 1. The solid curve is the normal distribution predicted by probit analysis of probit (% viability) = 0·198 (seed MC − 20·9). Since probit (50%) = 0, and the inverse of the slope of the probit line is the standard deviation, the model predicts that the MC reducing viability by 50% would be 20·9%, with a standard deviation of 5·1%.

Viability versus MC: dehydration at ambient temperatures

During the course of the experiments described above, wild rice seeds were also collected and simply stored in paper bags under ambient laboratory conditions. Based on the results for dehydration at 5 °C followed by TZ testing (Fig. 2), these seeds were expected to be non-viable. However, some survival was detected in these lots and viability appeared to increase as the imbibition duration prior to TZ testing was increased (Table 1). For example, seed stored under ambient laboratory conditions for 106 d (8% MC) scored only 13% viability after 8 d of imbibition, but the same lot had 60% viability when tested after an additional 21 d of imbibition. After 126 d of storage, some staining was evident in the TZ test after 1 d of imbibition, but none of the seeds were scored as viable due to cracking of the embryos. However, 54% of the seeds were scored as viable after a 34 d imbibition period. This lot still had 59% viability after 154 d of storage when allowed to imbibe for 27 d before TZ testing, but no seeds survived to 270 d under ambient conditions (Table 1). That the whole seed MC values accurately reflect embryo dehydration is illustrated by the measured embryonic MC of 6% for the 154 d sample. The reliability of the TZ test after extended imbibition was confirmed in another test (124 d ambient storage) which had 26% survival according to the TZ test after dehydration to 8·8% MC and 22% germination after the pericarp was removed over the embryo to break dormancy (Table 1).

Imbibition time-course

These rather serendipitous results were intriguing and in contrast to our results (Figs 1, 2) and those of Probert and coworkers (Probert and Brierley, 1989; Probert and Longley, 1989) with desiccation at low temperatures (≤ 15 °C). Since the TZ test results were influenced by the duration of imbibition, the time-course of imbibition of dry wild rice seeds was determined. Dry intact seeds were imbibed and the MC of the whole seeds and of the excised embryonic axes were determined at intervals. In agreement with Simpson's (1966) observations, the wild rice pericarp has a low permeability to water, as a period of 3 weeks was required to achieve full imbibition at 20 °C (Fig. 3). This high resistance to water uptake is consistent with the long period required to achieve MC equilibrium during dehydration as well (Fig. 1A). Whole seed MC (lemma and palea intact) has been used in Figs

TABLE 1. *Viability of wild rice seeds after storage under ambient or low humidity conditions*

Field-grown wild rice seeds were harvested by hand (MC = 35%) and stored in a paper bag under ambient laboratory conditions (approximately 23 °C and 40 to 50% RH) for up to 270 d. Viability was then tested by the TZ test after varying periods of imbibition. Seeds stored for 124 d, but imbibed at a higher temperature, were also tested by TZ and by germination after removing the pericarp over the embryo. Moisture contents were determined on the whole seed except where indicated.

Duration of storage (d)	MC before imbibition (% fresh wt. ± s.e.)	Duration of imbibition (d)	Temperature of imbibition (°C)	Number of replicates and seeds/rep	Viability (% ± s.e.)
106	8·1 ± 0·3	8	20	3 (20)	13 ± 4
		29	20	3 (10)	60 ± 17
126	8·8 ± 0·1	1	20	3 (50)	0 ± 0
		34	20	3 (50)	54 ± 1
154	6·3 ± 0·2[a]	27	20	3 (30)	59 ± 7
270	7·1 ± 0·5[a]	26	20	3 (50)	0 ± 0
124	8·8 ± 0·1	26	25	3 (30)	26 ± 10
		25	25	3 (30)	22 ± 6[b]

[a] Embryo MC.
[b] Viability determined by germination test after removal of pericarp over embryo.

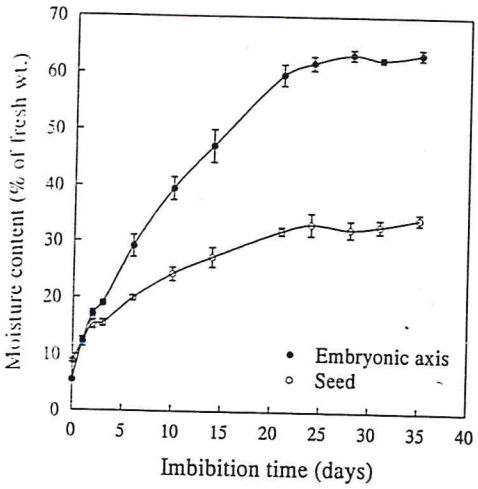


FIG. 3. Imbibition kinetics of intact wild rice seeds and embryonic axes. Wild rice seeds that had been stored under ambient conditions at room temperature for 3·5 months were submerged in water at 20 °C and the MC of both embryonic axes and whole seed segments were determined periodically using the CKFT method. Means are based on five replications of individual embryonic axes or whole seed segments. Error bars indicate ±s.e.

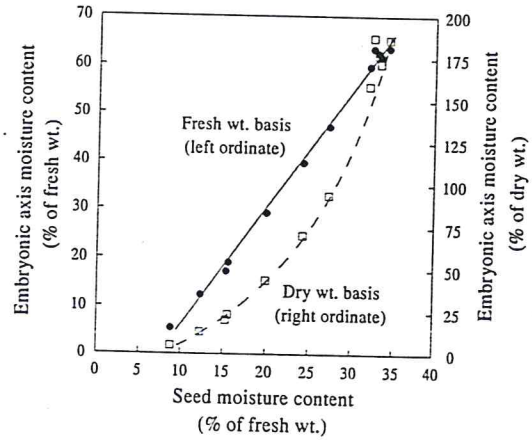


FIG. 4. The relationship between whole seed MC (fresh weight basis) and embryonic axis MC on a fresh weight basis (solid symbols, left ordinate) or dry weight basis (open symbols, right ordinate), derived from the imbibition time-course shown in Fig. 3. The linear regression equation for the embryonic axis MC (fresh weight basis) as a function of seed MC is MC_{embryo} (fresh weight basis) $= -17{\cdot}9 + 2{\cdot}42(MC_{seed})$, $r^2 = 0{\cdot}996$ ($P < 0{\cdot}001$). The dashed curve for the embryonic axis MC (dry weight basis) as a function of seed MC is derived by converting the MC values predicted by this regression equation on a fresh weight basis to a dry weight basis according to MC_{embryo} (dry weight basis) $= [-17{\cdot}9 + 2{\cdot}42(MC_{seed})]/[1 - (-0{\cdot}179 + 0{\cdot}0242(MC_{seed}))]$.

1 and 2 because it is easily monitored and would be the measurement used in a practical seed storage programme. However, since it is the embryonic MC that would be critical for survival, conversion of these whole seed MC values to embryonic axis moisture contents on both fresh and dry weight bases would facilitate comparison with other data concerning the desiccation tolerance of wild rice seed. Assuming that the distribution of water within the seed is the same during desiccation as during slow imbibition, the relationship between whole seed MC and embryonic axis MC was determined from the data of Fig. 3. Embryonic axis MC (fresh weight basis) was linearly related to whole seed MC (fresh weight basis), but the embryonic axis MC was greater than that of the whole seed over most of the MC range (Figs 3, 4). Embryonic axis MC on a dry weight basis is also shown as a function of whole seed MC (Fig. 4). Figure 4 can be used to convert whole seed MC on a fresh weight basis to the corresponding embryonic axis MC on either a fresh weight or dry weight basis.

Viability versus MC: effect of dehydration and rehydration temperatures

The increase in viability with extended imbibition time (Table 1) and the very slow imbibition rate of intact seeds (Fig. 3) suggested the possibility that for seeds dried to low MC, the rapid imbibition of split seeds or of seeds with slit pericarps (Probert and Longley, 1989) might cause injury during the TZ or germination tests. This could explain the poor TZ viability of seeds dried to low MC at 5 °C (Figs 1, 2), as the split seeds were imbibed directly in the TZ test solution. On the other hand, it is also well known that rapid imbibitional injury to plant tissues is more severe at low temperatures (Crowe, Hoekstra, and Crowe, 1989; Wolk, Dillon, Copeland, and Dilley, 1989). If this is also the case for desiccation injury, another explanation for the different results in Fig. 1 versus Table 1 could be that dehydration occurred at 5 °C in the former, but at ambient temperatures in the latter. We have recently shown that low temperatures are not required to maintain viability of hydrated wild rice seeds, but only to break dormancy (Kovach and Bradford, 1992). We therefore tested whether the dehydration and rehydration temperatures would influence survival of wild rice seed. Freshly harvested wild rice seeds (embryonic axis MC = 49%) were dehydrated under four RH conditions at six temperatures from 5 to 30 °C (Fig. 5). After dehydration, the seeds from each temperature and RH condition were then imbibed for 1 month at each of the six temperatures. Following the imbibition period, all seeds were transferred to water at 2·5 °C for 5 months to break dormancy, then tested for germination and viability at 20 °C.

Both the rate of moisture loss and the final embryonic axis MC were influenced by the RH and temperature of dehydration (Fig. 6). At 12 and 33% RH, seeds achieved near-equilibrium MC at all temperatures within the

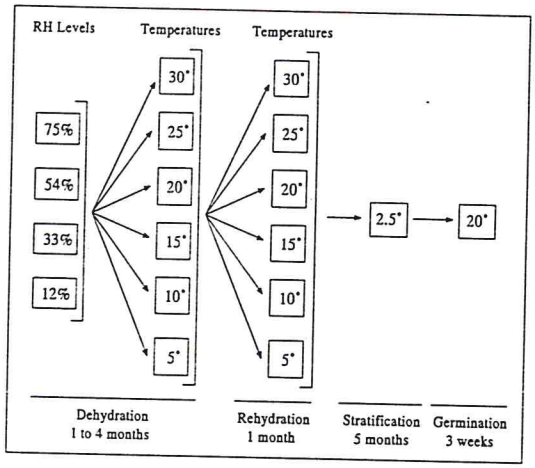


FIG. 5. Diagram of an experiment to test the effects of dehydration and rehydration temperatures on viability of wild rice seeds. Seeds were dehydrated in each of four RH levels at each of six temperatures for 1–4 months. Seeds from each of those conditions were then rehydrated at each of the six temperatures for one month. All seeds were then stratified for 5 months at 2·5 °C, then tested for germination at 20 °C under fluorescent light.

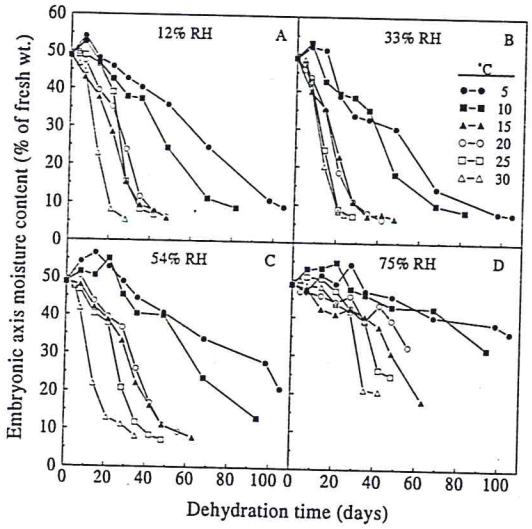


FIG. 6. Dehydration time-courses of wild rice embryonic axes in 12 (A), 33 (B), 54 (C), or 75% (D) RH. The temperatures of dehydration are indicated by the symbols. Seeds were dehydrated intact, and embryonic axes were excised and their MC determined by CKFT on five replicate axes per sample. Error bars are omitted for clarity, but are of a relative magnitude similar to those shown for embryonic axis MC in Fig. 3.

experimental period (Fig. 6A, B). At 54% RH, seeds at 5 and 10 °C did not reach equilibrium after 105 and 97 d, respectively (Fig. 6C). At 75% RH, moisture loss was slow and MC equilibrium was not achieved at any temperature (Fig. 6D). The rates of moisture loss (negative slopes of the dehydration curves) increased linearly with temperature under all RH conditions. The final points shown for each dehydration curve in Fig. 6 are the times and embryonic axis MC at which the seeds were transferred to the imbibition conditions.

Survival of wild rice seeds was sensitive to RH, dehydration temperature, and rehydration temperature (Fig. 7). Analysis of the arcsin-transformed percentages indicated highly significant main effects and interactions among RH, dehydration temperature, and rehydration temperature ($F=2{\cdot}06$, df $=75$, $P<0{\cdot}0001$ for the three-way interaction). At 12 and 33% RH, viability was progressively reduced as the dehydration temperature was reduced (Fig. 7A, B). Survival was $>70\%$ when seeds were dehydrated at 25 or 30 °C and imbibed between 10 and 25 °C, even though embryonic axis MC had reached 6–8%. Dehydration at 15 or 20 °C reduced viability by about half, and less than 20% of the seeds survived dehydration at 5 or 10 °C (Fig. 7A, B). In contrast, dehydration temperature did not significantly influence survival of seeds dried in 54 or 75% RH (Fig. 7C, D). In addition to the interaction between RH and dehydration temperature, rehydration temperature also affected survival. At all RH levels except 75%, survival was reduced when imbibition occurred at either 5 or 30 °C (Fig. 7). It is likely that seeds dehydrated in 75% RH, and seeds dehydrated at 5 °C in 54% RH, did not reach MC low enough to experience imbibitional damage at the low and high temperatures (Fig. 6C, D).

These results confirm the preliminary findings in Table 1

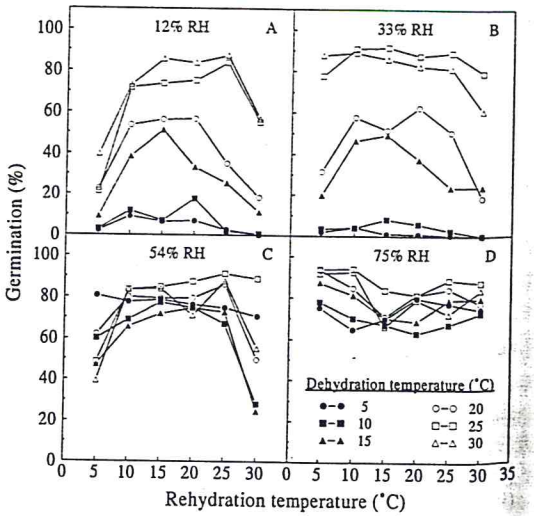
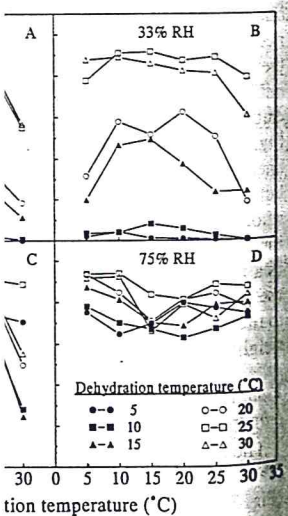


FIG. 7. Viability of wild rice seeds after dehydration in 12 (A), 33 (B), 54 (C), or 75% (D) RH at a range of temperatures (symbols), followed by rehydration at different temperatures (shown on the abscissa).

rates of moisture loss (negative
curves) increased linearly with
H conditions. The final points
on curve in Fig. 6 are the times
at which the seeds were trans-
onditions.
ls was sensitive to RH, dehydra-
ıydration temperature (Fig. 7).
ısformed percentages indicated
ffects and interactions among
ıture, and rehydration temper-
< 0·0001 for the three-way inter-
RH, viability was progressively
ion temperature was reduced
> 70% when seeds were dehyd-
imbibed between 10 and 25 °C,
axis MC had reached 6–8%.
°C reduced viability by about
the seeds survived dehydration
n contrast, dehydration temper-
influence survival of seeds dried
C, D). In addition to the inter-
dehydration temperature, rehy-
affected survival. At all RH
ıl was reduced when imbibition
30 °C (Fig. 7). It is likely that
RH, and seeds dehydrated at
ıot reach MC low enough to
damage at the low and high

e preliminary findings in Table 1



that wild rice seeds can survive desiccation to very low MC if dehydration and rehydration occur at warm temperatures. Even the seeds dehydrated at 75% RH, which did not show temperature sensitivity of dehydration or rehydration, achieved minimum embryonic axis MC (20–35%; Fig. 6D) below those that were apparently lethal when the TZ test was used (below ≈40% MC; Figs 2, 4). It seems likely that slow imbibition, along with the stratification period to break dormancy, can account for this discrepancy. This was examined in another experiment (Table 2) where samples of the seeds that had been dehydrated in 12 or 33% RH at 25, 15 or 5 °C and stored at −20 °C for 8 to 10 months were tested for viability according to several protocols, including the TZ test, slitting the pericarp above the embryo and incubating in 50 μM GA_{4+7} at 10/25 °C alternating temperatures (i.e. the protocol of Probert and Longley, 1989), and scraping the pericarp from above the embryo and incubating in 1·0 μM fusicoccin (FC) at 20 °C (a protocol we have found to be effective in breaking dormancy). These tests were conducted on the dry seeds and also after the seeds had been imbibed at 20 °C for 21 to 24 d. Viability was low in these seed lots when the tests were conducted directly on dry seeds (Table 2). After extended pre-imbibition, the TZ test indicated 10, 49, and 97% viability in the seeds dehydrated at 5, 15, and 25 °C, respectively, in good agreement with the results of Fig. 7A, B and with the total germination percentages in the scraped + FC treatments (Table 2). However, only about 35% of the seeds that initially germinated in the scraped + FC treatments subsequently developed into normal seedlings with good chlorophyll development and continued growth. This apparent loss of vigour could be due to the extended storage period at −20 °C before the test was conducted, or to the presence of deep dormancy that requires some chilling treatment for vigorous seedling growth, despite scarification and hormonal treatments (E.A. Oelke, personal communication; our unpublished results). These possibilities are currently under investigation. The method of Probert and Longley (1989) was apparently only partially effective in breaking dormancy of pre-imbibed seeds, as maximum germination was only 39% and variability was high even after extended imbibition (Table 2). These data emphasize that dormancy-breaking treatments or TZ tests resulting in rapid imbibition of dry wild rice seeds do not accurately reflect their true viability when they are allowed to imbibe slowly at moderate temperatures and undergo cold stratification. This experiment also illustrates that dry wild rice seeds survive freezing temperatures that are lethal to the hydrated seeds (Kovach and Bradford, 1992), and that dormancy is not broken under these conditions.

DISCUSSION

Our initial results on desiccation tolerance of wild rice seeds, based upon dehydration at 5 °C followed by TZ tests on dry seeds, were in good agreement with those of Probert and coworkers (Probert and Brierley, 1989; Probert and Longley, 1989). Converting our values for seed MC (Fig. 2) to embryonic axis MC (Fig. 4), 50% loss of viability corresponded to 32% MC, compared to a value of 29% from Probert and Longley (1989). Our subsequent experiments (Fig. 7; Tables 1, 2), however, make it clear that while this relationship between viability and MC is consistent and reproducible, it is not due solely to an intolerance of desiccation, but rather is dependent upon the particular dehydration and rehydration conditions employed. In our own data (Figs 1, 2), the rapid imbibition of dry split seeds in the TZ test can account for the loss of viability (Table 2). We believe that the viability curve of Fig. 2 actually represents the MC dependence of imbibitional damage, not the MC depend-

TABLE 2. *Effect of the pre-imbibition period on viability of wild rice seeds determined by the tetrazolium test or dormancy-breaking treatments*

Wild rice seeds dehydrated in 12 or 33% RH at 5, 15, and 25 °C to the indicated embryonic axis MC (fresh weight basis) were stored at −20 °C for 8 to 10 months. They were then tested for viability, without pre-imbibition, by the TZ test, by slitting the pericarp over the embryo and incubating in 50 μM GA_{4+7} at 10/25 °C alternating temperatures (slit + GA), or by scraping the pericarp from above the embryo and incubating in 1·0 μM fusicoccin at 20 °C (scraped + FC). Additional seeds from the same lots were also pre-imbibed in water for 21–24 d prior to conducting the same tests. Germination tests were extended for 21 d in all cases. Intact seeds imbibed in water did not germinate after up to 42 d of imbibition due to dormancy.

Dehydration temperature (°C)	Initial MC (%)	Imbibition period (d)	Viability test		
			TZ (% ± s.e.)	Slit + GA (% ± s.e.)	Scraped + FC (% ± s.e.)
				Total (Normal)[a]	
5	7·7	0	0 ± 0	9 ± 3 (2 ± 2)	0 ± 0 (0 ± 0)
		21	10 ± 4	7 ± 4 (1 ± 1)	2 ± 1 (0 ± 0)
15	6·0	0	0 ± 0	14 ± 7 (5 ± 4)	0 ± 0 (0 ± 0)
		21	48 ± 6	35 ± 4 (6 ± 3)	49 ± 6 (18 ± 1)
25	7·2	0	0 ± 0	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)
		24	97 ± 0	39 ± 18 (34 ± 16)	89 ± 5 (31 ± 16)

[a] Total germination percentages are indicated along with normal seedling percentages (in parentheses).

ence of desiccation injury. In some of their experiments, Probert and Brierley (1989) also slit the pericarp to cause more rapid desiccation, and the pericarp was apparently slit or removed from above the embryo prior to germination tests in the studies of Probert and Longley (1989). These treatments would also result in rapid imbibition during the germination tests. In their TZ tests, Probert and coworkers (Probert and Brierley, 1989; Probert and Longley, 1989) pre-imbibed the seeds for 1 or 2 d before splitting the seeds longitudinally and incubating in the tetrazolium solution. Our imbibition time-course (Fig. 3) and direct tests (Table 1) indicate that this might be insufficient time for the embryo to hydrate sufficiently to avoid imbibitional damage. Cereal grains are often pre-imbibed for a day prior to the TZ test and would be fully imbibed at that time, but this practice is 'necessary only to facilitate the removal of the embryo' (Lakon, 1949) or to prevent cracking of the dry embryo. There is no indication in the literature that other cereal grains would suffer imbibitional damage under the conditions used in the TZ test (Grabe, 1970) or if the pericarp were slit, especially at the relatively high initial MC where injury is seen in wild rice (Fig. 2). Wild rice embryos appear to be particularly sensitive to rapid imbibition and we have as yet had little success using alternatives such as prehydration of scraped seeds in high RH or short pre-imbibition periods prior to testing. We consistently find that highest viability and vigour of dried wild rice seeds is attained by allowing intact seeds to imbibe slowly for 21–24 d at moderate temperatures before slitting or scraping the pericarp to break dormancy. However, others (C. Aldridge and R. Probert, personal communication) have had success using 1 to 3 d pre-imbibition periods prior to the TZ test. Further work is needed to determine the critical MC at which imbibitional damage occurs in wild rice seeds and to develop more convenient alternatives to the extended imbibition protocol.

Even without imbibitional damage, dehydration at 5 °C to MC below about 8% would be sufficient to kill 90% of the seeds (Fig. 7). In the study of Probert and Brierley (1989), seeds did not survive dehydration at 15 °C in 15% RH and rehydration and stratification at 2 °C. According to our results, this combination of treatments would result in $<10\%$ germination (Fig. 7A). However, when wild rice seeds are dehydrated at warm temperatures and allowed to rehydrate slowly at temperatures between 10 and 25 °C before cold stratification to break dormancy, essentially complete survival of desiccation to MC as low as 6–8% is possible (Fig. 7). This conclusion is in agreement with the results of Oelke and coworkers (Oelke *et al.*, 1990; Oelke and Stanwood, 1988), and has recently been confirmed by C. Aldridge and R. Probert (personal communication). Thus, if dehydration occurs at warm temperatures and imbibitional damage is avoided, wild rice seeds are tolerant of desiccation.

Realizing now the specific requirements for survival of desiccation by wild rice seeds, we can re-examine the studies over the past 90 years that had led to the belief that wild rice seeds are recalcitrant. Brown and Scofield (1903) reported no experiments on drying of wild rice seed, but stated that 'practically all attempts to germinate thoroughly dried seed have proved unsuccessful.' It is implied, however, that most wild rice seeds available at that time may have already been processed for consumption, which involves heating and parching that would undoubtedly be lethal. They also reported that successful overwintering of seed can be achieved by storing the fresh, dormant seeds in cold water, which would break dormancy. Duvel (1906) noted that 'it is now very generally known that the seed of wild rice, if once allowed to become dry, will not germinate, save possibly an occasional grain.' However, he did not report any experiments on this point. Muenscher (1936) compared seed stored in water with seeds stored dry at either 1–3 °C or at ambient laboratory temperatures, but did not stratify the seeds after dry storage. This would agree with our observations that dormancy is not lost during dry storage (e.g., intact seeds in the experiments of Tables 1 and 2 did not germinate during extended imbibition). It is likely that lack of appreciation of the requirement for stratification led to misinterpretation of many instances of failure of dried seeds to germinate, since the dried seeds would require a further hydrated cold period in order to break dormancy, as has been noted by Ellis *et al.* (1985). Even if dormancy was not present, the very slow imbibition of dried wild rice seeds (Fig. 3) would require extended germination tests, as is the case for *Citrus limon* L. seeds (King and Roberts, 1980). In some studies, the possibility of imbibitional damage cannot be excluded. Simpson (1966) dried seeds for either 14 or 90 d at room temperature and found 33 and 8% germination, respectively, for intact seeds. Pricking the seed coat to speed imbibition and break dormancy resulted in 77 and 0% germination for the same seeds. The seeds apparently survived 2 weeks of dehydration well but were partially dormant. According to our dehydration curves (Fig. 6), 14 d under ambient conditions might not have been sufficient to reduce embryo MC to a level where imbibitional damage would occur in the pricked seeds, whereas 90 d would be. This experiment was interpreted as evidence for intolerance of desiccation, but may be confounded by imbibitional damage due to the method used to break dormancy.

Some studies have found that wild rice seeds survived dehydration for varying periods. These studies have all allowed an extended imbibition and stratification period prior to germination tests. Fyles (1920) reported several experiments where wild rice seeds were allowed to dry for up to several weeks before storing submerged in a lake through the winter. Although no MC values after drying are reported, up to 100% germination was

observed under these conditions. In addition, she found that seeds that had dried naturally on the plant to the point of shattering were 100% viable after stratification. These results agree with our unpublished observations on seed development, where viability was unaffected by drying during maturation to MC below 10%, if an extended warm imbibition period and stratification followed. Oelke *et al.* (1990) dried wild rice seeds under ambient conditions (21–24 °C, 40% RH) for up to 12 d to seed MC as low as 9%. The dry seeds were then stored at −2 or +3 °C for up to 21 months, transferred to water at 3 °C for 3 months stratification, then germinated. Although viability began to decline after 12 months, it remained at about 50% for up to 9 months of dry storage at 9% MC. This agrees reasonably well with our data for seeds dehydrated to similar MC at 20 °C and rehydrated at 5 °C, which had 61% viability (Fig. 7C). We have also confirmed the desiccation tolerance of wild rice embryos by excising hydrated embryonic axes, dehydrating them in low RH, rehydrating in 100% humidity, and germinating on minimal agar medium. Excised axes can survive dehydration to MC as low as 5% and subsequently develop into normal seedlings according to this protocol (our unpublished results).

Although our results demonstrate clearly that under the proper dehydration, rehydration, and stratification conditions wild rice seeds are not 'recalcitrant', neither are they 'orthodox.' 'Recalcitrant' seeds actually exhibit a wide range of tolerance to water loss among species (Farrant *et al.*, 1988) and some seeds exhibit intermediate categories of seed storage behaviour with some characteristics of both orthodox and recalcitrant seeds (Ellis, Hong, and Roberts, 1990). Even when imbibitional damage was avoided, survival of wild rice seeds dried to <8% MC was dependent upon the temperature of dehydration (Fig. 7A, B). In the present experiment, the temperature of dehydration is confounded with the rate of dehydration (Fig. 6). Thus, it is possible that it is actually the extended period of dehydration rather than the temperature that is damaging. Berjak, Pammenter, and coworkers (Berjak, Farrant, Mycock, and Pammenter, 1990; Pammenter, Vertucci, and Berjak, 1991) have shown that embryonic axes of some recalcitrant seeds survive rapid dehydration to MC that are lethal if the embryos are allowed to dry slowly within the intact seed. We do not believe that this is the case with wild rice, as the rates of dehydration at 5 and 10 °C were similar in 12, 33, and 54% RH (Fig. 6), but the seeds in 54% RH survived well while those in 12 and 33% RH did not (Fig. 7). Our additional unpublished results indicate that survival may actually be improved by slower, rather than more rapid dehydration at optimal temperatures. Instead, it appears that dehydration temperature was critical only when seed MC fell below 8–10%. This situation would be analogous to that for imbibitional damage, where rapid imbibition of seeds or pollen from low MC at low temperatures is more damaging than imbibition at warm temperatures or from higher MC (Crowe *et al.*, 1989; Wolk *et al.*, 1989). This interaction between temperature and MC during imbibition has recently been explained on the basis of the dependence of the liquid crystalline to gel phase transition temperature of membrane lipids upon their level of hydration (Crowe *et al.* 1989). Imbibition at low temperatures and MC causes the membrane lipids to pass through a phase transition during water uptake, while at warmer temperatures or higher MC the phase transition has occurred before rapid water uptake begins. This mechanism likely explains the poor survival of wild rice seeds rehydrated at 5 °C (Fig. 7). Our observations of intolerance of wild rice seeds to desiccation below 8–10% MC at low temperatures, but tolerance at warm temperatures, would be consistent with a similar mechanism operating during dehydration as well. Specific tests of this hypothesis are in progress. The reason for poor survival of rehydration at 30 °C (Fig. 7A, B, C) is less obvious, but could be related to accelerated ageing of the seeds during the initial stages of imbibition.

Tolerance of wild rice seeds to desiccation, at least when dehydrated at warm temperatures, is consistent with the finding that wild rice seeds and embryos contain 'dehydrin-like' proteins at maturity and are capable of synthesizing them in response to dehydration (Bradford and Chandler, 1992). Such proteins are highly conserved across species, are induced by water loss or abscisic acid and their presence has been correlated with desiccation tolerance of seeds (Blackman, Wettlaufer, Obendorf, and Leopold, 1991; Skriver and Mundy, 1990). Bradford and Chandler (1992) proposed that the presence of dehydrin-like proteins might be diagnostic for whether seeds of a given species are actually intolerant of desiccation, or whether conditions might be found for their successful desiccation and rehydration. In the case of wild rice, the seeds do accumulate dehydrin-like proteins during development and we were able to identify conditions for the maintenance of viability during dehydration and rehydration. Seeds lacking dehydrin-like proteins might be incapable of desiccation under any conditions. Further studies on truly recalcitrant species will be required to test whether the correlation between desiccation tolerance and presence of specific proteins is supported. However, even if dehydrin-like proteins are necessary for desiccation tolerance, they apparently are not sufficient, since wild rice seeds are intolerant of desiccation at low temperatures even though dehydrin-like proteins are already present and their synthesis is induced even at low temperatures in dehydrating excised embryonic axes (Bradford and Chandler, 1992). Blackman and coworkers (Blackman *et al.*, 1991) also concluded that the presence of heat-stable maturation proteins (which would include the dehydrin-like proteins) is not sufficient to prevent the loss

of desiccation tolerance during germination of soybean (*Glycine max* L.) seeds.

The temperature sensitivity of desiccation tolerance in wild rice seeds represents a unique relationship among seed MC, temperature, and viability. Experiments are in progress investigating the longevity of wild rice seeds under various MC and storage conditions to determine whether once dried, their storage behaviour conforms to the orthodox pattern. Dry wild rice seeds (12% MC) retained their initial viability after freezing in liquid nitrogen and extended imbibition at 20 °C (our unpublished results), so long-term storage of wild rice seeds in germplasm banks should be possible. Until non-dormant genotypes or large-scale methods of uniformly breaking dormancy are developed, a period of cold hydrated storage will still be required before wild rice seeds can be planted for agricultural purposes. However, the ability to dehydrate the seed, store it dry, and test it for quality before cold storage will greatly simplify handling and reduce the costs associated with seed production of this crop. In addition, seed lots of insufficient germination quality to use for planting seed can be diverted to the commodity market, which is not possible for seed after hydrated storage due to the development of unacceptable flavours. Understanding the requirements for maintaining viability and breaking dormancy of wild rice seed will open new practical opportunities for handling planting seeds and germplasm stocks. Further study of the mechanism of the low MC/temperature interaction causing damage to wild rice seeds should also provide new insights into the molecular requirements for desiccation tolerance.

ACKNOWLEDGEMENTS

Supported by United States Department of Agriculture Competitive Research Grants Office grant 89–37264–4797, by grants from the California Wild Rice Program and by Regional Research Project W-168.

LITERATURE CITED

AIKEN, S. G., LEE, P. F., PUNTER, D., and STEWART, J. M., 1988. *Wild rice in Canada.* NC Press, Ltd., Toronto.

ATKINS, T. A., THOMAS, A. G., and STEWART, J. M., 1987. The germination of wild rice seed in response to diurnally fluctuating temperatures and after-ripening period. *Aquatic Botany*, **29**, 245–59.

BERJAK, P., FARRANT, J. M., MYCOCK, D. J., and PAMMENTER, N. W., 1990. Recalcitrant (homoiohydrous) seeds: the enigma of their desiccation-sensitivity. *Seed Science and Technology*, **18**, 297–310.

BLACKMAN, S. A., WETTLAUFER, S. H., OBENDORF, R. L., and LEOPOLD, A. C., 1991. Maturation proteins associated with desiccation tolerance in soybean. *Plant Physiology*, **96**, 868–74.

BRADFORD, K. J., and CHANDLER, P. M., 1992. Expression of 'dehydrin-like' proteins in embryos and seedlings of *Zizania palustris* and *Oryza sativa* during dehydration. *Plant Physiology* (in press).

BROWN, E., and SCOFIELD, C. S., 1903. *Wild rice: its uses and propagation.* Bulletin No. 50, U.S. Department of Agriculture, Bureau of Plant Industry. Government Printing Office, Washington, D.C.

CARDWELL, V. B., OELKE, E. A., and ELLIOTT, W. A., 1978. Seed dormancy mechanisms in wild rice (*Zizania aquatica*). *Crop Science*, **70**, 481–4.

CROWE, J. H., HOEKSTRA, F. A., and CROWE, L. M., 1989. Membrane phase transitions are responsible for imbibitional damage in dry pollen. *Proceedings of the National Academy of Sciences, U.S.A.*, **86**, 520–3.

DUVEL, J. W. T., 1906. *The storage and germination of wild rice seed.* Bulletin No. 90, Miscellaneous Papers. U.S. Department of Agriculture, Washington, D.C. Pp. 5–14.

ELLIS, R. H., HONG, T. D., and ROBERTS, E. H., 1985. *Handbook of seed technology for genebanks. Vol. II. Compendium of specific germination information and test recommendations.* International Board for Plant Genetic Resources, Rome.

—— —— —— 1990. An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany*, **41**, 1167–74.

FARRANT, J. M., PAMMENTER, N. W., and BERJAK, P., 1988. Recalcitrance–a current assessment. *Seed Science and Technology*, **16**, 155–66.

FYLES, F., 1920. *Wild rice.* Bulletin No. 42. Department of Agriculture, Dominion of Canada, Ottawa.

GRABE, D. R., 1970. *Tetrazolium testing handbook for agricultural seeds.* Association of Official Seed Analysts, Springfield, Illinois, USA.

GREENSPAN, L., 1976. Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards*, **81A**. No. 1 Jan–Feb.

INTERNATIONAL SEED TESTING ASSOCIATION, 1985. International rules for seed testing. Rules 1985. *Seed Science and Technology*, **13**, 299–355.

KING, M. W., and ROBERTS, E. H., 1980. The desiccation response of seeds of *Citrus limon* L. *Annals of Botany*, **45**, 489–92.

KOVACH, D. A., and BRADFORD, K. J., 1992. Temperature dependence of viability and dormancy of *Zizania palustris* seeds stored at high moisture contents. *Annals of Botany*, (in press).

LAKON, G., 1949. The topographical tetrazolium method for determining the germinating capacity of seeds. *Plant Physiology*, **24**, 389–94.

LARUE, C. D., and AVERY JR., G. S., 1938. The development of the embryo of *Zizania aquatica* in the seed and in artificial culture. *Bulletin of the Torrey Botanical Club*, **65**, 11–21.

MUENSCHER, W. C., 1936. *Storage and germination of seeds of aquatic plants.* Bulletin 652, Cornell University Agricultural Experiment Station, Ithaca, New York.

OELKE, E. A., MCCLELLAN, M., and LEIF, J., 1990. Wild rice production research. In *Minnesota Wild Rice Research 1989*, Miscellaneous Publication 64–1990, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul. Pp. 1–15.

—— and STANWOOD, P. C., 1988. Wild rice seed moisture content and viability. *Agronomy Abstracts*, American Society of Agronomy, Madison, Wisconsin, p. 146.

PAMMENTER, N. W., VERTUCCI, C. W., and BERJAK, P., 1991. Homeohydrous (recalcitrant) seeds: dehydration, the state of water and viability characteristics in *Landolphia kirkii*. *Plant Physiology*, **96**, 1093–8.

PROBERT, R. J., and BRIERLEY, E. R., 1989. Desiccation intolerance in seeds of *Zizania palustris* is not related to developmental age or the duration of post-harvest storage. *Annals of Botany*, **64**, 669–74.

—— and LONGLEY, P. L., 1989. Recalcitrant seed storage physiology in three aquatic grasses (*Zizania palustris*, *Spartina anglica* and *Porteresia coarctata*). Ibid. **63**, 53–63.

ROBERTS, E. H., 1973. Predicting the storage life of seeds. *Seed Science and Technology*, **1**, 499–514.

SIMPSON, G. M., 1966. A study of germination in the seed of wild rice (*Zizania aquatica*). *Canadian Journal of Botany*, **44**, 1–9.

SKRIVER, K., and MUNDY, J., 1990. Gene expression in response to abscisic acid and osmotic stress. *The Plant Cell*, **2**, 503–12.

WOLK, W. D., DILLON, P. F., COPELAND, L. F., and DILLEY, D. R., 1989. Dynamics of imbibition in *Phaseolus vulgaris* L. in relation to initial seed moisture content. *Plant Physiology*, **89**, 805–10.

WOODS, D. L., and GUTEK, L. H., 1974. Germinating wild rice. *Canadian Journal of Plant Science*, **54**, 423–4.