ıfluence of Chemical Seed Treatments on Germination of Dormant Wild Rice Seeds[1]

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ABSTRACT

Wild rice (*Zizania palustris* L.) seeds have a dormancy eriod of 3 to 5 months. This dormancy period can be artially broken right after harvest by dehulling the eeds and scarifying them. However, this is time conming and the resulting seedlings are weak. The purose of this research was to search for chemical means o decrease the dormancy period and increase the seedng vigor of wild rice.

Seeds which had been stored in water at 3 C for 120 iays were soaked in 0, 28, 43, 57, 71, 86, and 95% ethanol or various lengths of time up to 24 hours. Germination was increased from 15% with no chemical treatment to 51% with a 7-hour soaking in 48% ethanol.

Seeds which had been harvested for 1 week were shaken in 95% ethanol for 15 sec, then in chloroform for 1 min and again for 15 sec in 95% ethanol. After treatment the seeds were stored in water at 3 C and germination samples taken over a period of 180 days. Germination at the end of 60 days was 42% for the treated seed compared to 16% for untreated seed.

Freshly harvested seeds were scarified by tumbling with granite grit for 60 min and then placed for 24 hours in all combinations of 0, 0.005, 0.05, 0.5, and 5.0 m*M* gibberellic acid (GA_3) and 0, 0.063, 0.125, 0.25, and 0.5 m*M* 6-benzyl adenine (BA). A combination of the highest concentration of both resulted in the highest germination after 21 days in water; 64% compared to 7% with no chemical treatment. However, seedling survival in the greenhouse, 51 days after treatment, was the highest with the lowest concentration of GA_3 and the highest concentration of BA. Treating freshly harvested dehulled and scarified seed with 2.63% sodium hypochlorite for 2 hours increased germination from 2 to 19% and seedling survival from 50 to 87%, compared to no chemical treatment.

Pretreating wild rice seeds with ethanol in combination with chloroform can decrease the dormancy period without dehulling and scarifying seeds. Seedling vigor of scarified seeds can be increased by treating them with GA_3 and BA.

Additional index words: Zizania palustris, Ethanol, Chloroform, Gibberellic acid, 6-Benzyl adenine, Seedling survival.

SEED dormancy for a period of 3 to 6 months in wild rice (*Zizania palustris* L.) is a severe problem in the domestication of this species as a cereal grain. This prolonged dormancy is particularly a problem in the rapid development of new cultivars. Germination of wild rice seeds immediately after harvest increased from 0 to 85% by removing part or all of the pericarp from directly above the embryo (3, 12). However, this is time consuming, and often results in a low percent of vigorous seedlings, thus, treating seeds chemically could be more suitable.

A number of chemicals have been used successfully to break seed dormancy. Thiourea stimulated the germination of dormant cocklebur *Xanthium pensylvanicum* Wallr.) seeds (5). Roberts (10) found that hydrogen peroxide, hydrogen sulfide, ammonium hydroxide, sodium oxide, potassium cyanide, sodium nitrate, and potassium nitrate promoted germination of dormant rice (*Oryza sativa* L.) seeds. Hydrogen peroxide, sodium hypochlorite, and potassium nitrate were effective in increasing the germination of pearl millet [*Pennisetum typhoides* (Burm.) Stapf and C. E. Hubb] seeds (2). Soaking wild rice seeds, which had been stored for 90 days in water at 3 C, in a 50% ethanol solution for 240 min. increased germination from 0 to 14% (6).

Seedlings from seeds forced to germinate by scraping or tumbling often have reduced vigor and a low survival rate. This could be the result of an inhibitor present in the seed or low amounts of cytokinins and gibberellins or both (1, 3). Applications of cytokinins and gibberellins are known to substitute for chilling requirements in dormant seeds of several plants (4, 5, 8). Germination was increased from 29 to 76% by germinating freshly harvested scarified wild rice seeds in solution of GA_3 and kinetin (3). However, no seedling survival information was obtained.

Immediate germination after harvest and subsequent seedling survival is necessary for rapid cultivar development in wild rice. This necessity led to experimentation with various chemicals and growth hormones in an attempt to decrease the dormancy time period for seeds and to increase seedling survival of seeds forced to germinate before completion of the 3 month dormancy period.

MATERIALS AND METHODS

Ethanol. Fifty wild rice seeds (cultivar 'K2'), which had been stored at 4 C in water for 120 days, were placed into solutions of 95% ethanol and water and removed after 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours. The ethanol concentrations were 0, 28, 43, 57, 71, 86, and 95% and the temperature during ethanol treatment was 21 C. The pericarp of 50 additional seeds placed into water was ruptured with a dissecting needle. Each treatment was replicated four times. After removal from the solutions, the seeds were placed into water at constant 23 C and germination counts taken after 14 days.

Chloroform, Acetone and Ethanol. Wild rice seeds (K2), which were stored in water at 23 C for 7 days after harvest, were removed from the water and placed into and shaken with one of the following: i) 95% ethanol for 4 min, ii) acetone for 1 min, iii) 95% ethanol for 15 sec, chloroform for 1 min, ethanol for 15 sec or, iv) water for 4 min. The temperature during treatment was 21 C. After treatment, all seeds were thoroughly rinsed with water and then stored in water at 3 C. While in storage, 2 replications of seeds treated with the various solvents were continuously aerated by bubbling air through the storage water. The remaining 2 replications were left unaerated. Storage water of the aerated treatments was changed every 15 days, but not for the unaerated ones. Germination tests were conducted on lots of 1,000 seeds at 30-day intervals for 120 days and a final germination count was made at 180 days.

Gibberellic Acid and 6-Benzyl Adenine. Freshly harvested seeds were dehulled and scarified by tumbling for 60 minutes with granite grit in a rock tumbler according to the method

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Table 1. The effect of ethanol soaking on germination of *Z. palustris* seeds stored for 120 days in water at 4 C.

Soaking time	Percent ethanol						
	0	28	43	57	71	86	95
hour	% germination						
0.5	15†	13	25	22	31	32	35
1		27	19	22	37	34	29
2		41	31	40	36	45	44
3		35	28	56	45	53	54
4		38	42	46	55	53	43
5		34	48	41	45	45	24
6		49	52	52	48	42	12
7		43	61	52	41	41	9
8		39	59	36	25	9	2
24		23	3	7	4	2	0

L.S.D.$_{.05}$ = 12.1

† This treatment was not repeated for each soaking time; puncturing the pericarp several times with a dissecting needle resulted in 70% germination in water.

described by Oelke and Albrecht (9). The scarified seeds were placed into solutions of all combinations of 0, 0.005, 0.05, 0.5, and 5.0 m*M* gibberellic acid (GA_3) and 0, 0.063, 0.125, 0.25, and 0.5 m*M* 6-benzyl adenine (BA). After 24 hours of soaking at 23 C, the seeds were thoroughly rinsed with water and placed in water at an alternating temperature regime of 23 C for 16 hours and 18 C for 8 hours. A photosynthetic photon flux density (PPFD) of 6 nE $sec^{-1}m^{-2}$ was supplied by a fluorescent lamp during the 16-hour period. Germination counts were taken 21 days after treatment. Seedlings were planted into flooded soil in the greenhouse and the number of surviving plants counted 51 days after treatment. Four replications of 100 seeds were used for each treatment.

Hydrogen Peroxide and Sodium Hypochlorite. Freshly harvested seeds were dehulled and scarified as above. The scarified seeds were placed into 2.5, 5.0, and 10.0% solutions of hydrogen peroxide for 2 and 8 hours. Others were placed into 0.65, 1.31, 2.63, and 5.00% solutions of sodium hypochlorite for 1 and 2 hours. After removal from the solutions, the seeds were thoroughly rinsed with distilled water and placed in water at the temperature and light regime described above. Germination counts were taken 28 days later. Seedlings were planted in a greenhouse and seedling survival counts were taken 56 days after chemical treatments were made. Three replications of 100 seeds were used in each treatment.

RESULTS AND DISCUSSION

Germination increased and then decreased with time of soaking seeds in ethanol for all concentrations of ethanol (Table 1). However, as the concentration of ethanol increased from 28 to 95%, the highest germination occurred in a shorter period of time. The maximum germination was obtained by soaking the seeds in a 43% solution of ethanol for 7 or 8 hours. The germination was 61% compared to 15% with water soaking. Ethanol may have influenced the permeability of the pericarp since puncturing the pericarp of the same lot of seeds with a dissecting needle increased the germination from 15 to 70%.

Since ethanol appeared to have some effect on the germination of partially dormant seeds (120 days storage in water at 4 C), it, along with chloroform and acetone was tried on freshly harvested seeds. The effects of pre-storage treatments of seeds with the 3 organic solvents, aeration during storage, and length of storage time in water at 3 C on germination are presented in Table 2. Very little germination was obtained after 30 days of storage for any of the solvents used or aeration treatments. However, after 60 days of storage, the ethanol-chloroform-ethanol wash treatment

Table 2. The influence of organic solvents, aeration, and length of storage time in water at 3 C after solvent treatments on germination of freshly harvested *Z. palustris* seeds.

Solvent wash‡	Days in water storage	Storage water not aerated	Storage water aerated
		% germination†	
Water	0	0	0
	30	1	1
	60	16	22
	90	48	48
	120	78	63
	180	81	74
Ethanol	0	0	0
	30	1	1
	60	12	23
	90	52	51
	120	76	60
	180	81	65
		L.S.D.$_{.05}$ = 6.5¶	
Acetone	0	0	0
	30	1	1
	60	16	26
	90	50	52
	120	79	55
	180	78	62
Ethanol and chloroform	0	0	0
	30	1	1
	60	42	35
	90	63	58
	120	73	59
	180	71	61
		L.S.D.$_{.05}$ = 11.5§	

† Germination counts taken after 4 weeks of incubation.
‡ Treatment before storage, see Material and Methods for details.
§ Least significant difference for comparison of different solvent treatments at the same date and aeration level. ¶ Least significant difference for comparison of different aeration treatments at the same date and solvent treatment.

resulted in 42% germination compared to 12 to 16% for the other wash treatments when the storage water was not aerated. The increase in germination at 60 days for the ethanol-chloroform-ethanol treatment was also evident when the seeds were aerated, but the difference was not as great. After 90 days of storage, the differences were still observed, but were decreasing. After 120 days of storage, the effect of the solvent wash treatments disappeared, probably because of natural release of dormancy due to cold storage temperatures.

Chloroform affected germination differently than the other solvents. Chloroform promoted early germination, especially in the non-aerated water. After longer periods of storage, however, germination of chloroform-treated seeds dropped below that of other solvent treatments in both aerated and non-aerated storage. The early germination increase could be a result of chloroform dissolving the waxy layer surrounding the pericarp, thereby increasing its permeability. The later low germination percentages were a result of seed decay, again, possibly a result of increased pericarp permeability.

Aeration tended to increase germination percentages after 60 days of storage regardless of the solvent treatments, had little effect after 90 days of storage, and decreased germination after 120 and 180 days compared to non-aerated storage (Table 2). There was a visible difference in microbial growth in these two treatments. A mat of mold developed on the water surface of the non-aerated treatments. No mold grew on

ble 3. Effect of pregermination treatments with BA and GA, on germination of scarified, freshly harvested *Z. palustris* seeds.

concentration	GA, concentration (*M*)				
	0	5×10^{-4}	5×10^{-3}	5×10^{-4}	5×10^{-3}
M	% germination†				
	7.0	19.5	18.0	23.5	41.0
$\times 10^{-5}$	18.5	31.5	30.5	32.5	45.5
$\times 10^{-4}$	33.0	46.5	47.5	43.0	60.0
$\times 10^{-4}$	47.0	47.0	44.5	54.5	55.0
$\times 10^{-4}$	50.0	55.5	48.5	55.5	64.0
$L.S.D._{.05}$ = 9.2					

†ermination counts taken 21 days after treatment.

Table 4. Effect of pregermination treatments with BA and GA, on plant survival.

BA concentration	GA, concentration (*M*)				
	0	5×10^{-4}	5×10^{-3}	5×10^{-4}	5×10^{-3}
M	% survival†				
0	36.2	19.8	47.0	53.0	16.2
6.0×10^{-5}	61.5	75.2	80.5	48.5	17.8
1.2×10^{-4}	67.3	62.8	59.5	54.2	16.5
2.5×10^{-4}	63.0	75.8	70.8	50.0	25.0
5.0×10^{-4}	61.8	76.5	67.5	45.2	10.8
$L.S.D._{.05}$ = 21.8					

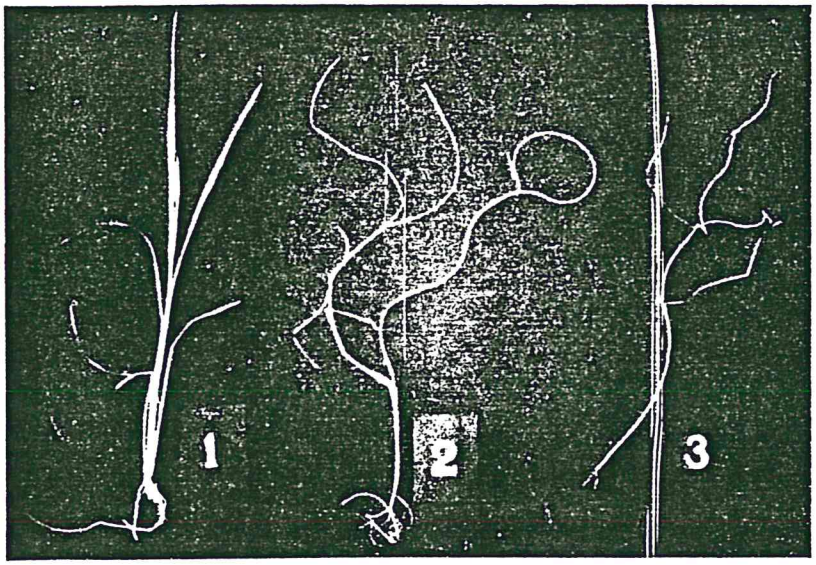
† Survival counts were taken 51 days after treatment.

e water surface of the aerated treatments, but microal growth was present on most of the seeds. After days of storage, there was a considerably higher proortion of decayed seeds in the aerated than in the -aerated treatments. One explanation for the germation differences due to aeration treatments might that microorganisms began breaking down the perirp early in storage causing an increase in germinaon under aerated conditions but after 90 days of orage, microbial breakdown of the embryo in aerated eatments caused a lower proportion of viable seeds compared to the non-aerated treatments. Physiolocal processes within the wild rice seed itself may also affected by aeration as reported for cockleur (*Xanium pensylvanicum* Wallr.) seeds (5) and for seeds Eastern Redbud (*Cercis canadensis* L.) (7). From ese data it appears that pre-storage treatment with loroform followed by non-aerated storage in water at C may be an effective means of decreasing the cold orage period necessary to release seeds from dorancy.

Since many dormant seeds have low levels of GA_3 d cytokinins, an experiment was designed to dermine if applications of these growth promoters to eshly harvested, *scarified* seeds would increase germination. Concentrations of GA_3 ranging from 5×10^{-6} *M* to 5×10^{-3} *M* and of BA ranging from 6×10^{-5} to 5×10^{-4} alone or in combination enhanced the germination of freshly harvested, scarified wild-rice seeds (Table 3). There were significant increases in germination in response to increasing concentrations of both of these grower regulators with the highest germination percentages observed in a combination of 5×10^{-3} *M* GA_3 and 5×10^{-4} *M* BA.

This is in contrast to some of our preliminary work which showed no effect of GA_3, $GA_{4,7}$, isopentenyl adenine, BA, or kinetin on the germination of *unscarified* dormant wild-rice seeds. Apparently, there is a need to disrupt the pericarp to allow penetration of growth regulators into the seeds.

Although a pregermination treatment with a combination of 5×10^{-4} *M* BA and 5×10^{-3}*M* GA_3 gave the highest germination percentages, it had negative effects on plant survival (Table 4). The greatest plant survival resulted from pregermination treatments of 5×10^{-6} or 5×10^{-5} *M* GA_3 combined with BA at concentrations greater than 6×10^{-5} *M*. The low survival rate at the high GA_3 concentrations was probably due to the excessive shoot elongation and poor root development (Fig. 1). The promotive effects of



g. 1. Growth of wild rice seedlings 51 days after seed treatment with GA_3 and BA. Seedlings 1, 2, and 3 developed from seeds treated with 5×10^{-6} *M* GA_3 + 5×10^{-4} *M* BA, 5×10^{-3} *M* GA_3 + 5×10^{-4} *M* BA, and 5×10^{-3} *M* GA_3, respectively.

Table 5. The influence of hydrogen peroxide and sodium hypochlorite on germination of freshly harvested *Z. palustris* seeds and subsequent seedling survival when planted in the greenhouse.

Chemical	Concentration	Time in solution	Germination after 28 days	Plant survival after 56 days
	%	hour	%	%¶
Hydrogen peroxide	2.5	2	5.5	20.8
	5.0	2	6.5	20.8
	10.0	2	14.0	20.0
	2.5	8	13.5	17.6
	5.0	8	17.0	14.6
	10.0	8	17.5	21.4
Sodium hypochlorite†	0.65	1	9.0	22.5
	1.31	1	12.0	10.3
	2.63	1	18.0	57.2
	5.25	1	0	0
	0.65	2	10.5	23.8
	1.31	2	14.0	56.6
	2.63	2	19.0	87.5
	5.25	2	0	0
Water			2.0†	50.0
			0 ‡	0
			64.0§	61.3
		L.S.D.$_{0.05}$ =	6.1	18.2

† Dehulled seeds scarified by tumbling with crushed granite for 60 min.
‡ Intact dehulled seeds. § Pericarp removed from above the embryo. ¶ (Number of plants survived/number of seeds germinated) × 100.

GA_3 and BA on germination of dormant wild-rice seeds are consistent with results of similar research conducted on dormant seeds of other grasses (2, 4, 8, 11).

Other chemicals evaluated for effects on germination and seedling survival in the greenhouse included: hydrogen peroxide, sodium hypochlorite, potassium cyanide, sodium oxide, sodium nitrate, and thiourea. Of these chemicals, only hydrogen peroxide and sodium hypochlorite had promotive effects on the germination of freshly harvested wild-rice seeds (Table 5). Germination increased as hydrogen peroxide concentrations increased from 0 to 10% and was greater with 8 hour treatments than with the respective 2 hour treatments. Although hydrogen peroxide increased germination percentages over the water control, seedling root and shoot growth were slow, a probable cause for poor survival after planting these seedlings in the greenhouse. The reason for the promotive effect on germination is not known but could be due either to physical changes in the pericarp or oxidative reactions within the seeds as was reported to be the case in rice (*Oryza sativa* L.) (10).

Sodium hypochlorite had increasing promotive effects on germination with increasing concentrations from 0 to 2.63% when used as a 1 or 2-hour soak. Sodium hypochlorite concentrations of 5.25% apparently caused excessive damage to the pericarp and embryo since germination failed. Starch from the endosperm was observed oozing from these seeds and there was no germination. Greater germination and greater survival were observed in the 2-hour treatments than in the 1-hour treatments. The reason for increased germination is not known, but change in pericarp permeability could be a factor. The presence of bacteria or fungi was not noted in the water control. Therefore, increased germination and seed survival probably was not the result of seed sterilization by the chemicals.

Burton (2) suggested that the promotive effect of hydrogen peroxide and sodium hypochlorite on germination of dormant pearl millet seeds might be due to oxidation of germination inhibitors. Roberts (10) presented evidence indicating that high oxygen levels and oxidizing agents promote a possible non-enzymatic oxidation reaction in rice seeds that breaks their dormancy. Oxidation reactions such as these could possibly occur in wild rice seeds.

Soaking dormant wild rice seeds for 1 hour in a 2.63% sodium hypochlorite solution appears to be a good way to obtain up to 20% germination followed by good seedling survival without using the time consuming method of dehulling and scarification of seeds. Also, pretreating wild rice seeds with ethanol in combination with chloroform is suggested as a way to decrease the dormancy time period by 30 days for wild rice seeds which are to be stored in water at 2 C. This research also suggests that to increase seedling survival from seeds forced to germinate without a cold treatment by scarification, treating scarified seeds with 5×10^{-6} *M* GA_3 and 5×10^{-4} *M* BA solutions for 24 hour will substantially increase seedling survival.

REFERENCES

1. Albrecht, K. A., E. A. Oelke, and M. L. Brenner. 1979. Abscisic acid levels in the grain of wild rice. Crop Sci. 19: 671-676.
2. Burton, G. W. 1969. Breaking dormancy in seeds of pearl millet, *Pennisetum typhoides*. Crop Sci. 9:659-664.
3. Cardwell, V. B., E. A. Oelke, and W. A. Elliott. 1978. Seed dormancy mechanisms in *Zizania aquatica* L. Agron. J. 70:481-484.
4. Chen, S. S. C., and J. L. L. Chang. 1972. Does gibberellic acid stimulate seed germination via amylase synthesis? Plant Physiol. 49:441-442.
5. Esashi, Y., Y. Hata, and H. Katoh. 1975. Germination of cocklebur seeds: Interactions between gibberellic acid, benzyladenine, thiourea, KNO_3 and gaseous factors. Aust. J. Plant Physiol. 2:569-579.
6. Halstead, E. H., and B. T. Vicario. 1969. Effect of ultrasonics on the germination of wild rice (*Zizania aquatica*). Can. J. Bot. 47:1638-1640.
7. Hamilton, D. F., and P. L. Carpenter. 1975. Regulation of seed dormancy in *Cercis canadensis* L. J. Am. Soc. Hortic. Sci. 100:653-656.
8. Lado, P., F. Rasi-Caldogno, and R. Colombo. 1974. Promoting effect of fusicoccin on seed germination. Physiol. Plant. 31:149-152.
9. Oelke, E. A., and K. A. Albrecht. 1978. Mechanical scarification of dormant wild rice (*Zizania aquatica* L.) seed. Agron. J. 70:691-694.
10. Roberts, E. H. 1964. A survey of the effects of chemical treatments on dormancy in rice seed. Physiol. Plant. 17: 30-43.
11. Simpson, G. M. 1965. Dormancy studies in seed of *Avena fatua*. 4. The role of gibberellin in embryo dormancy. Can. J. Bot. 43:783-816.
12. Woods, D. L., and L. H. Gutek. 1974. Germinating wild rice. Can. J. Plant Sci. 54:423-424.