Seed Storage and Viability of *Zizania texana* and *Zizania palustris*

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Texas wild rice (*Zizania texana* Hitchc.), a perennial aquatic grass, is native to a 2.5 mile length of the San Marcos River within the city of San Marcos in southcentral Texas. This species is endangered due to its limited habitat or range, decline in plant numbers over the past 60 years since it was first identified by Hitchcock (1933), and low seed production in native stands. Flowering will occur throughout the year if subfreezing temperatures do not occur frequently. Fresh seed has been reported to have dormancy (Power, 1990). Seed that was removed from plants and stored immediately in water with a low oxygen concentration started to germinate in about three weeks. In addition, freshly harvested seed that was stored in cold (3°C/37°F) water for four months was reported to germinate quickly in four days when placed in water with a low oxygen concentration. Dormancy may be due perhaps to embryo immaturity or presence of growth inhibitors within the seed. However, the slow germination may have been the result of an inappropriate oxygen concentration or temperature of the water, or an impermeable seed coat; and not due to an endogenous (internal), physiological dormancy.

The major objective of this study was to investigate whether seed viability of Texas wild rice was affected by drying seed to different moisture contents. Another goal of this study was to determine whether seed will germinate immediately after removal from dry storage when placed in water at a temperature of 20°C (70°F). The presence of dormancy in fresh seed of this species is similar to *Z. palustris* that must have dormancy requirements satisfied prior to germination by stratification for three months in cold water.

Materials and Methods

Seed of Texas wild rice was aquired from Southwest Texas State University (Power, 1990). The wild rice used for cultivation (*Z. palustris*) served as a control treatment or reference standard in the first run of this experiment, since previous seed-storage research used this species. The

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second run of this experiment used two sources of *Z. palustris* due to an insufficient amount of seed for Texas wild rice. A seed source ('K2(1)C6') that was very dormant and another source ('K2') with less dormancy were used.

The different seed moisture contents evaluated in the first run of this study were obtained from five storage treatments. The initial samples collected for whole-seed moisture content (MC) determinations and germination tests came from cold, hydrated storage except for the cold, dry conditions used for K2(1)C6 in the second run. Three replications (separate containers) were evaluated for each storage treatment. The first treatment consisted of cold (3°C), hydrated conditions, which have been used usually for stratification (Oelke et al., 1990). The cold water was aerated continuously with an aquarium aerator to obtain a high oxygen content to prevent the germination of Texas wild rice. The second treatment involved air drying the seed for seven days in the laboratory at an ambient air temperature of 21°C. The air-dried seed was then placed in sealed vials and stored at 3°C until needed for MC determinations and germination tests.

The three remaining treatments involved seed storage in sealable containers or chambers with different relative humidities (RH) that were placed in an incubator at a constant 21°C. Seed samples were put on filter paper that was placed on fine stainless steel wire mesh (Kovach and Bradford, 1992). The first storage treatment with RH chambers was a control treatment that used fresh seed with a MC of 35% placed over deionized water for 24 and 42 days prior to the MC determinations and germination tests. The other two RH treatments stored seed over saturated salt solutions of magnesium chloride ($MgCl_2$) for 24 days and sodium chloride (NaCl) for 42 days to produce relative humidities within the chambers of approximately 33 and 75% respectively. The expected moisture contents for seed in the $MgCl_2$ and NaCl treatments were approximately 7% and 20% respectively. The second run of the study used the same three RH storage treatments and cold (3°C), hydrated storage. An additional treatment, a cold (3°C), dry storage environment, was used only for the highly dormant K2(1)C6.

Seed viability was measured by germination tests that ran usually for 21 days at 21°C. For the germination tests of *Z. palustris*, 15 seeds per replication were used in the first run of the experiment. Seed of *Z. texana* (seven seeds per replication) was germinated in deionized water with a low oxygen concentration (<1.0 ppm) that was achieved by bubbling nitrogen gas through water in a five gallon container. Germination tests in the second run used 25 seeds per replication. The pericarps or seed coats of ungerminated seeds were scarified or slit above the embryo after 14 days if very little or no germination had occurred,

and incubated for another 21 days to check for viability of the seeds, which may have been dormant. Ungerminated seeds in samples with good germination were not scarified until after 21 days. Seed was considered to have germinated when the coleoptile had grown longer than the length of the seed. The whole-seed moisture contents were determined by drying seed for six hours at 130°C in a forced-air oven. Moisture content determinations used 15 seeds per replication in the first run and 25 seeds per replication in the second run. Data were recorded for the whole-seed MC, germination percentage prior to scarification, and total germination percentage that included germinated seeds before and after scarification.

Results

The Texas wild rice showed poor viability after the various storage treatments with a range of 0 - 19% germination (Table 1). The germination for *Z. palustris* in the first run was usually much better in all treatments with a range of 0 - 99%. However, neither seed source germinated after the air-dry treatment. The control treatment in RH chambers and cold, hydrated storage resulted in the best germination.

The K2 and K2(1)C6 seed evaluated in the second run usually showed a very large increase in germination after scarification (Table 2). Germination percentages were again lower in seed from treatments that produced the lowest moisture contents. Cold, hydrated storage resulted in the highest germination percentage for K2. The cold, dry treatment for K2(1)C6 resulted in a higher germination percentage before and after scarification than the cold, hydrated storage. The K2(1)C6 had the best viability of any seed source after the air-dry treatment.

Conclusions

Texas wild rice seed was not tolerant of dessication since viability ceased for any treatment when the MC went below the initial MC. The age of the seed, which was nine months old, may have contributed to the absence of or low germination that was observed before and after scarification in germination tests. Some seed may also have been immature and unable to germinate. The apparent satisfaction of dormancy requirements, or lack of dormancy in this species, may have contributed also to the poor seed viability that occurred after treatments which lowered the MC. Dormancy was clearly present in K2(1)C6 due to the great increase in germination after scarification. The K2(1)C6 consequently showed a better tolerance to desiccation and a greater seed vigor than had been observed with K2, which was harvested four months before the experiment and had less dormancy.

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The K2 and K2(1)C6 seed evaluated in the second run also showed low to high levels of dormancy based on the increase in germination after scarification (Table 2). Dormancy was apparently stronger in the K2(1)C6, and this condition might explain its higher germination percentages than were observed for K2 after the storage treatments that lowered the MC. The better viability of seed stored in cold, dry conditions showed that seed vigor could be maintained without storing seed in water. However, dormancy must be released with cold, hydrated stratificaition. The drying rate of seed in the different storage treatments as well as the final MC appeared to affect seed viability. The rapid drying of seed in seven days for the air-dry storage may have been a cause of the poor vigor that resulted after this treatment in both runs of the experiment. The slower drying rate of seed in the RH chamber for the $MgCl_2$ and NaCl treatments may have resulted in the higher survival at lower moisture contents. The storage duration may also have affected the seed viability as indicated by germination percentages of K2 in the control treatment at 24 and 42 days.

Future research should investigate the effect of different storage treatments on the viability of *Z. texana* seed at various moisture contents, if a sufficient quantity of seed can be obtained. The perennial growth habit of Texas wild rice may be a character that plant breeders would desire to incorporate into a future cultivar of *Z. palustris* for paddy cultivation or in habitat improvement of wetlands for waterfowl.

Further Reading

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Table 1. Seed moisture content (MC) and germination percentage (Germ.) obtained after completion of five storage treatments for *Zizania texana* and *Z. palustris* in first run of experiment.

Seed Source	Storage Treatment	Storage Duration (days)	MC (%)	Germ.[z] (%)	Total Germ. (%)
Z. texana					
	Initial	0	39.7	16.0	16.0
	Air-Dry	7	12.0	0.0	0.0
	$MgCl_2$-RH[y]	24	13.4	0.0	0.0
	Control-RH[x]	42	27.5	9.5	9.5
	NaCl-RH	42	16.0	0.0	0.0
	Cold-Hydrated	>42[w]	50.6[v]	14.0	19.0
Z. palustris					
	Initial	0	39.5	24.0	61.0
	Air-Dry	7	11.5	0.0	0.0
	Control-RH	24	29.2	0.0	90.0
	$MgCl_2$-RH	24	12.2	0.0	27.0
	Control-RH	42	29.8	3.0	93.0
	NaCl-RH	42	15.7	0.0	63.0
	Cold-Hydrated	>42	36.2	41.0	99.0

[z]Germination percentage prior to seed scarification. Total Germ. includes scarified seeds that germinated.
[y]RH= relative humidity chamber.
[x]No control treatment for 24-day duration due to insufficient seed.
[w]Seed was stored in standard storage conditions of 3°C water for about 6 months after harvest, but the samples used were placed in incubator for 42 days prior to MC and Germ. tests.
[v]Some seed was cracked and had imbibed water.

Table 2. Moisture content of seed (MC) and germination percentage (Germ.) obtained after completion of storage treatments for two seed sources of *Z. palustris* in second run of experiment. The 'K2(1)C6' seed was very dormant and 'K2' had less dormancy.

Seed Source	Storage Treatment	Storage Duration (days)	MC (%)	Germ.[z] (%)	Total Germ. (%)
K2	Initial	0	36.0	68.0	95.0
	Air-Dry	7	10.3	0.0	0.0
	Control-RH[y]	24	35.9	23.0	85.0
	$MgCl_2$-RH	24	10.9	2.7	27.0
	Cold-Hydrated	>24[x]	33.8	56.0	89.0
	Control-RH	42	18.3	5.0	32.0
	NaCl-RH	42	14.3	3.0	29.0
	Cold-Hydrated	>42	34.9	56.0	99.0
K2(1)C6	Initial	0	30.0	0.0	61.0
	Air-Dry	7	10.1	1.0	17.0
	Control-RH	24	43.0	1.0	60.0
	$MgCl_2$-RH	24	12.3	0.0	24.0
	Cold-Hydrated	24[w]	33.9	1.0	65.0
	Cold-Dry	>24[w]	26.0	5.0	79.0
	Control-RH	42	16.4	0.0	69.0
	NaCl-RH	42	14.1	0.0	44.0
	Cold-Hydrated	42	33.2	0.0	40.0
	Cold-Dry	>42	25.7	14.7	69.0

[z,y]See Table 1.
[x]Seed was stored in 3°C water for 6 months after harvest, but the samples that were used remained in 3°C water in an incubator for 42 days prior to MC and Germ. tests.
[w]Seed source stored dry at 3°C for at least 30 days after the winter-to-spring harvest of plants in greenhouse before start of the second run of experiment. Samples remained in 3°C water for treatments with 24- and 42-day durations, and those for cold-dry treatment remained in a dry condition.

PREDICTION OF TOPDRESS NITROGEN BY SOIL AND PLANT SAMPLING

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Laboratory and field investigations in 1990 and 1991 showed that fall applied nitrogen is subject to rapid losses due to nitrification, even when the soil temperature at application is less than 50°F. Field investigations in 1991 suggested that extractable soil ammonium sampled early in the season can be used to determine the quantity of plant available N carried over from the previous fall. This would allow growers to determine if topdress is necessary to make up for lost N. Also, in 1991 we showed that the SPAD 502 chlorophyll meter (Minolta Corp.) might be a valuable tool in assessing the N status.

Field and laboratory studies were undertaken in 1992 to further investigate the utility of using extractable soil ammonium along with SPAD data and plant tissue N for the assessment of soil and plant N status and the prediction of the need for topdress N. A laboratory study was needed because the procedure used for handling soil samples in 1991 resulted in high values if the samples were not analyzed within a day of sampling.

Laboratory Study of Soil Sample Preservation

A 2 molar (concentration) solution of potassium chloride was added to all soil samples taken in the field. This solution serves as a preservative and an extraction solution. When samples are delivered to the laboratory it is only necessary to separate the soil from the solution by filtration and analyze the solution for ammonium. The problem that was noted in 1991 was that the ammonium concentrations increased with time after sampling because microbial mineralization of ammonium from the soil organic matter was not sufficiently inhibited by the potassium chloride solution. The laboratory study was carried out to determine what bactericide agents would be the best to preserve the samples for transport to the laboratory. We hoped that we could find a chemical agent that was a good enough preservative to allow growers to send samples to a laboratory without putting the samples on ice.

Several organic toxins plus the heavy metals barium, cadmium and mercury were tried. The best inhibitor was merthiolate, added to the potassium chloride solution. This is the same compound that is used to inhibit infection in wounds, but we used it at much higher concentrations. The results in Table 1 show that merthiolate decreased the rate of ammonium released from the soil extracts during storage but only with refrigeration is the rate decreased sufficiently to make the sample stable enough for the expected 3-5 day delay from time of sampling until laboratory analysis. (Note: In peats 1 ppm is equivalent to 6 lb/ac of N.) Thus, unfiltered samples could be sent to a lab, but they would have to be on ice. This requires insulated shipping containers and much higher shipping costs.