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A Comparison of Selection Methods for Reduced Shattering in Wild Rice[1]

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ABSTRACT

Current wild rice (*Zizania palustris* L.) cultivars are considered to be recessive for a two-complementary-gene system in which complete seed shattering is dominant. The strength of seed retention in these nonshattering cultivars is not adequate to prevent substantial field losses. To test for the existence of quantitative genetic variability above that conditioned by the two-gene qualitative system and to compare selection systems, two forms of selection were conducted in the cultivar Netum. The selection criterion was strength of individual seed retention on plant main-stems at maturity as measured with a hand-held spring operated meter. Two cycles of mass selection in the greenhouse and one cycle of half-sib family selection in the field, both at a 10% selection intensity, produced two populations which were compared to each other and to Netum at Grand Rapids and Excelsior, Minn. Progress from selection for tensile strength was 49.6% for two cycles of mass selection and 15.9% for one cycle of half-sib family selection. Realized heritability estimates for seed retention were 0.58 and 0.55 at the two test locations. Selection for tensile strength had no effect on flowering or maturity date.

Additional index words: **Quantitative variation, Heritability, *Zizania palustris*, *Zizania aquatica*, Tiller maturity.**

CULTIVATION of wild rice, *Zizania palustris* L. (Dore, 1969), previously classified as *Zizania aquatica* L., is a recent development that is largely attributable to the discovery of genotypes which shatter less than those in natural stands. Reduced shattering was recognized in a few plants by Paul Yagyu and Erwin Brooks, Department of Agronomy, University of Minnesota in 1963.

Woods and Clark (1976) in a preliminary study reported that shattering is dominant to nonshattering, and that control of shattering is simply inherited, perhaps involving only one or two genes. Elliott and Perlinger (1977) reported that segregation in crosses involving shattering and nonshattering types fit ratios expected in a two-complementary-gene system, where seed retention is conditioned by homozygous recessive alleles at either locus.

Even though the discovery of this qualitatively inherited seed retention characteristic permits the use of combines to harvest wild rice, the strength of seed retention of current cultivars is considerably less than in modern cultivars of other grain crops, and shattering losses prior to or at harvest frequently are large. Strength of retention declines rapidly after seed maturation, and seed can be dislodged from panicles by wind, contact with other panicles, birds, and other mild disturbances if harvest is not timely. An increase in seed retention above that available with the qualitative gene system would permit a greater harvestable yield by allowing later harvest, when more seeds are mature, and by reducing losses from environmental hazards.

Numerous reports in the literature have considered the inheritance of shattering in grasses but only those dealing with the reduction or elimination of an abscission layer are reviewed here, because of their similarity to wild rice shattering. The histological studies by Hanten (1975) and Hanten et al. (1980) emphasized the importance of the abscission layer attaching the spikelet to the pedicel, in the control of shattering in wild rice. The two cell layer, which extended from the exterior surface to the supporting tissue of the vascular bundle, plasmolyzed and displayed some cell separation in both shattering and nonshattering types. However, further development of separation pockets and complete separation were observed only in the shattering type of wild rice. Hanten et al. (1980) also reported the existence of a cone of sclerified cells surrounding the vascular cylinder just proximal to the abscission zone, which did not appear as well developed in shattering as in nonshattering types.

Abscission layer formation appears to have been eliminated from modern cultivars of most grain crops (McWilliam, 1980) with the possible exception of rice (*Oryza sativa* L.). However, inheritance and selection studies involving older cultivars and wild relatives indicate a trend for major gene control (one to three loci). Intermediate shattering types often occur and indicate the existence of modifying genes.

Tunis grass [*Sorghum virgatum* (Hack.) Stapf], a wild relative of sorghum [*S. bicolor* (L.) Moench], forms an abscission layer while modern cultivars do not. Crosses of tunis grass with several forms of cultivated sorghum revealed a two-complementary-gene control of abscission with abscission being dominant (Karper and Quinby, 1947).

In crosses between tetraploid oat species *A. barbata* Pott ex Link (shattering) and *A. abyssinica* Hochst. ex A. Rich. (nonshattering), duplicate dominant epistasis was observed (Ladizinsky, 1975). In crosses between hexaploid *A. byzantina* K. Koch and *A. sativa* L. (Kehr and Hayes, 1950), shattering was found to be single gene recessive, but intermediate types were reported. Modifying genes as well as suppressor genes appear to be added with the third genome in hexaploid oats (Jensen, 1961; Coffman and MacKey, 1959; and Hafiz, 1981).

In rice (*Oryza sativa*) an abscission layer has persisted even into modern cultivars (Zee et al. 1979) although genotypes which do not develop an abscission layer have been known for 50 years (Chandraratna, 1964). Reported segregation ratios for shattering in rice vary considerably with the material studied and range from single gene inheritance with shattering dominant to two or three genes with nonshattering dominant (implying perhaps a suppressor gene as in oats), to polygenic inheritance (Chandrar-

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atna, 1964). The latter is most common, especially when a quantitative measure of shattering is used (Vidyadharan and Ponnaiya, 1963; and Ramiah and Rao, 1936).

The structure of the abscission layer in rice is similar to that in wild rice (Hanten et al., 1980). Studies with rice indicate that variation in shattering is related to the degree of development of the subtending cone of sclerenchyma (Zee et al., 1979; Srinivas et al., 1979). *O. sativa* var. *'fatua'*, a shattering type, does not have a lignified cylinder of cells around the vascular tissue that excludes the abscission zone (Chalam, 1942).

The primary purpose of the research reported herein was to explore the usefulness of quantitative variation within a population of wild rice which has major gene resistance to shattering. Previous work (unpublished) from our wild rice breeding project has indicated differences in strength of seed retention within nonshattering populations, but no systematic evaluation of this variation had been made. The first objective of this study was to determine, by progress from selection experiments, if quantitative genetic variation for seed retention exists in a cultivar which has shattering resistance conferred by two recessive genes. A second objective was to compare the efficacy of mass selection and half-sib family selection for seed retention. Also, we estimated realized heritability for quantitatively controlled seed retention and evaluated flowering date and harvest maturity for correlated response to selection.

MATERIALS AND METHODS

Consideration of the wild rice reproductive system, project facilities, labor availability, and alternative selection schemes led to the choice of mass selection and half-sib progeny testing for comparative evaluation as alternative selection methods.

Mass Selection

Two cycles of mass selection for seed retention were performed in the greenhouse, one each during the fall seasons of 1979 and 1980 in the cultivar Netum which has resistance to shattering conferred by two recessive genes. Plants were handled using standard greenhouse technique (Elliott, 1980) for both mass selection cycles and for the recombination generation of half-sib family selection. Two seedlings each were planted in 15 cm pots submerged in water to approximately 1 cm above the top of the pot. Plants were thinned to one per pot when they were approximately 20 cm tall. Supplemental fluorescent lighting with an 18-h photoperiod was provided throughout the growing period and the temperature was 25 ±5°C. Plants were fertilized with 40 kg ha^{-1} of N as NH_4NO_3 at early boot stage.

In the first cycle of mass selection 5 to 10 seeds per main culm of 750 plants were measured with a tensile strength meter (Dial push-pull gauge model DPP-1 kg, John Chatillon and Sons, Inc., Kew Gardens, New York[3]). All data are reported as decinewtons (dN). The gauge was rigidly attached to a modified hemostat clip which closed on the seed. The gauge and clip were held parallel to the long axis of the seed which was kept in its natural position relative to the axis of the panicle branch. The retention of seeds of a panicle was determined when approximately 40 to 50% of the seeds on a panicle had darkened and the seeds could not be indented with the thumbnail. Selections were made within groups of 100 to 200 plants located in different greenhouse subsections using a 10% selection intensity among materials in each subsection.

The open-pollinated plants selected in the first cycle produced an average of 11.75 seedlings for establishing the C_1 population (range was 4 to 17). A total of 500 plants were grown for the second cycle of selection. Greenhouse growth conditions and evaluation of tensile strength of kernel attachment were similar to the first cycle.

A composite of seeds from 50 selected plants was made for the evaluation phase in 1981. A degree of balance was achieved by including 36 seeds from each of 40 families and all available seeds (range 3 to 33, mean 22.5) from the remaining families. This composite was designated N(M)C2.

Half-Sib Family Selection

In 1979, 226 random open-pollinated plants of Netum from a grower's paddy were individually harvested and progeny of each open-pollinated plant constituted a half-sib family. The families were hand-planted in a research paddy at Excelsior, Minn. on 6 May 1980. The families were planted in single-row unreplicated plots grouped into five incomplete blocks; four of the blocks contained 40 families each and one block contained 66 families. The single-row plots were 1.8 m long, spaced 60 cm apart, and contained approximately 15 plants. Standard paddy procedures (Elliott, 1980) were used in growing the half-sib family selection trial in 1980.

Random plants were tagged at flowering and were reviewed for maturity at 2-day intervals. Seed retention measurements were made on five seeds of one panicle per plant (usually the main culm) on each of five tagged plants per row. A within-block selection intensity of 10% was used in each of the five blocks.

Remnant seeds from each of the selected families were germinated and planted in the greenhouse, fall, 1980. Recombination was accomplished by open pollination of approximately 26 plants per family randomly distributed through the population. A balanced composite of seed from the recombination generation was made for the 1981 evaluation and was designated N(HS)Cl.

Evaluation of Progress from Selection

The two selected populations, N(M)C2 and N(HS)Cl, a 1980 source of the parent cultivar Netum, an unrelated cultivar 'K2' and a related population, experimental 3, were grown in the 1981 field season. Experimental 3 was derived from the same source population as Netum but had been further selected for plant type, early maturity, and seed retention. The five entries were planted at Excelsior (21 May) and Grand Rapids, Minn. (7 May) each with 15 replicates in a randomized complete block design. Each plot consisited of two rows 1.8 m long, 30 cm between rows of a plot, and 60 cm between rows of adjacent plots. Strength of seed retention and date of measurement were recorded on 10 randomly selected and pretagged plants per plot by the senior author at Excelsior and by a technician at Grand Rapids. Data recorded were tensile strength and date of measurement. An approximate measure of length of grain filling period for each plot was calculated using mean heading date and mean date of seed retention measurement.

In an effort to determine if there was an effect of measuring the first or second tiller instead of the main culm

[3] Mention of a trademark of proprietary product does not constitute a guarantee or warranty of the product by the Univ. of Minnesota, and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Mean kernel tensile strengths of Netum, the two selected populations and check entries by location and combined over locations.

Entry	Location		
	Excelsior	Grand Rapids	Combined
	dN		
Netum	8.4	12.0	10.2
N(HS)C1	10.5	13.2	11.8
N(M)C2	14.1	16.4	15.3
K2	8.1	13.2	10.7
Experimental 3	14.2	18.3	16.2
LSD (0.05)			
One-tailed t†	1.1	1.1	1.8
Two-tailed t	1.4	1.3	2.3

† For comparison of Netum with the selected populations.

when necessitated by breakage or sterility, strength of seed retention and date of measurement were recorded for the main-stem and two tillers of each of 50 plants of the Netum half-sib families in 1980, and 44 plants of the N(HS)Cl population and 52 plants of the K2 cultivar from the evaluation of selection progress experiment at Excelsior in 1981.

Data Analysis

Data were analyzed using the Statistical Analysis System (Statistical Analysis Institute, 1979). The progress from selection experimental results were analyzed using the General Linear Models procedure because of imbalance in the number of plants available per plot at Excelsior. The analyses presented were calculated on plant means using a sequential fitting of variables in the model (type I sum of squares). In each case the sum of squares (type IV) obtained by fitting each variable last in the model revealed virtually no change in estimates and thus the type I sum of squares analyses of variance were used.

Gain from selection was computed as the difference in kernel tensile strength mean of the selected population and the parental population. Estimates of realized heritability from mass selection were computed by location as the ratio of actual gain in kernel tensile strength to an estimate of cumulative selection differential.

To assess possible correlated response in flowering date or harvest maturity, flowering date, and harvest maturity means of the selected populations were compared to the original population.

RESULTS AND DISCUSSION

The 1981 evaluation trial at Grand Rapids was in generally good condition except for some stalk breakage due to wind. Tillers or alternate plants in the plot were measured to provide data when broken main-stems were encountered on the randomly selected plants. Ducks and deer at Excelsior reduced stands enough to introduce imbalance in the data. Some plants were available in all plots but 10 plants per plot were not always available.

Progress from Selection

Comparison of single location analyses indicated homogeneity of error and a significant entries effect for seed retention. The combined analysis indicated significant entries and locations (confounded with operator) effects as well as a significant entries × locations effect.

The mean tensile strengths of the entries at each location and combined over locations are given in Table 1. Based on means over locations, progress from selection was 15.9% with the single cycle of half-sib selection and 49.6% from two cycles of mass selection.

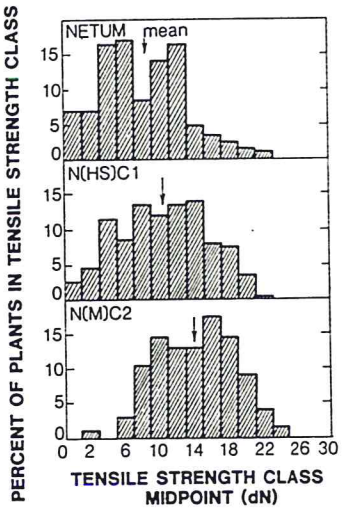

Fig. 1. Distribution of plant mean kernel tensile strength at Excelsior for Netum, N(HS)C1, and N(M)C2.

Netum and its subpopulations may be compared using the protected LSD based on a one-tailed t-test; all other comparisons should be based on two-tailed tests. For comparisons among the combined locations means, the entries × locations interaction mean square (4 df) was used in the F-test of entries and as the mean square error for the LSD. This explains the insignificant combined locations result for progress from selection for N(HS)Cl whereas the individual locations showed significance.

Progress from half-sib selection was probably real since N(HS)Cl was significantly greater than Netum at each location. However, the estimate of actual gain could be questioned because of genotype × environment interaction.

Gain from two cycles of mass selection was clearly superior to that from one cycle of half-sib family selection. The superiority of experimental 3, an unreleased population which had been mildly selected several generations for tensile strength and other agronomic traits, over Netum also indicated the effectiveness of single plant selection for seed retention.

Frequency distributions of plant mean kernel tensile strengths (Fig. 1 and 2) within each location indicate normal distributions of the seed retention of plants within populations except for Netum at Excelsior. The selected populations were not skewed and did not show any indication of reduced variance due to selection.

Correlated Response to Selection

Flowering date on a plot basis (emergence of the first floret of the main-stem of 50% of the plants), mean date of main-stem maturity and length of grain filling period were evaluated to detect possible correlated changes due to selection for seed retention. Because of deer grazing, flowering date data from four replicates at Excelsior were omitted. The combined locations analysis indicated significant entries and locations effects but not entries × locations. As was known from other trials (Stucker, 1981) K2 was

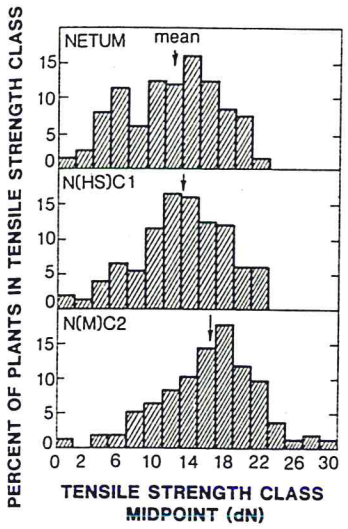

Fig. 2. Distribution of plant mean kernel tensile strength at Grand Rapids for Netum, N(HS)C1, and N(M)C2.

later than Netum (an average of 5 days in this trial) and experimental 3 was earlier (an average of 3 ½ days). Selection for tensile strength did not alter the flowering date of the selected populations compared to Netum, the parent cultivar (Table 2).

An analysis of mean date of main-stem maturity indicated significant locations and entries effects but no interaction. Mainstem maturity means (Table 2) paralleled flowering dates in that there was no correlated response to selection for tensile strength. Since consistent seed maturity was used to determine the date of tensile strength measurement, no shift in population maturity date with selection was expected.

An approximate measure of the grain filling period on a locations basis was obtained by computing the difference between mean flowering dates and mean main-stem maturity dates. An unprotected LSD was constructed using twice the pooled error variances of flowering dates and maturity dates for comparison of entries. There appear to be no real differences among entries for grain filling period at this level of resolution even though the Netum derived populations differed in maturity from K2 and experimental 3.

Tiller Evaluation

In the tiller evaluation experiments the analysis of variance of seed retention for Netum in 1980 indicated no differences (P = 0.14) among means of the main-stems, tiller #1, and tiller #2, across plants. In the 1981 evaluation of N(HS)Cl and K2, there was marginal significance (P = 0.08) for differences in means of main-stems and tillers. Across the three populations, stem rankings were not consistent (Table 3). The lack of consistent differences among mainstems and tillers justified the substitution of a tiller measurement when a main-stem was lost.

There was no entry by stem type interaction in the analysis of the 1981 data (Table 3). The difference in mean tensile strength of kernel attachment between the two entries based on means over stem type was significant (P = 0.002).

Tillers of a plant do not usually mature on the same day and are thus measured on different days with consequent operator and climatic variation introduced. Consequently the tiller-to-tiller within-plant variance can be used as an estimate of plant-to-plant environmental variance. The contribution of among-stem variation was calculated by using a nested classification analysis of the 1981 Excelsior data (Table 4). The variance component for plant-to-plant variation was about equal to that for tiller-to-tiller within-plant variation, and each was slightly less than 20% of the total variation within an entry on a seed basis.

Of interest also are the sequence periods of tiller maturation which were available from the 1980 Excelsior tiller measurement sample in Netum and the 1981 Excelsior tiller sample in N(HS)Cl and K2 (Table 5). Period #1 was the number of days between the maturity date of the main-stem and the first tiller, and period #2 was the number of days between maturity of the first and second tillers. In all three populations the average for period #1 was more than twice that for period #2 and ranged from 7 to 9 days. The variance for both periods was high, however, with a range of about 12 days for period #1 and 9

Table 2. Mean flowering date and mean date of kernel strength measurement (maturity) by location and combined over locations.

Entry	Location				Combined	
	Excelsior		Grand Rapids			
	Flowering	Maturity	Flowering	Maturity	Flowering†	Maturity‡
	days after 1 July					
Netum	14.5	42.7	19.0	48.4	16.8	45.5
N(HS)C1	14.7	42.0	18.0	48.7	16.4	45.4
N(M)C2	15.0	42.0	18.2	47.3	16.6	44.6
K2	20.3	47.1	23.1	53.2	21.7	50.2
Experimental 3	11.2	39.2	15.2	46.2	13.2	42.7

† LSD 0.05 combined over locations = 0.8.
‡ LSD 0.05 combined over locations = 0.9.

Table 3. Mean kernel tensile strength for main-stems and tillers in three populations measured at Excelsior.

Stem	Netum (1980)	N(HS)C1 (1981)	K2 (1981)†
	dN		
Main stem	10.7	9.3	8.0
#1 tiller	9.2	11.3	8.9
#2 tiller	8.8	9.8	8.7

† LSD 0.05 = 1.8.

Table 4. Analysis of variance of kernel tensile strength for N(HS)C1 and K2 in the tillers evaluation trial, assuming all sources of variation nested.

Source	df	Mean square	Variance component (dN)² ± SE
Entries	1	896.7	
Plants/entries	94	165.4	7.61 ± 2.99
Tillers/plants	180	56.0	6.09 ± 1.44
Seeds/tillers	1104	25.6	25.56 ± 1.19

Table 5. Tiller sequence periods† for N(HS)C1 and K2 (1981) and Netum (1980) at Excelsior.

Entry	Period #1	SD	95% C.I. of mean	Period #2	SD	95% C.I. of mean
Netum	9.0	3.8	±1.1	3.3	2.5	0.8
N(HS)C1	7.3	3.8	±1.0	2.8	2.7	1.0
K2	8.3	3.6	±1.0	3.0	2.7	1.0

† Period #1 = date of tensile strength measurement (maturity) of tiller #1 minus that of the main stem. Period #2 = date of measurement of tiller #2 − tiller #1.

Table 6. Components of error variance of kernel tensile strength in the evaluation of the selection progress experiment.

Variance component	Value of variance component (dN)²	SE
Among plots within entries and locations	1.04	0.23
Among plants within plots	16.45	0.80
Among seeds within plots	28.97	0.30

days for period #2 in the Netum and Netum derived populations. Period #1 approximated a uniform distribution more than a normal distribution.

The long average period of time between maturity of the main-stem and first tiller (7 to 9 days) relative to that between first and second tillers (3 days) is a major contributing factor in the field yield loss. The rapid loss in kernel tensile strength of the main-stem during the interval between maturity of the main-stem and the several tillers before harvest leads to the common observation of almost total seed loss from the main-stem. It also suggests that if genetic variation exists for length of this maturation synchrony period, selection for shorter period could be useful in reducing crop losses. There is a high degree of phenotypic variability for both tiller synchrony periods which suggests an exploitable genetic component, an observation also made by Foster and Rutger (1980).

Realized Heritability in Mass Selection

Substantial gain from two cycles of mass selection and a smaller but probably real gain from one cycle of half-sib family selection for kernel tensile strength were observed in this experiment. This progress can only be possible if substantial additive genetic variance for this trait exists in the population and if its heritability on a single plant basis is high. Since actual gain from half-sib selection was not significant, realized heritability was not computed for half-sib selection.

A form of realized heritability can be computed by location from the 1981 evaluation trial data. Ignoring block effects, which were small, the mean of the top 10% of the plants of Netum, minus the mean of the population provided an estimate of the selection differential (9.63 at Excelsior, 8.10 at Grand Rapids). Actual gain (two cycles) is the mean of N(M)C2 minus the mean of Netum at each location. Narrow sense heritability (h^2) on a plant mean basis is (actual gain per cycle/selection differential) × 2 since only one parent was controlled. The estimates are $h^2 = 0.58$ (Excelsior) and 0.55 (Grand Rapids).

Effect of Variability on Choice of Selection Method

Components of the error variance were calculated for the evaluation of selection progress experiment (Table 6). The largest components are those of seed-to-seed and plant-to-plant variance. In the tiller evaluation experiment it was observed that about half of the plant-to-plant variance could be accounted for by tiller-to-tiller (nongenetic) variation and thus perhaps half is genetic, which would be expected from the high realized heritability observed with mass selection.

Plot-to-plot variance was small and would not justify replicated family testing. Only one cycle per year is possible with half-sib family testing. However, one advantage of this method is the opportunity for multiple trait evaluation.

Mass selection appears to be superior for improving seed retention in wild rice. The effect of the principal component of error variance, that of seed-to-seed, was reduced by multiple seed measurement per plant and at least equal gain per cycle was observed in comparison with half-sib family selection. Mass selection in the greenhouse allows two cycles per year and is labor efficient since it does not compete with labor demands of fall field harvest.

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Seed Dormancy Mechanisms in Wild Rice (*Zizania aquatica*)[1]

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ABSTRACT

Freshly harvested seeds of wild rice (*Zizania aquatica* L.) require 3 to 5 months of moist storage at 1 to 3 C to induce germination. Dormancy lasting more than 1 year has been noted. These periods of dormancy pose problems to plant breeders desiring multiple generations per year and to growers desiring to change varieties in establish fields. The purpose of this research was to determine the role of the pericarp in seed dormancy, the existence of germination inhibitors, and the influence of gibberellin and kinetin on germination of dormant wild rice seeds.

The pericarp of seeds harvested the previous day was scraped off, punctured, or cut at several locations on the seed. Germination occurred only when the treatments were made directly over or very near the embryo, indicating mechanical resistance by the pericarp. Soil collected from fields 1 and 2 years out of production was screened for seed. The pericarps of nongerminating seeds were randomly punctured, increasing germination 33 and 79% respectively. This suggests an impermeable pericarp.

Freshly harvested seeds from which the pericarp was either scraped or not scraped were germinated in aqueous extracts of the pericarp, and hulls (lemma and palea) from freshly harvested seeds. Aqueous extracts of the pericarp reduced germination 77% while aqueous extracts of the hulls reduced germination 84%, compared to scraped seed germinated in water. Aqueous extracts of the hulls from seeds stored for 1 year had little influence on germination when used as germination media for freshly harvested scraped seeds. The hulls were removed from some freshly harvested seeds and not from others before storing for 1, 2, and 4 weeks in water at 1.5 C. Seedling survival after 30 days was significantly reduced when lemmas and paleas were left on the seeds 1 or 2 weeks during storage in water at 1.5 C. These experiments support the contention of growth regulators in the hulls of freshly harvested seed.

All combinations of 0, 0.01, 0.1, 1, and 5μM solutions of gibberellic acid (GA_3) and kinetin were applied to germinated dehulled, punctured seeds. Before seeds were dehulled and punctured, they were stored in plastic containers at 1.5 C for 90 days. Germination increased from 36 to 51% as GA_3 concentrations increased. Kinetin alone had little influence on germination except in combination with GA_3. Less etiolated seedlings were obtained when kinetin was included in GA_3 treatments. The addition of 5μM GA_3 + 1μM kinetin increased germination of freshly harvested, scraped seeds from 29 to 76%.

Wild rice appears to have multiple mechanisms of seed dormancy. The seed pericarp exhibits mechanical resistance and impermeability. Water soluble germination inhibitors appear to be present in hulls and pericarp, and gibberellic acid concentrations are low in freshly harvested seed. Freshly harvested seed can be germinated by dehulling and scraping, permitting multiple generations per year in breeding programs. Persistence of dormant seeds in fields will present problems in introducing new varieties.

Additional index words: Wild rice, Inhibitors, Germination, Impermeability, Growth regulators.

THE ability of wild rice (*Zizania aquatica* L.) to delay germination for prolonged periods after harvest was first reported by Brown and Scofield (1903) and Duvel (1906). Failure of seeds to germinate under conditions normally favorable to germination is termed dormancy. Historically, domestication of plants has resulted in selection of less dormant types. Wild rice represents a wild species in the process of being domesticated. Barton and Crocker (1953) have suggested that dormancy may be due to: (1) impermeability of seed coats to water (alfalfa [*Medicago sativa* L.], clovers [*Trifolium* spp.], and other legumes) or gases (cocklebur [*Xanthium pensylvanicum,* Wallr], cucumber [*Cucumis sativa* L.], and wild oats [*Avena fatua,* L.]); (2) mechanical resistance of the seed coat to expansion of the embryo (waterplantain [*Alisma trivale* Pursh], pigweed [*Amaranthus* retroflexus L.], and raspberry [*Rubus idaeus* L.]); (3) morphologically incomplete embryos (holly [*Ilex opaca* Ait.]); (4) physiological dormancy requiring stratification (swamp persiaria [*Polygonum coccineum* Muhl.], water pepper [*Polygonum hydropiperoides* Michx.], sugar pine [*Pinus lambertiana* Dougl.], and Chinese maple [*Acer truncatum* Bunge], or light of appropriate intensity, wavelength or duration (lettuce [*lactuca sativa* L.], birch [*Betula* sp], and Canadian hemlock [*Tsuga canadensis* Carr.]; and (5) chemical inhibition of metabolic events (sugar beets [*Beta vulgaris* L.], lovegrass [*Eragrositis lehmanniana,* Nees], and wild oats [*Avena fatua* L.]).

Muenscher (1936) observed no germination of wild rice after 60 days of storage in water at 1 to 3 C, but near maximum germination in samples taken after 150 days of storage. Simpson (1966) observed initial loss of dormancy began after 96 days of storage at 1 to 3 C in water. Maximum germination was reached after 192 days of storage. This suggests a physiological form of dormancy but Simpson (1966) observed no effect from gibberellic acid treatments. Using wet seed stored 128 days, Simpson increased the germination percentage four to 10-fold by a single puncture in the scutellar region of the seed coat of dehulled seeds with a dissecting needle, a response suggestive of seed coat impermeability as a dormancy factor. Further support for seed coat impermeability as a factor of dormancy was presented by Halstead and Vicario (1969) who reported no germination for untreated seeds removed from 1 to 3 C wet storage 90 days after harvest. Treating seeds with ultrasonic vibrations at 70 kc/s for 10 min resulted in 74% germination.

LaRue and Avery (1938) were able to germinate immature seeds on agar and Woods and Gutek (1974) report germination of freshly harvested wild rice following removal of the lemma and palea and scraping the pericarp from above the embryo with a scalpel. This would apparently rule out the possibility of morphologically immature embryos as a cause of dormancy.

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Table 1. Effects of treatments on germination of freshly harvested *Z. aquatica* seeds.

Treatments	Germination after 7 days
	%
Check	0
Pericarp punctured	0
Pericarp removed; dorsal side distal to embryo	0
ventral side opposite embryo	0
over embryo	55
Pericarp cut over and parallel to embryo	10
Pericarp cut on either side of embryo	25
L.S.D. 0.05	11.0

The objectives of these investigations were to explore the role of the pericarp on seed dormancy, determine if inhibitors are present, and study the influence of gibberellin and kinetin on germination of dormant wild rice seeds.

MATERIALS AND METHODS

Role of Pericarp in Seed Dormancy

Experiment 1. In 1973,, wild rice seeds were dehulled (lemma and palea removed by hand) and the pericarp partially removed, punctured, or cut within 24 hours after harvest. Pericarp tissue was removed by scraping with a razor blade over the embryo, on the ventral side of the seed opposite the embryo, and the end opposite the embryo. Other treatments in which pericarp tissue was not removed included puncturing the pericarp with a dissecting needle three times on each side of the embryo area, cutting through the pericarp with a razor blade immediately over and parallel to the embryo, and cutting through the pericarp on both sides of the embryo. Four replications of 10 seeds of each treatment and a control were germinated in petri dishes on the laboratory bench (22 ± 2 C) in deionized water.

Experiment 2. Soil samples were taken in late June from three wild rice fields for a period of 2 years after they had been in wild rice. Wild rice seeds were removed from the peat soil by sieving and picking by hand from 0.9 m^2 × 30 cm deep samples and allowed to germinate for 3 weeks in water at 26 C. The pericarp of any seeds that did not germinate after a period of 3 weeks was punctured three times on each side of the embryo. These were allowed to germinate an additional 3 weeks. Seeds failing to germinate after puncturing were tested for viability using 1% solution of 2,3,5-Triphenyl-2H-tetrazolium chloride (tetrazolium). The tetrazolium procedures suggested by Grabe (1970) for large-seeded grasses was followed, modified using a 1% solution rather than the suggested 0.1%.

Presence of Germination Inhibitors

Experiment 1. To determine if germination inhibitors were present, freshly harvested seeds were germinated in water with hulls or pericarp scrapings added. Ten scraped seeds and their hulls were placed in a petri dish for germination in distilled water. A second treatment consisted of returning pericarp scrapings from 50 seeds to the germination media (water) containing 10 scraped seeds. The check treatments included dehulled nonscraped and dehulled scraped seeds. Germination counts were taken after 7 days. Seeds failing to germinate when the hulls were included in the petri dish were rinsed in and placed in distilled water without the hulls for another 7 days before taking final germination counts. Each treatment was replicated three times using a randomized complete block design.

Experiment 2. The hulls of freshly harvested seeds and hulls of seeds stored in water at 1.5 C since the previous fall (approximately 1 year) were air-dried or dried for 24 hours at 68 C and then ground through a 50μ (30-mesh) screen. Samples weighing 3.2 g (hulls of approximately 1,000 seeds) were soaked 24 hours in 100 ml of distilled water. After filtering through Whatman No. 1 filter paper, a one-tenth dilution series, starting with 3.2 g/100 ml and continuing to 0.0032 g/100 ml, was established. Ten dehulled, freshly harvested seeds, scraped as described by Woods and Gutek (1974) were germinated in each dilution and a water check. The treatments were replicated five times using a randomized split plot design with sources of hulls or hull treatment as main plots and dilutions as subplots.

Table 2. Effect of pericarp scrapings and hulls of *Z. aquatica* added to germination media on germination of freshly harvested scraped seeds.

Treatments	Germination†	Treatment-1
	%	
1. Seeds scraped—distilled water	43.3	100.0
2. Check—no scraping—distilled water	0.0	0.0
3. Seeds scraped—hulls added	6.7	15.5
4. Seeds scraped—pericarp scrapings added	10.0	23.1
5. Ungerminated seeds of treatment 3—distilled water‡	50.0	115.5
L.S.D. 0.05	28.8	

† 7-day germination. ‡ Germination count taken 7 days after removing hulls.

Experiment 3. To determine if leaving the hulls on the seed during storage in water influences seedling growth and survival, the hulls were removed from some freshly harvested seeds and not from others before storing for 1, 2, and 4 weeks in water at 1.5 C. In addition, a third group of seeds were dehulled and scraped and also stored in water at 1.5 C for 1, 2 and 4 weeks. After storage in water for the prescribed length of time, the seeds were removed and those which were not scraped before storage were scraped before testing for germination in water at 24 C. The resulting 1-week-old seedlings were transplanted into flooded soil in the greenhouse. The seedlings were measured for height and leaf number before transplanting, and again 30 days later. Tiller number was determined 30 days after transplanting. Each treatment was replicated four times with 25 seeds per replication, using a randomized complete block design.

Gibberellin and/or Kinetin Influence on Germination of Dormant Seeds

Experiment 1. Wet seeds (40% moisture) stored without water at 1.5 C for 90 days in plastic covered containers were dehulled and surface sterilized with 0.5% solution of sodium hypochlorite for 15 min. The pericarp of each seed was then punctured several times with a dissecting needle and the seeds were germinated in sterilized petri dishes containing solutions of GA_3 and kinetin alone and in combination. All procedures were conducted under aseptic conditions and the seeds were germinated in an incubator kept at 26 C. All combinations of 0, 0.01, 0.1, 1, and 5μM solutions of GA_3 and kinetin were used. Each treatment was replicated four times using a randomized complete block design with 20 seeds per replication. Germination counts were taken at the end of 14 days.

Experiment 2. Freshly harvested seeds and seed stored for 130 days at 1 to 3 C were dehulled and surface sterilized with 0.5% solution of sodium hypochlorite. Freshly harvested seeds were scraped under aseptic conditions. All seeds were germinated in sterilized petri dishes containing a solution with 5μM of GA_3 and 1μM of kinetin. One set was germinated on the laboratory bench at ambient room temperature (22 ± 2 C) and another set was germinated at 24 C day and 14 C night temperature in an illuminated incubator. Florescent lighting was supplied during the 16 hour day period. Four replications of 50 seeds each were arranged in a randomized block design.

RESULTS AND DISCUSSION

Role of Pericarp on Seed Dormancy

Removal of the pericarp over the embryo resulted in a germination of 55% compared to 0% when the pericarp was left intact on freshly harvested seeds (Table 1). Removal of the pericarp directly above the embryo was necessary, since removing it from the dorsal side distal to the embryo or from the ventral side opposite the embryo did not result in any germination. Punc-

Table 3. Effect of aqueous extracts of hulls of freshly harvested and 1-year-old seeds of *Z. aquatica* on germination percentage of freshly harvested scraped seeds.

Weight of hulls extracted	Hulls (lemma and palea)		Fresh hulls		
	Dried at 68 C				
	Old	Fresh	Air-dried	Dried at 68 C	Mean†
g/100 ml	%				
3.2×10^{-2}	69	48	35	40	41
3.2×10^{-3}	73	62	51	72	62
3.2×10^{-4}	73	67	72	67	69
3.2×10^{-5}	68	74	73	76	74
0 (water check)	69	66	66	66	66
L.S.D. 0.05—dilutions	NS	NS	16	16	11
L.S.D. 0.05—hulls at one dilution	10			NS	
L.S.D. 0.05—hulls × dilutions	NS			NS	

† Mean of all freshly harvested hull extraction experiments.

turing the pericarp three times on either side of the embryo did not cause germination. Weakening the pericarp by making a cut over or on either side of the embryo allowed some seeds to germinate, but maximum germination was obtained by removing the pericarp over the embryo as described by Woods and Gutek (1974). The lack of germination from treatments other than the direct removal of pericarp over the embryo suggests the possibility of some mechanical resistance to emergence of the coleoptile and radicle or germination inhibitor in the pericarp of freshly harvested seed.

The earlier study of Simpson (1966) working with wet stored seeds having undergone at least a 90-day postharvest "after ripening" period suggested the pericarp acted as an impermeable structure restricting diffusion of gases or water. We found supportative evidence for this because it was necessary to puncture the pericarp of seed that had been in the soil for 1 or more years before some of the seeds would germinate. Thirty-three percent of the viable seeds found in fields 1 year after the last crop and 79% of seeds found in the soil after 2 years had to be punctured before they would germinate. Puncturing old dormant seeds stimulated germination, whereas disruption of the pericarp of freshly harvested seeds produced no germination unless the pericarp over the embryo was removed. These data are supportive of the hypothesis that pericarp permeability to gases is a major cause of dormancy after the postharvest after-ripening period is completed but factors other than temperature, oxygen, water, and light restrict germination during the initial postharvest period.

Presence of Germination Inhibitors

Although many dormant seeds will germinate when the pericarp is removed above the embryo, often seedling vigor is poor resulting in a low survival rate of the seedlings. This prompted a check for inhibitors in the hulls (lemma, palea) and pericarp. A significant reduction (6.7 vs. 43.3%) in germination of freshly harvested scraped seeds resulted when hulls or pericarp scrapings were added to the germination media clearly indicating the presence of a water soluble inhibitor (Table 2). Removing the hulls after 7 days,

Table 4. Percent germination and seedling growth of *Z. aquatica* after 1 week in water as influenced by hull removal before storage in water at 1.5 C.

Storage period	Prestorage treatment of seeds	Seedling measurements		
		Length	Leaves/plant	Germination
Days	Hulls	cm		%
7	On†	2.9	1.3	39
	Removed†	4.4	1.6	51
	Removed and scraped‡	3.6	1.5	46
	L.S.D. 0.05	NS	NS	NS
14	On	1.5	1.3	37
	Removed	2.3	1.3	47
	Removed and scraped	2.6	1.6	46
	L.S.D. 0.05	NS	NS	NS
28	On	3.2	2.0	61
	Removed	3.5	1.9	49
	Removed and scraped	1.7	1.5	77
	L.S.D. 0.05	0.4	0.2	NS

† Pericarp removed above embryo after removal from storage.
‡ Pericarp removed above embryo before storage period.

rinsing the seeds, and placing them in distilled water for another 7 days resulted in a total germination equal to the scraped check. Pericarp scrapings restricted germination of scraped seed to levels similar to those obtained when hulls were present.

Aqueous extracts from hulls of freshly harvested seeds reduced the germination of dormant scraped seeds at the highest concentration of the hull extract (Table 3). Extract from the hulls of freshly harvested seeds reduced germination an average of 37% when the extract of approximately 100 hulls was used as the germination media. A similar extract from the hulls of 1-year-old seed had no inhibitory effects on freshly harvested scraped seeds. This loss of inhibition may be associated with the natural breaking of dormancy after 3 or more months of storage in water at 1 to 3 C as reported by Muensher (1936) and Simpson (1966). Drying hulls at 68 C had no effect on activity of inhibitors.

Evidence of germination inhibitor(s) in the hulls prompted the removal of hulls from same freshly harvested seeds before storage in water at 1.5 C for different time intervals to determine if the inhibitor(s) diffused from the hulls into the pericarp. The mean germination percent was lower and seedling length was less when the hulls were left on 7 or 14 days, compared to dehulled seeds stored for the same length of time, but differences were non-significant (Table 4).

Growth and survival measurements for seeds stored for different intervals of time were made 30 days after transplanting (Table 5). Height, tillers per plant, and leaves per plant were non-significant; however, transplanting survival percent was significantly reduced when the hulls were left on the seed for 7 and 14 days. After 28 days of storage, survival percent was the same whether the hulls were left on or not during this interval of storage. The data are not conclusive, but suggest that the germination inhibitor(s) located in the hulls may diffuse into the seed and surrounding water media during storage. During the initial stage of postharvest dormancy, the pericarp appears to exert its influence through chemical inhibitors and

Table 5. Seedling growth and percent survival 30 days after transplanting into flooded soil of *Z. aquatica* seedlings obtained from seeds with or without hulls during different storage periods in water at 1.5 C.

Storage period	Prestorage treatment of seeds	Plant measurements			
		Height	Tillers/ plant	Leaves/ plant	Survival
Days	Hulls	cm			%
7	On†	60	0.4	5.5	16
	Removed†	72	0.6	6.2	46
	Removed and scraped‡	62	0.4	6.1	38
	L.S.D. 0.05	NS	NS	NS	10
14	On	48	0.3	3.4	13
	Removed	72	0.9	7.4	33
	Removed and scraped	63	0.7	6.5	31
	L.S.D. 0.05	NS	NS	NS	15
28	On	73	0.7	6.9	43
	Removed	79	0.9	8.2	43
	Removed and scraped	73	1.1	8.2	36
	L.S.D. 0.05	NS	NS	NS	NS

† Pericarp removed above embryo after removal from storage.
‡ Pericarp removed above embryo before storage period.

mechanical resistance rather than permeability restrictions as found in older seeds.

Gibberellin and/or Kinetin Influence on Germination of Dormant Seeds

Germination response of dormant (freshly harvested), partially dormant (stored 90 days), and non-dormant (stored 130 days) wild rice treated with gibberellic acid (GA_3) was differential. GA_3 treatments stimulated germination in freshly harvested seed and partially dormant seeds, but had no effect on non-dormant seeds (Tables 6 and 7). Simpson (1966), using seed stored 182 days, observed no response to GA. The data are not conclusive but indicate either endogenous GA levels are low in freshly harvested seeds and build up during the period of low temperature storage or, the effect of natural inhibitors is suppressed by the application of exogenous sources of GA.

Kinetin alone in the absence of GA_3 had no significant effect on germination percent, but in combination with GA_3 differences were noted (Table 6). Kinetin at 1μM in combination with GA_3 produced less etiolated seedlings with more normal root development than were obtained with GA_3 alone, thus kinetin was included in GA treatment reported in Table 7.

In summary, inhibition of germination in freshly harvested wild rice seed stored less than 90 days is primarily a function of chemical (physiological) dormancy imposed by inhibitors located principally in the lemmas and paleas and to a lesser extent in the pericarp. There may also be some mechanical resistance of the pericarp. This dormancy can be broken by scraping the pericarp over the embryo as shown in our experiments and by Woods and Gutek (1974). Stratifying the seeds at 1 to 3 C for 90 days will normally break dormancy. Dormancy which persists after 3 to 5 months of storage or in the field is probably imposed by impermeability of the pericarp since germination is markedly increased by puncturing the seeds as shown in this study and the earlier work of Simpson (1966). Reduced seedling vigor and survival of seedlings from freshly harvested seeds forced to break dormancy by scraping may be due to inhibitors and interaction with growth regulators in the seeds. Gibberellic acid at 5μM stimulates germination and in combination with kinetin gives normal appearing seedlings. Response to GA is not observed in non-dormant seeds. Plant breeders can obtain multiple generations per year by scraping and treating with GA and kinetin, but dormant wild rice seeds in fields will still be a problem to growers wanting to change varieties.

Table 6. Influence of GA_3 and kinetin on germination percentage of partially dormant (stored 90 days at 40% seed moisture and 1.5 c) seeds of *Z. aquatica*.

Kinetin levels	GA_3 levels (μM)					
	0	0.01	0.1	1	5	Average
μM	%					
0	36.2†	31.5	46.2	45.0	51.2	42.0
0.01	25.0	37.5	43.8	36.2	50.0	38.5
0.1	35.0	27.5	46.2	50.0	48.8	41.5
1	43.8	43.8	51.2	50.0	48.8	47.5
5	37.5	46.2	50.0	51.2	46.2	46.2
Average	35.5	37.2	47.5	46.5	49.0	

† L.S.D. 0.05 for treatment means = 14.6%.

Table 7. Influence of GA_3 and kinetin on germination of freshly harvested *Z. aquatica* seeds (dormant) and seeds stored in water at 1 to 3 C for more than 130 days (nondormant).

Germination temperature	Dormancy of seed	Germination medium	
		Water	GA_3 + kinetin†
		%	
Constant 21 C	Dormant	24	59*
	Nondormant	44	43
Alternating 14–24 C	Dormant	29	76*
	Nondormant	55	57

* Significant at the 0.05 confidence level.
† 5 μM GA_3 + 1 μM kinetin.

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