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Temperature Dependence of Viability and Dormancy of *Zizania palustris* var. *interior* Seeds Stored at High Moisture Contents

DAVID A. KOVACH and KENT J. BRADFORD*

Department of Vegetable Crops, University of California, Davis, California 95616–8631, USA

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Seeds (caryopses) of North American wild rice (*Zizania palustris* var. *interior*), a temperate aquatic grass, have been thought to require storage at low temperatures and high moisture contents to preserve viability. The seeds are also deeply dormant at maturity and require up to 6 months of stratification to break dormancy. We report here that wild rice seeds can retain viability at moisture contents ≥ 30% (f. wt. basis) for up to 6 months at temperatures as high as 30 °C, and for at least 1 year at temperatures below 20 °C. Dormancy is not broken at temperatures above 10 °C, but subsequent stratification requirements are unaffected by prior warm storage. Cold storage is therefore not required to maintain viability of wild rice seeds, but is necessary to break dormancy. Hydrated wild rice seeds can be frozen to −10 °C without damage, but dormancy is not lost at subfreezing temperatures. These results provide new options for long-term storage of wild rice seeds.

Key words: *Zizania palustris* var. *interior* (Fassett) Dore, wild rice, seed, germination, dormancy, storage, moisture content.

INTRODUCTION

Cultivated North American wild rice [Poaceae, *Zizania palustris* var. *interior* (Fassett) Dore] is native to shallow lakes and streams of the northern United States and southern Canada (Warwick and Aiken, 1986; Aiken *et al.*, 1988). The seeds (caryopses) normally abscise at a relatively high moisture content (> 30%, f. wt basis) and fall into the water, sinking to the bottom and germinating the following spring. Early efforts to establish wild rice from seeds in new areas generally failed, and it was soon determined that viability was apparently lost unless the seeds were stored in water (Brown and Scofield, 1903; Duvel, 1906; Muenscher, 1936). The consensus from this work was that wild rice seeds must be stored submerged at near-freezing temperatures to maintain viability. However, a complication to the early work on wild rice seed storage is the presence of a deep dormancy at maturity, requiring 6 months or longer of stratification (cold hydrated storage) before all viable intact seeds will germinate (Simpson, 1966; Cardwell, Oelke and Elliott, 1978; Atkins, Thomas and Stewart, 1987). Unless total viability is assessed by the tetrazolium (TZ) test (Grabe, 1970) or by scraping the pericarp from over the embryo (perhaps with the addition of gibberellic acid) to break dormancy (Woods and Gutek, 1974; Cardwell *et al.*, 1978; Huang, 1978), viability can be underestimated in germination tests because of the presence of dormant seeds. It has been pointed out that early comparisons of the survival of wild rice seeds in wet versus dry storage may have been confounded by the absence of stratification in the dry seeds, resulting in the maintenance of dormancy rather than in loss of viability (Ellis, Hong and Roberts, 1985). The seeds may therefore have 'orthodox' storage physiology, with their apparent 'recalcitrant' behaviour in storage (Roberts, 1973) resulting from dormancy rather than from intolerance of desiccation. Probert and coworkers (Probert and Brierley, 1989; Probert and Longley, 1989), using both stratification and other techniques to break dormancy, reported that some wild rice embryos lost viability if their moisture content (MC) declined below 45%, with 50% of the embryos failing to survive dehydration to 30% MC (equivalent to a whole seed MC of approx. 20%). They concluded that wild rice seeds are truly recalcitrant and that hydrated storage is necessary to preserve viability. On the other hand, some reports indicate that wild rice seeds can tolerate desiccation to as low as 6–19% MC under certain dehydration and rehydration conditions (Oelke, McClellan and Leif, 1990; Kovach and Bradford, 1992). Until reliable storage regimes can be developed based upon these latter findings, wild rice planting seeds and germplasm must be stored under cold submerged conditions. Storage life under these conditions is limited, however, since the seeds can begin to germinate in cold storage once dormancy has been broken (Muenscher, 1936; Simpson, 1966). We have therefore investigated the effects of temperature and seed moisture content on viability and dormancy of wild rice seeds stored at high moisture contents.

Although the requirement for stratification of wild rice seeds is now well established, there is still little information on the exact temperatures required to break dormancy or on possible interactions between seed MC, temperature and release from dormancy. Due to the requirement for stratification, most studies of wild rice seed storage have been conducted at low temperatures, leading to the

* For correspondence.

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conclusion that low temperatures are required to maintain viability as well as to break dormancy (Duvel, 1906). The longevity of hydrated wild rice seeds at warm temperatures has not been tested, however. Simpson (1966) proposed that a warm aquatic environment immediately after seed maturation might deepen and prolong the state of dormancy, although this point was not addressed experimentally. Maintenance of dormancy could be a means of extending the total storage period of wild rice seeds by preventing germination in storage.

Storage of wild rice seeds at freezing temperatures has been attempted, but the results have been equivocal. Fyles (1920) and Moyle and Krueger (1964; cited in Simpson, 1966) reported that freezing did not harm wild rice seeds, while Duvel (1906) and Simpson (1966) found that the seeds did not survive freezing. The latter authors attributed the freezing damage to the tissue dehydration that would accompany ice formation. A potentially lethal freezing event at −18 °C was detected by differential thermal analysis in wild rice seeds at MC > 19% (Oelke and Stanwood, 1988), suggesting that freezing temperatures above −18 °C may not be lethal to hydrated seeds. Simpson (1966) found no survival of seeds stored moist but not submerged at −20 °C, but seeds frozen in water survived at the same temperature. Duvel (1906) did not determine the viability of seeds that failed to germinate after storage at −12·5 °C, so they may have been dormant rather than dead.

The objective of this study was to investigate the viability and dormancy of wild rice seeds in relation to moisture content and temperature in storage. We show that both viability and dormancy can be maintained for extended periods at warm temperatures without altering subsequent responsiveness to stratification.

MATERIALS AND METHODS

Long-term storage of wild rice seeds

Field-grown wild rice seeds (cultivar NC1) were harvested by hand and immediately put into water at room temperature. Empty and light seeds and plant debris that floated to the surface were removed. The remaining seeds were kept in water at room temperature for 2 d and given a 2-h treatment in 1% NaOCl solution to reduce fungal growth during storage. Seed MC had increased from 28% at harvest to 37% after the 2-d soaking period. Sublots of this seed were air-dried under ambient conditions to 34 and 31% MC. Seeds were then placed in 20-l plastic containers, sealed, and stored at 2·5, 5, 7·5, 10, 12·5, 15, 20, 25, and 30 °C. Seeds submerged in water were also stored at each temperature, and the water was changed at approximately monthly intervals. Seed MC were determined monthly during storage and the seeds were misted with water or 1% NaOCl solutions when necessary to maintain approximately constant MC. Seed MC averaged over all temperatures were 41·0 ± 0·3, 34·7 ± 0·4, and 31·9 ± 0·4% in the submerged, '34%' and '31%' MC treatments, respectively, over the first 6 months of storage. During the second 6 months of storage, viability was progressively lost at the warmer temperatures and seed MC values increased to 40–60%. However, at temperatures ⩽ 15 °C seed MC averaged over the second 6 months of storage were 44·9 ± 0·6, 33·9 ± 0·4, and 30·6 ± 0·3%, respectively, in the submerged, '34%' and '31%' MC treatments. Some of the non-submerged treatments developed extensive fungal growth during storage, depending upon the storage temperature. This did not appear to cause appreciable damage to the seeds, however, until after approximately 6 months of storage. Germination and viability were determined monthly for 1 year under all storage conditions.

To determine the kinetics of release from dormancy after storage at various temperatures and MC, samples of seeds stored at all temperatures and MC from the above experiment were transferred to water at 2·5 °C after 2, 4, and 6 months. The germination and viability of these seeds were then tested monthly for 5 months after transfer to 2·5 °C.

Germination and viability tests

Seed viability was determined by tetrazolium (TZ) tests. Fifty seeds were cut longitudinally through the embryo and soaked in 0·15% (w/v) 2,3,5-triphenyltetrazolium chloride solution for 20–24 h at 20 °C in the dark, then scored according to intensity and location of stain using criteria similar to those for other grains (Grabe, 1970). Germination tests were conducted for 21 d with seeds submerged under 3 cm of de-ionized water at 20 °C in the dark. Seedlings were scored as germinated if they had a normal shoot of at least 1·5 cm length. Seeds showing protrusion of the epiblast and swelling of the mesocotyl and coleoptile (Aiken *et al.*, 1988) were not scored as germinated unless further growth occurred. Due to the very large number of treatment combinations and tests required, only a single replicate of 50 (TZ test) or 200–300 (germination test) seeds per sampling per storage condition could be accommodated within our limitations of personnel and germination incubator space. Although this precludes formal statistical analysis, the large individual sample size and the consistency of results across similar treatments and with repeated sampling over time gives credence to the results. The intent of this experiment was to survey a wide variety of storage environments and identify overall trends, rather than to compare absolute survival values among specific individual treatments. Only differences in germination or viability of sufficient magnitude that statistical analysis is hardly needed were considered relevant. Subsequent experiments, that included a similarly wide range of treatments and employed replicated ($n = 3$) germination tests, had coefficients of variation of approximately 13% (Kovach and Bradford, 1992). This can be considered a reasonable estimate of experimental error under our standard germination conditions for the data of Figs 1 and 2.

Moisture content determinations

Whole seed (including the lemma and palea) MC was determined by oven drying 3 g of seeds at 130 °C for 6 h. This method was found in comparison tests to agree with the standard two-step method, where ground seeds are oven dried at 130 °C for 1 h (International Seed Testing Association, 1985).

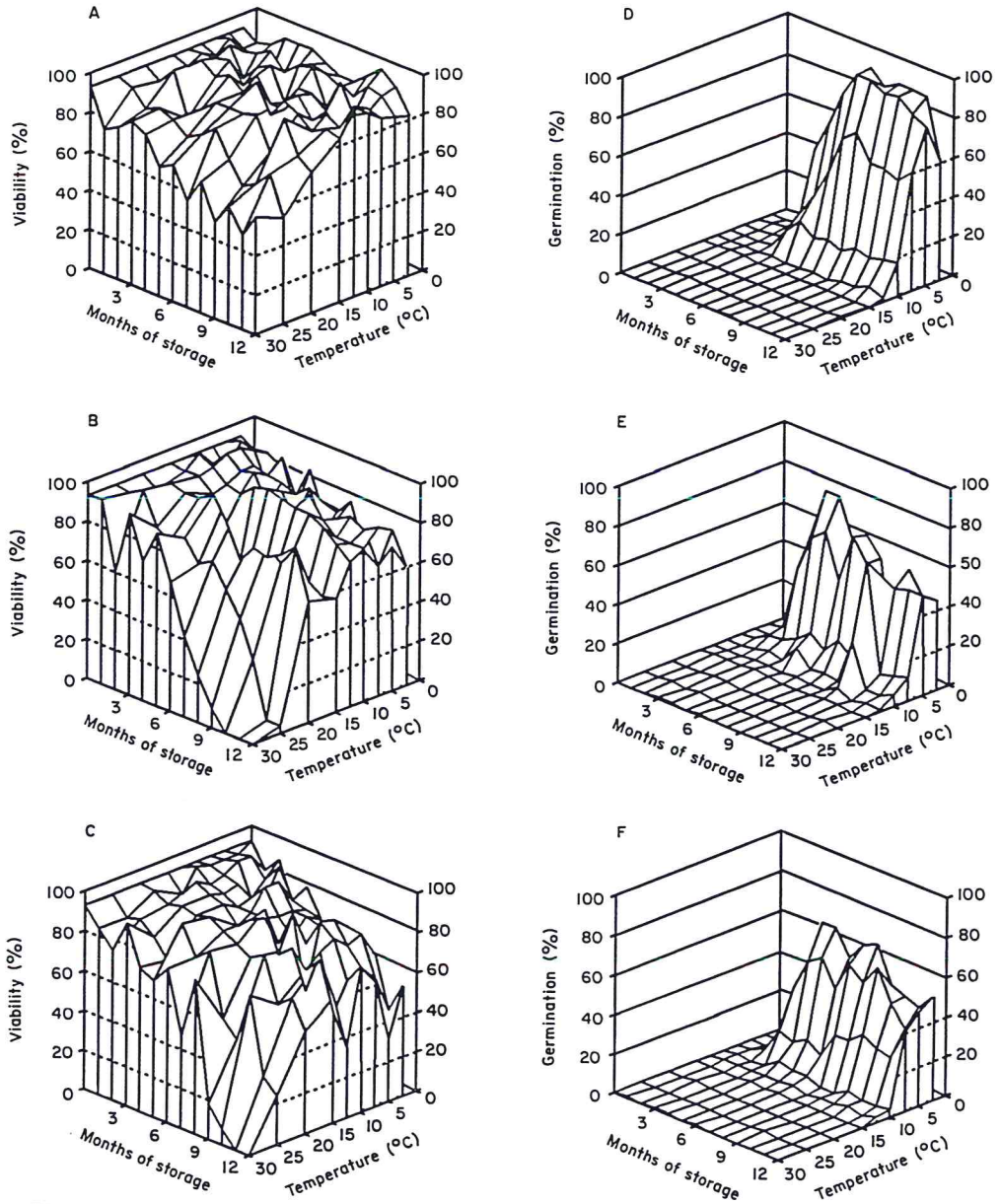

FIG. 1. Viability (TZ test) (A, B, C) and germination (D, E, F) of wild rice seeds stored in water (A, D) or at approximately 34% (B, E) or 31% (C, F) moisture content (f. wt basis) at 2·5 to 30 °C for 1 year.

Storage of wild rice seeds at freezing temperatures

Fully imbibed, non-dormant wild rice seeds (stored in water at 15 °C for 4·5 months, then in water at 2·5 °C for an additional 4·5 months) were brought to 35 and 30% MC by surface-drying or air-drying for 1 d at room temperature. The seeds were then stored progressively at 0, −5, −10, −15, −20, and −30 °C. After 24 h at each temperature, a sample was removed for germination and viability tests, and the remaining seeds were transferred to the next lower temperature. Germination percentages are based on three replications of 30 seeds each for each temperature.

The influence of long-term storage at −3 °C on dormancy was also assessed. Fresh dormant seeds were stored in ice at −3 °C for 9 months, and germination and viability were tested at 20 °C. Germination percentages are based on three replications of 150 seeds each, and viability percentages are based on TZ tests with three replicates of 50 seeds each.

RESULTS AND DISCUSSION

Viability and dormancy in relation to MC and storage temperature

When stored in water, wild rice seeds maintained > 80% viability for 1 year at temperatures below 15 °C (Fig. 1A). Viability was lower at warmer storage temperatures, and the rate of loss of viability increased with increasing temperature. Rather surprisingly, approx. 50% of the seeds survived 12 months of submerged storage at 30 °C. Survival was reduced during extended aerobic storage at lower MC

levels, with viability declining markedly after 6 months storage at temperatures above 20 °C (Fig. 1 B, C). Below 20 °C, survival under aerobic conditions was less than that in submerged storage, but remained at 60–70 % after 1 year at 34 % MC. At 31 % MC, there was a continuous decline in viability even at the lowest temperatures. Microbial growth was difficult to control in the aerobic storage regimes, so some of the loss of viability of seeds under these conditions may be attributed to fungal or pathogenic attack, rather than to physiological intolerance of the MC and temperature conditions. Even so, these data indicate that a considerable fraction of the seed population can survive for up to a year at temperatures as high as 20 °C and MC as low as 31 %. Thus, near-freezing temperatures are not necessary to maintain viability of wild rice seeds.

Low temperatures are required, however, to break dormancy of wild rice seeds. Seeds stored in water began to germinate (when removed from storage and tested at 20 °C) after 3–4 months at temperatures below 10 °C, and dormancy was completely broken after 7 months at 2·5 and 5 °C (Fig. 1 D). Seeds became germinable 1 month earlier under aerobic storage at 34 or 31 % MC than in submerged storage, in contrast to the results of Simpson (1966). However, germination percentages subsequently declined in the aerobically-stored seed, and dormancy was maintained in 20–30 % of the viable seed population (compare Fig. 1 B, C with Fig. 1 E, F). Dormancy was also maintained in seeds stored at or above 15 °C under all MC conditions (Fig. 1 D, E, F).

Since viability could be maintained for long periods at temperatures where dormancy was not broken, we determined whether dormancy could be broken by exposure to low temperature after a period of storage at warmer temperature. Subsamples of seeds from each initial storage temperature and MC condition in Fig. 1 were transferred to 2·5 °C water after 2, 4 and 6 months. For those seeds stored initially at dormancy-breaking temperatures, there was no effect of this transfer on the timing of release from dormancy (data not shown). For seeds initially stored at dormancy-maintaining temperatures, the pattern of release from dormancy after transfer to 2·5 °C water was consistent for all temperatures and MC levels, and is illustrated by the example of seeds initially stored in water at 15 °C (Fig. 2). Regardless of the initial period of warm storage, an additional 5–6 months at 2·5 °C was required to fully break dormancy. Thus, the total storage time was simply extended by the initial time at temperatures > 10 °C, and the period of stratification required to break dormancy was not altered by up to 6 months of prior storage at temperatures as high as 30 °C. The suggestion by Simpson (1966) that a period of hydrated storage at warm temperature would prolong or deepen the state of dormancy was not supported, at least for this population of wild rice seeds. However, it does appear that hydrated storage at 15 °C can extend the total storage duration by maintaining dormancy and delaying the occurrence of germination in store that occurs with continuous low temperature storage. Storage at 15 °C also provides a means of maintaining dormant seed for research purposes over an extended period, which is not possible with low temperature storage.

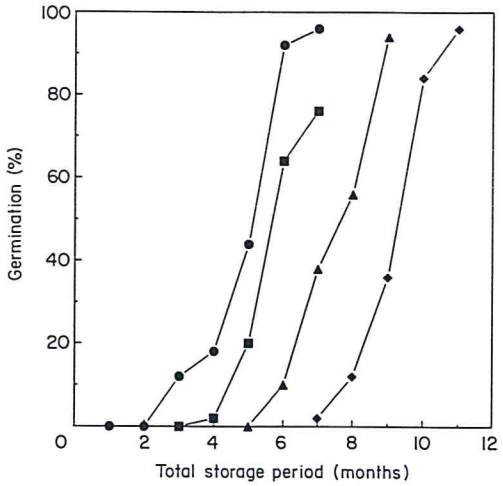

FIG. 2. Germination of wild rice seeds after various periods of storage in 15 °C water prior to transfer to water at 2·5 °C. Seeds were stored continuously at 2·5 °C [0 months at 15 °C (●)] or at 15 °C for 2 (■), 4 (▲), or 6 (◆) months before transfer to 2·5 °C, then tested for germination at monthly intervals.

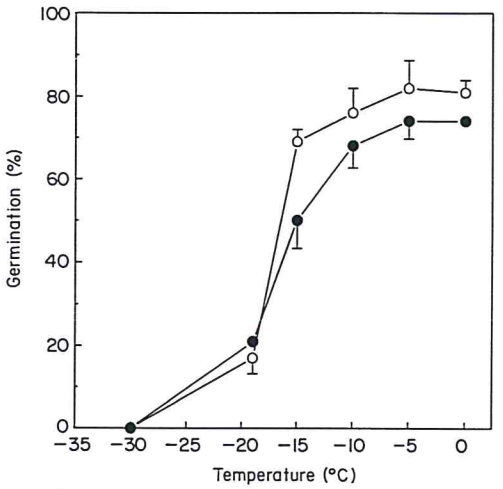

FIG. 3. Tolerance of wild rice seeds to subzero temperatures at two MC. Non-dormant wild rice seeds were transferred to 0 °C after surface drying [35 % MC (●)] or after dehydration to 30 % MC (○) (f. wt basis). The seeds were then transferred progressively to lower temperatures at 24 h intervals, with a sample taken for germination tests prior to each transfer. Error bars indicate s.e. ($n = 3$) where they exceed the size of the symbol.

Storage at sub-zero temperatures

Storage at sub-zero temperatures might also be a way of preventing germination in storage of hydrated seeds. The initial germination percentage of non-dormant seeds was maintained after cooling to −10 °C, but fell sharply after cooling to between −15 and −20 °C (Fig. 3). There was no significant difference in freezing tolerance between seeds at 30 and 35 % MC. These results are in agreement with differential thermal analysis data that showed an exotherm at −18 to −22 °C for seeds above 19 % MC (Oelke and Stanwood, 1988; P. Stanwood, K. Bradford, D. Kovach, unpub. res.). In a separate experiment, dormant seeds stored at −3 °C for 9 months were 87 % viable, as

compared to 92% for seeds stored for the same period in water at 2·5 °C (data not shown). However, only 2% of frozen seeds germinated after transfer to 20 °C, while 86% of seeds stored in water at 2·5 °C germinated. Therefore, both viability and dormancy were maintained at the freezing temperature. Elliott (1975) reported that 27–57% of wild rice seeds germinated following 9 months of hydrated storage at −2 °C. Although he did not determine total viability, Elliott's results suggest that some loss of dormancy may occur at temperatures just below freezing, in contrast to our observations. Storage of wild rice seeds in ice at temperatures just below freezing may be an attractive alternative to the standard regime for long-term germplasm preservation, as neither microbial growth nor germination occurred during storage. However, a subsequent stratification period or dormancy-breaking treatment would be required to obtain maximum germination.

In summary, the information presented here provides new options for storage of hydrated wild rice seeds. The total storage time can be adjusted by a period of warm storage (optimally 15 °C) that maintains both viability and dormancy, followed by a stratification period to break dormancy. Alternatively, seeds can be stored for extended periods frozen in ice at temperatures from −10 to −3 °C with little loss of viability or dormancy.

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