WILD RICE BREEDING AND GERMPLASM IMPROVEMENT

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Variety Trial

As in past years, variety trials are an important component of the breeding project. In them we test new potential cultivars against released cultivars. We are especially interested in how Franklin continues to perform, especially in terms of shattering resistance. We continue to test pistillate entries for yield, and this year we tested some pistillate population hybrids, as well, to observe whether hybrid vigor is significant in this type of hybrid.

Materials and Methods

Although we planted variety trials in two locations, only one location yielded data this year--Waskish (Rennemo farm). As usual, we planted each entry in 4-row plots, with 12 inches between rows and 36 inches between adjacent plots. Rows were 10 feet long, and alleys were 6 feet wide to allow maneuverability with the binder (plot harvester). Pre-plant fertility was consistent with the rest of the production paddy: 120 lb/A of potassium, 30 lb/A of phosphorus, and 50 lb/A of nitrogen as urea. One topdress equivalent to 30 lb/A of N as urea was applied to all plots by hand at late boot-early flowering.

Table 1 lists the entries harvested along with the results of the trial. Sunshine is a new entry, being a selection from K. Petrowske, who developed Petrowske Bottlebrush. The other checks originally came from the sources listed in parentheses, and are being grown out in isolation at NCES as a seed source for the following year's trials in order to maintain consistency. This is being done in response to the suggestions of the Crop Variety Review Committee and other University breeders. Several of the entries in this year's variety trial were hybrids of two distinct pistillate populations. The hybrids were formed in 1993 by interplanting one parent in an isolated stand of the other and roguing out normal (male-fertile) plants, leaving only pistillate plants to be pollinated by the surrounding population. Reciprocal crosses were made in this fashion between either K-1 Pi or K-2 Pi as one parent and PM3(E)C3 as the other parent. In addition, a cross of non-pistillate K-1 with PM3 had been reselected in the F_2 and F_3 generations for pistillate plants. We would expect this population, PM3 x K-1C4 F4, to show little (if any) heterosis (hybrid vigor) by the F_4 generation, even though it should have about 50% pistillate plants like the F_1 hybrids.

Flowering date (50% of mainstems with at least some male branches emerging) was noted for each plot and averaged to determine relative maturity. Based on this information and on observations of the incidence of dark seed in a plot, we harvested the earliest two entries, Sunshine and Voyager, on August 24, then the remaining entries on August 29. The latest entries were mostly ones containing 50% pistillate plants (except for SSC5, which was later than even the pistillate entries). Although we thought these late entries could have benefitted from more maturing time, many of the plants were lodging by the time of the second harvest, so we harvested all the rest of the plots at the second harvest date.

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Results and Conclusions

This year we obtained data for processed yield, based on percent recovery as analyzed by a private laboratory used by the wild rice growers (Northern Rice Labs, Inc.). Percent recovery is multiplied by actual yield to estimate the amount of finished wild rice (i.e., after processing). Table 1 shows yield, shattering, and maturity related variables (flowering date and moisture) in the six replicates harvested, as well as recovery and processed yield in the two replicates used for this analysis.

Table 1 Yield and shattering losses of wild rice variety trial entries at Waskish, 1994.

Entry	Flowering date	Yield	Shattering	Moisture	Recovery	Processed yield
	days[1]	lb/A[2]	%[3]	%[4]	%[5]	lb/A[6]
First Harvest						
Sunshine (Petrowske)	78	1024	19	38	42	326
Voyager (Gunvalson)	79	1091	11	38	43	362
Second Harvest						
NACH-B (Manomin)	82	1339	18	31	44	542
K2 (Godward)	82	1364	23	29	46	356
Franklin (certified)	82	1516	13	30	42	436
K-1 Pi[7]	81	1507	16	29	42	429
K-2 Pi[7]	82	1317	17	29	43	408
Petrowske BB (Imle)	81	1699	15	31	45	532
PLAR Pi[7]	83	1516	17	25	45	396
PM3 x K-1C4 F4[7]	85	1398	15	31	40	406
PM3(E) x K-1Pi[7]	81	1902	12	29	41	566
K-1Pi x PM3(E)[7]	84	1293	12	31	39	377
PM3(E) x K-2Pi[7]	83	1416	14	30	40	390
K-2Pi x PM3(E)[7]	84	1636	11	31	40	602
PM3(E)C3 Pi[7]	85	1370	14	30	41	392
SSC5	87	800	12	34	36	262
mean	82	1387	15	31	42	424
LSD 5%	2	341	6	3	4	161

1 Days after May 1

2 Adjusted to 40% moisture (mean of 6 reps)

3 Expressed as a percentage of harvested plus shattered grain (mean of 6 reps)

4 Grain moisture at harvest--usually related to maturity (mean of 6 reps)

5 Estimated by Bob Schwob, Northern Rice Labs, Inc. (mean of 2 reps)

6 Actual yield x percent recovery (measured in only 2 of 6 reps)

7 Populations containing approximately 50% pistillate plants

Not all of the later entries were at optimum maturity at harvest, as indicated by percent moisture and percent recovery. Therefore, they probably did not reach their full yield potential by the time of harvest. Nevertheless, two of the pistillate entries were in the top three entries for yield and were also the top two for processed yield. Franklin yielded about 180 lb/A less unprocessed grain than Petrowske Bottlebrush, and about 400 lb/A less processed grain than PM3(E) x K-1Pi. In this year's trial, Franklin's shattering losses were not the lowest, probably because of differing maturity of entries. Sunshine, Voyager, and SSC5 were harvested slightly earlier than optimum maturity as indicated by higher percent moisture and lower yield, probably resulting in less shattering losses relative to other entries.

Table 2 shows that the actual proportion of pistillate plants in pistillate hybrids and their parents ranged from 40 to 51%, and that yield ranged from 1293 to 1902 lb/A (at 40% moisture). When yield of each hybrid is contrasted to the mid-parent mean, only one F_1 hybrid's contrast shows a significant difference (i.e., increase in yield associated with the hybrid)--PM3(E) x K-1Pi. The difference is large (32% or 463 lb/A). We expected the reciprocal of this F_1 hybrid (K-1Pi x PM3(E)) to also show positive heterosis, but it showed a 10% decrease. As expected, the only F_4 of a hybrid (PM3 x K-1C4 F4) showed no residual heterosis. The F_1 hybrids with K-2 Pi as a parent have positive values for heterosis (5 and 22%) but they are not significant. So, although evidence for heterosis is not consistent, there is an indication of heterosis in at least one of the F_1 hybrids. These hybrids will be tested again in 1995.

Table 2 Yield of wild rice population hybrids and their parents, and heterosis of hybrids with respect to the mid-parent mean--Waskish, 1994

Entry	Pistillate plants	Yield	Midparent yield	Yield difference	Heterosis
	%[1]	lb/A[2]	lb/A[2]	lb/A[2]	%[3]
K-1 Pi	46	1507	--	--	--
K-2 Pi	51	1317	--	--	--
PM3(E)C3 Pi	45	1370	--	--	--
PM3 x K-1C4 F4	46	1398	1439	-41	-3
PM3(E) x K-1Pi	48	1902	1439	463	32 **
K-1Pi x PM3(E)	40	1293	1439	-145	-10
PM3(E) x K-2Pi	48	1416	1344	72	5
K-2Pi x PM3(E)	44	1636	1344	292	22

1 Calculated from whole-plot counts in 2 reps

2 Adjusted to 40% moisture (mean of 6 reps)

3 (Hybrid yield - Midparent Yield) ÷ Midparent Yield expressed as a percentage

** Statistical contrast of hybrid vs. parents is significant at the 0.01 level

Table 3 shows the comparison between Franklin and three other currently grown cultivars during a four year period, in seven trials at four different locations. Franklin's yield was exceeded slightly by Petrowske Bottlebrush, but Franklin had significantly less shattering loss than the other three entries. This confirms the information given upon release of Franklin describing it as "more shattering resistant than K2 or other currently grown varieties, especially retaining more seed when harvest is delayed."

Table 3 Yield and seed shattering losses of wild rice varieties, 1991-94.

Entry	Grand Rapids[1]		Waskish[1]		Clearbrook[1]		Aitkin[1]		1991-94 Average[1]	
	Yield	Shat	Yield	Shat	Yield	Shat	Yield	Shat	Yield	Shat
	lb/A[2]	%[3]	lb/A[2]	%[3]	lb/A[2]	%[3]	lb/A[2]	%[3]	lb/A[2]	%[3]
Franklin	1450	12	1284	14	1319	16	1819	13	1417	13
K2	1449	21	1143	23	1391	21	1488	22	1350	19
Petrowske bottlebrush	1571	25	1254	31	1249	27	1862	14	1430	24
Voyager	1210	18	865	29	1110	32	1854	11	1175	23
LSD 5%	139	3	118	3	116	3	363	6	128	3

From Varietal Trials of Selected Farm Crops, 1995 Edition. Minnesota Report 235-1995. Minnesota Agricultural Experiment Station.

1 Means of trials at Grand Rapids in 1991 and 1992; Clearbrook, 1992 and 1993; Waskish, 1992 and 1994; Aitkin, 1993; and all 7 trials, respectively.

2 Adjusted to 40% moisture

3 Expressed as a percentage of harvested plus shattered grain

Controlled Pollinations

A large amount of our research time and effort this year went into crossing and selfing plants in the field and in the greenhouse. We have been making improvements in the materials and techniques made in these controlled pollinations. The new pollination bags and improved methodology are described below.

Materials

Past efforts to practice controlled pollinations in the field have met with little success. In the past the breeding personnel normally used a long pleated bag (18 in. by 2 in. by 1 in.) which had been treated for moisture repellence. Although this type of bag was long enough to contain the entire panicle in most cases, we observed that the bag would collect moisture from rain or dew. This moisture-laden bag would weigh the panicle down as it elongated, causing the stem to bend or break over into the water and making the panicle essentially useless. We tried lighter-weight glassine bags because they were smaller, were made of thinner paper, and were more water-proof. However, we observed two problems: first, the paper of the bags would still soak up water, but would be slower to dry out; second, stigma exsertion would sometimes be delayed, possibly due to accumulation of heat and humidity inside these relatively impermeable bags. These two problems made pollination difficult to carry out.

Last winter we made prototypes of a new smaller bag formed from lighter paper which was left untreated and was therefore more breathable than either of the bags used before. The bag was long enough (10 in. by 2 in.) to include a few male branches if selfing were desired, but it was not pleated, reducing the weight. The untreated thinner paper would not soak up as much water and would dry out more easily, reducing the time the panicles were weighed down and allowing pollinations in the field to begin earlier in the day.

Once we had tested a large number of the prototypes in the greenhouse and were satisfied with the dimensions we had the bags custom made by Lawson Pollination Bags. We then proved them on a larger scale in our field pollination activities.

Methodology

We have also changed the methods of controlled pollinations using of the new bags. The changes are oriented toward increasing efficiency of pollinations (enabling more crosses to be made), and increasing the number of seeds per pollination (improving reliability). The new methodology is summarized below.

Crossing: When the plant is still in the boot stage, we place a lightweight pollination bag over the boot and flag leaf. This prevents outcrossing because once the panicle emerges, stigmas are exserted from the pistillate (female) florets and may be subject to pollination unless protected. When the pistillate part of the panicle has emerged fully into the bag, we exclude the staminate (male) florets from the bag before they open and shed pollen. Thus, only the pistillate branches remain in the bag. The opening of the pollination bag is folded over and stapled to seal it off. Stapling through one of the pistillate branches inside the bag enables the bag to remain in place through the wind and rain. We then identify the individual plant by marking the family number (or row) and plant number on the bags with a permanent marker or pencil. The pistillate portion of the panicle will be ready to be pollinated within 5 days after the stigmas are exserted from the florets--the sooner after exsertion, the better.

We bag panicles to be used as a pollen source in the afternoon before the pollination. Since the pollen is short-lived, the foreign pollen should lose its viability by the time the fresh pollen is collected from the bagged panicle. The pistillate inflorescence is clipped off or folded over inside the bag in order to make room inside the bag for most or all of the staminate branches. The row and plant number are recorded on the bag.

After the morning dew has evaporated, the pollen is collected by shaking or tapping the bagged panicle over small cups placed under the opening, or by inverting the bagged panicle and shaking the pollen into the bag itself, which is then removed to carry the pollen to the female. The pistillate bag is not removed for pollination in order to minimize its exposure to incidental pollen in the air, especially pollen from staminate florets of the same panicle which are usually opening by that time. Instead, the pistillate bag is slit with scissors at the top, the pollen is poured in, and the top of the bag is folded over and stapled shut as soon as possible. The pollen source is then recorded on the pistillate bag.

Selfing: For self-pollinations, we leave some staminate branches inside the bag instead of excluding them as described above. We staple the bag shut with some male florets inside. Once staminate florets begin to shed pollen, we usually invert and shake (or tap) the bagged panicle to facilitate pollen travel inside the bag from male florets to female florets.

Results

Using the prototype bags in the 93-94 greenhouse season, we were able to make 459 crosses and selfs which resulted in at least one seed. The actual number or weight of seeds was not recorded. Using the new custom-made bags and new crossing methodology, breeding personnel made 528 controlled crosses which resulted in at least one seed, and 888 selfs. Figures 1 and 2 show the distribution of the number of seeds resulting from a sample of 56 of the crosses and 56 of the selfs. The 56 representative crosses resulted in an average of 37 seeds, ranging from 2 to 124 for any given cross. The 56 representative selfed plants produced an average of 39 seeds per panicle, ranging from 1 to 133 seeds per panicle. In both cases, a majority of the pollinations resulted in 20 or more seeds (32 of the crosses and 37 of the selfs). Although no data are shown for seed set using the old pollination methods, nevertheless the results we show are indicators that successful pollinations can be made with the improvements. Twenty or more seeds is enough for a breeder to work with in most cases. Even one seed resulting from a self or a cross can be useful to a breeder.

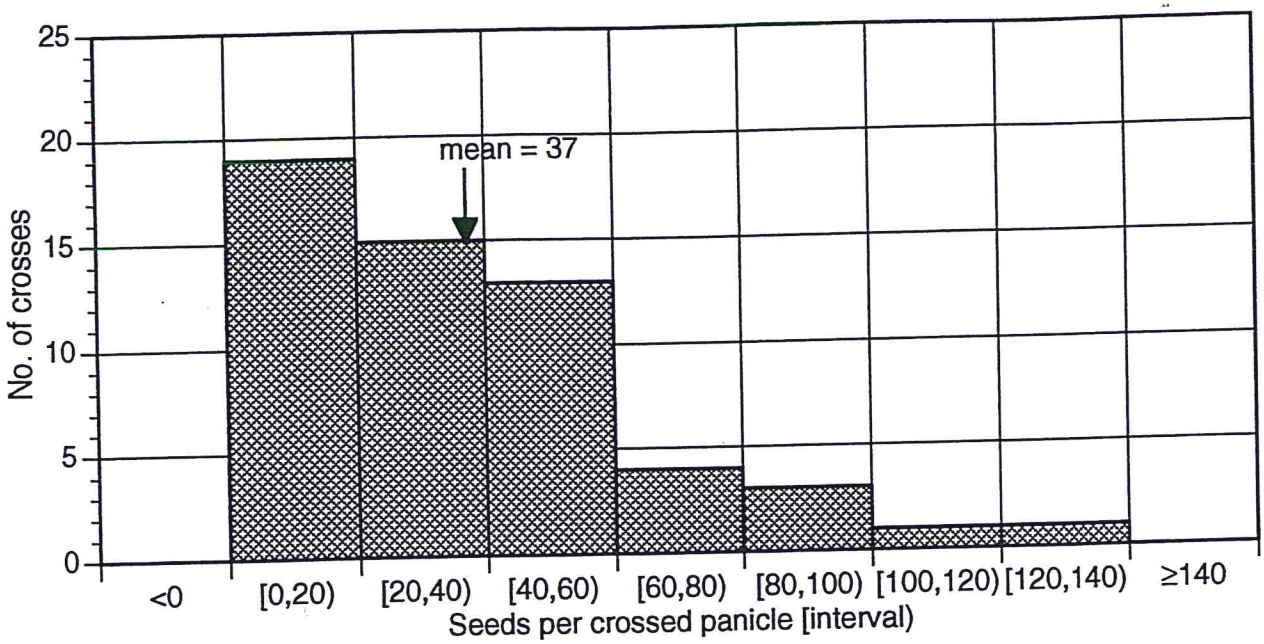

Figure 1 Frequency distribution of number of seeds resulting from 56 crosses of plants from diverse populations--1994 field nursery.

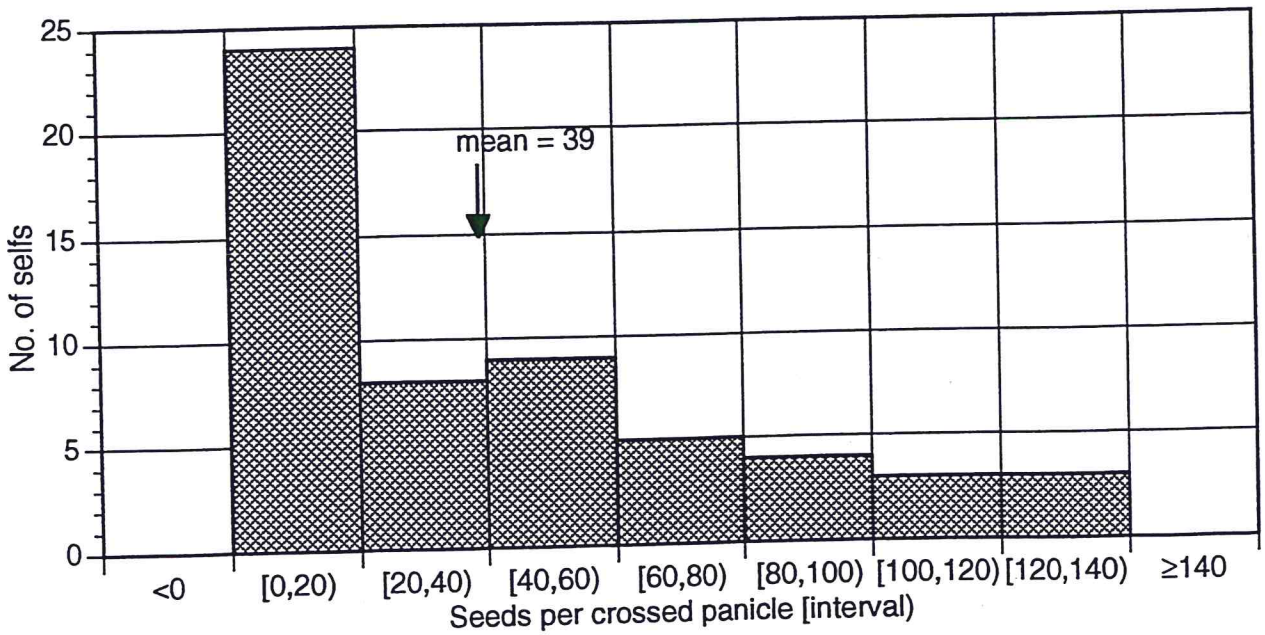

Figure 2 Frequency distribution of number of seeds resulting from 56 selfs of plants from diverse populations--1994 field nursery.

Disease Ratings (With R. Nyvall)

We continue investigating disease resistance in wild rice populations. This year we concentrated on disease ratings of half-sib families inoculated with Bipolaris spores at the Aitkin research site at the Vomela farm. This site has provided a good environment for disease development in the past, but we still inoculated in order to achieve uniformity of infection.

Materials and Methods

We planted 160 half-sib families of the population MK-1 at the Aitkin site. Row spacing alternated between 10 inches and 20 inches. Rows were 6 feet long with 4 foot alleys. Each row-plot was a different family which was replicated twice. The families were divided into 5 sets of 32 families each, each set planted as a contiguous block, so that the experiment could be analyzed as a "Sets-in-Reps" design. This allowed for variability within replicates to be separated out by blocking into sets. Plants at the late boot to early flowering stage were inoculated once with Bipolaris oryzae spores at dusk on July 31. Spore concentration was 100,000 per ml in a 0.05% gelatin solution as a "sticker." Inoculation was carried out using a CO_2 sprayer, covering as many leaves as possible in each row. Families were rated on September 1 in two ways. First, each family-row was rated for average lesion length, estimated in mm. Then each family was given a subjective evaluation for the strength of the chlorotic halos around the lesions--a 1-5 scale was used, 1 meaning absence of chlorotic halo and 5 meaning very pronounced halos. Because of earlier plant mortality, only 79 families which had plants to rate in both reps. These were used in the analysis.

In addition to the MK-1 families, 180 families of Petrowske Bottlebrush (Clearwater's 'P2') were grown in a similar "Sets-in-Reps" design, with 5 sets of 36 families. These were inoculated on the same date as the MK-1 families, except that a mixture of Bipolaris oryzae and Bipolaris sorokiniana spores was used. The concentration was 50,000 spores per ml of each species. Plants were rated for lesion size on September 2 as described above.

Results

The analysis of halo rating showed no significant differences among the ratings. Table 4 shows the results of the analysis for lesion size in the MK-f families. All sources of variation were significant, but most importantly, families within sets were found to be significantly different from each other. Since there was a significant difference between sets, variability within the replicates was great enough to discourage comparisons between families of different sets.

Table 4 Analysis of Variance of lesion size ratings among MK-1 half-sib families

Source	df	Sum of squares	Mean square	F-value	P-value
Rep	1	1.759	1.759	5.380	.0231
Set	4	5.689	1.422	4.349	.0033
Rep x Set	4	4.346	1.086	3.322	.0147
Fam (Set)	74	44.804	.605	1.852	.0044
Error	74	24.199	.327		

Based on the mean squares, we calculated heritability (narrow-sense) to be 56%, which is moderate. However, the estimate of additive genetic variance upon which heritability is

based may be inflated by any genotype by environment effect, since the experiment was carried out at only one location. From the same analysis, we calculated the expected gain from selection among half-sib families to be approximately 0.25 rating units (or mm) per cycle. To our knowledge, these are the first estimates of heritability and gain from selection for Fungal Brown Spot Disease rating. Now that we have a reliable methodology for inoculating and rating wild rice families, we hope to continue to investigate this trait for heritability, expected and actual gain from selection, while concurrently carrying out our recurrent selection program in several populations for resistance to these diseases.

Thirteen of the 79 families rated in both replicates were also rated by Jason Brantner for sensitivity to the toxin ophiobolin. However, there was a very low correlation between his ratings and either lesion size or halo rating in the field (J. Brantner, personal communication).

The analysis of Petrowske Bottlebrush families showed no statistically significant differences among families. Nevertheless, we selected the best 17 of 89 families (average rating of 2.5 or less) and harvested the seed for a second cycle of selection.

Shattering Testers

We continue to devote a large share of our time in the winter greenhouse to developing tester lines which have one or the other major shattering gene. This winter we have planted 129 nonshattering S_3 lines which are derived from 18 S_2 nonshattering lines, as well as 102 shattering S_3 lines which are derived from 10 S_2 shattering lines. All the current lines originated from 8 S_0 parent plants.

Once these tester lines are identified, they can be crossed to plants of a population or cultivar, and progeny can be evaluated to identify which lines from the population or cultivar are free of both shattering genes. In order to identify the tester lines, we are crossing different nonshattering lines to each other and observing the progeny. If the crossing of two phenotypically nonshattering lines results in shattering progeny, the lines are complementary. We are currently evaluating 37 progeny from 5 such crosses made in the 93-94 greenhouse season. So far, none of the crosses has identified complementary lines, but new combinations are being made this season. In addition, we plan to increase and test some of these noncomplementary crosses (i.e. all nonshattering) to accelerate the release of a cultivar which is fixed for at least one of the shattering genes.

In conjunction with this work, we will continue to work with Dr. Phillips' laboratory to identify RFLP markers which are associated with the shattering genes. Either or both of these two ways of identifying shattering genes should prove useful in future efforts to develop and release cultivars which lack major shattering genes. Populations lacking these shattering genes may also prove easier to improve for quantitative shattering resistance.

Nondormancy

We are also continuing to select for nondormancy as we carry the trait by backcrossing from a Florida Zizania aquatica population to Z. palustris cultivars. We are completing the fifth backcross generation, which should result in a population which contains 97% Z. palustris genes while still showing the nondormancy trait. So far, we have made over 100 backcrosses in the 94-95 greenhouse season, many of which are BC_5, but some of which are BC_2, BC_3, and BC_4, as well.

Germplasm Collection and Preservation

Last summer, several teams from the breeding personnel were armed with canoes, poles, and special permits, and sent to collect seed from 9 natural stands in the Grand Rapids area: Laura Lake, Big Rice Lake, Moose Lake, White Elk Lake, Bowstring River, Rice Lake, Prairie Lake, Dora Lake, and Squaw Lake. In addition to collecting bulk seed from throughout each stand, we also collected seed from plants with unusual characteristics, such as nonshattering, in hopes of finding new genes for nonshattering, or other new traits. Also, in several of these stands, the teams collected subsamples from different parts of the stand in order to compare the genetic differences within the stands with the differences among stands. These will be evaluated in field plots next year. A sample of seed from each collection was sent to Dr. Chris Vertucci of the National Seed Storage Laboratory in Ft. Collins, CO, in order to begin a pilot germplasm preservation project. When wild rice germplasm can be successfully stored to preserve viability over a long term, a repository of genes will be available for future breeding efforts.

Dr. Porter and Dr. Oelke have also collaborated with Dr. Vertucci in her research on Zizania seed storage. Using 'Franklin' and 'Johnson', she has developed a 'phase diagram' which identifies possible combinations of moisture content and temperature for optimum long-term storage. Details of this work can be found in the paper cited as follows:

Vertucci, C.W., Crane, J., Porter, R.A. and Oelke, E.A. 1994. Physical properties of water in Zizania embryos in relation to maturity status, water content and temperature. Seed Science Research 4:211-224.

We will continue to collaborate with Dr. Vertucci's lab to develop reliable seed pre-treatment protocols and storage conditions for maintaining maximum seed viability.

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