

Genetics and Utilization of Seed Nondormancy in Wild Rice

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Nondormancy is a trait that will aid the domestication of wild rice, as it has been key in the domestication of other cereals (Harlan 1992). Seed dormancy in wild rice is typically overcome by a three-month cold treatment but may last as long as seven years. Due to dormancy of fallen seed, growers have difficulty switching varieties in a paddy unless it has been fallow for at least three years or otherwise treated to kill the seed. Selection for nondormancy is therefore necessary to allow complete seed replacement by the grower each year. In addition, since dormant seeds which fall into paddy soil are the result of shattering, nondormant cultivars should reverse the natural selection for shattering which currently exists under continuous cultivation.

Because seed nondormancy is a key trait in domestication, selection for seed nondormancy within cultivars of *Z. palustris* has been attempted on several occasions. Stucker et al. (1987 and 1989) describe two previous unsuccessful attempts to initiate a selection program for reduced seed dormancy. These populations were not maintained, but the apparent progress from selection is an indication that there is some genetic variability for dormancy. Still, additive genetic variability, heritability, and expected response to selection were not estimated. Estimates of parameters would be useful to a breeder in weighing the potential for success of a recurrent selection program for reduced dormancy. Furthermore, since dormancy involves plant growth regulators (phytohormones), and shattering is a result of abscission, which may also involve some of the same plant growth regulators, there could be some correlation between shattering and dormancy. Such a correlation, if it exists, should be known by the breeder planning to select for both reduced dormancy and shattering resistance.

In 1990, Porter and Schumer (1991) obtained seeds from a *Z. aquatica* population in Florida which germinated within 1 week after harvest. Since Duvall and Biesboer (1988b) reported that *Z. aquatica* and *Z. palustris* crosses produced viable seeds if *Z. palustris* were the pollen parent, they crossed the Florida population with K2 and obtained F₁ seeds, which germinated within 1 week after harvest. That initial hybrid has since been backcrossed to *Z. palustris* cultivars available in the greenhouse (Porter and Schumer 1992; Porter et al. 1993 and 1994). Five generations of backcrossing have suggested that this nondormancy is highly heritable and not recessive. These results were similar to studies on dormancy in *Oryza sativa*, where 1 or 2 dominant genes have been shown to control the trait (Seshu and Sorrells 1985). Therefore, both quantitative and qualitative nondormancy need to be investigated. RFLP analysis of these independently derived BC₄ or BC₅ isolines should allow the identification of linked markers. Rice (*Oryza sativa*) probes linked to nondormancy also can be used on the backcross lines.

A nondormant cultivar might be expected to germinate soon after harvest, compromising its ability to survive the winter. Ideally, none of the seeds would survive and a new cultivar (or harvested seeds of the same cultivar) could be planted for the following season. If conditions were unfavorable for germination in late fall, this nondormant cultivar might be fall planted, survive until spring, and germinate when the soil is warm enough and saturated. Alternatively, it could be spring-planted, but fall-planting is more reliable for most growers. In order to determine the usefulness of a nondormant cultivar, research must address the survival of nondormant seeds from one season to the next.

Our objectives in this study of nondormancy were to: 1) quantify genetic variability for dormancy in *Zizania palustris* populations and estimate the correlation between dormancy and quantitative shattering resistance; 2) understand the inheritance of qualitative nondormancy using *Z. aquatica* x *Z. palustris* backcrosses to find RFLP markers for the trait; 3) determine the effectiveness of nondormancy in reducing or eliminating seed survival from one season to the next.

PROGRESS

Objective 1: Quantifying dormancy in Z. palustris

We addressed the potential for reducing dormancy levels within cultivated *Z. palustris* by estimating heritability of dormancy and gain from selection. Seeds from 345 half-sib families of 'Franklin' and K-2Pi (a pistillate breeding population derived from Franklin) were tested last winter and spring, removing them from cold storage and germinating them at 3 one-month intervals. The number of seeds germinating after one month in storage was used as the measure of dormancy. (Other measurements involving the ratio of germination in the first month to the other two were less informative.) Heritability was calculated to be 66% (although any GxE variability was not separated from genetic variability). The average germination after 1 month of storage was 26 seeds per 5 g (150 seeds), or 17%. Gain from selection (assuming half-sib family selection, no pollen control, 10% selection intensity) was calculated to be 10.8% per year (equivalent to a gain of 3 seeds per year). At this rate, 80% germination would be reached in 34 cycles of selection, making this approach less desirable than the qualitative gains likely from the *Z. aquatica* nondormancy. Because of the time required to prepare the seed and carry out the germination tests, these families could not be planted in the field to estimate correlation with shattering resistance.

Objective 2: Understanding the inheritance of qualitative nondormancy

Nondormant *Z. aquatica* collected from the Suwannee River in Florida was originally crossed in 1990 as the donor parent to the dormant *Z. palustris* cv. K2. Backcrosses to *Z. palustris* were carried out for four to five generations. Individuals were deemed nondormant if they came from seeds in which germination occurred within two weeks of cold storage. Three backcross lines are being used for RFLP mapping: two BC5S1 lines 95G-74-1, 95G-220-11, and the sister BC4S2 lines 95G-19-1 and 95G-21-1. These lines are segregating for nondormancy, except for 95G-19-1, which appears to be fixed for nondormancy. They were tested for germination at two week intervals by removing from cold storage (4°C) and placement at room temperature (20°C).

RFLPs linked to genes controlling nondormancy are being identified by comparing 4 nondormant individuals from each backcross line, 5 individuals of *Z. aquatica*, and representatives of the recurrent-parent *Z. palustris* cultivars. DNA were isolated from the tissue of each plant; four enzymes *EcoRI*, *EcoRV*, *DraI*, and *HindIII* were used to digest DNA and screen for polymorphisms. We have currently screened the backcross lines using 31 probes of known location throughout the rice genome (*Oryza sativa*). The majority of these probes (21 of 31) detect a unique polymorphism between *Z. palustris* and *Z. aquatica* with at least one of the four restriction enzyme digests. Thus, RFLP probes are useful for the detection of donor parent *Z. aquatica* DNA in the *Z. palustris* background. Of the 21 probes that detected unique RFLPs, 4 probes detected unique *Z. aquatica* restriction fragments in one of the three BC lines and one probe detected unique *Z. aquatica* restriction fragments in two of the three BC lines. Using binomial probabilities and assuming the donor parent genome is reduced by 0.5 each backcross generation, the probability that *Z. aquatica* introgression occurred by chance in one of the three BC lines is $P=0.186$ and in two of the three BC lines is $P=0.003$. Thus, one probe (RZ698) has a strong likelihood to be linked to the gene controlling nondormancy. A test of this marker linkage will proceed with the analysis of dormant BC5 individuals as they should not retain the donor *Z. aquatica* RFLP allele. Further confirmation will proceed by testing other probes that are linked to this marker. We will use the existing *Oryza sativa* linkage map and the emerging wild rice map for candidate markers that may be more tightly linked to the gene controlling nondormancy.

Objective 3: Effectiveness of nondormancy in the field

In moving the backcross lines from last winter's greenhouse generation to the field this spring, there was a high mortality rate early in the season, probably due to a combination of transplant stress and midge larval feeding on the seedlings. As a result, we did not achieve the seed numbers needed to carry out the field testing stage this fall. However, we are now increasing and selecting open-pollinated progeny in the greenhouse this winter for testing next fall.

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