Progress in Mapping Shattering and Nondormancy Genes.

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Introduction and objectives.

The primary objective of the molecular genetics project is to increase the understanding of wild rice genetics and facilitate breeding objectives through the application of genetic markers. Our primary research areas involve: i. the development of a saturated genetic linkage map of wild rice for breeding applications, ii. comparative mapping to understand the relatedness of the wild rice genome to white rice and other cereals, iii. identification of markers linked to genes controlling shattering, nondormancy, and the pistillate trait, and iv. the development of lines via marker-assisted selection.

Development of a genetic linkage map.

Currently the wild rice map is unfolding. To develop a mapping population we have generated several F_2 populations from several controlled crosses of nonshattering by shattering plants. Three F_2 populations with greater than 150 individuals were evaluated for shattering, Johnson X Dora Lake (1 population) and K2-Vomela X Dora Lake(2 populations)and segregation ratios for shattering versus nonshattering were 125:63, 143:61, and 122:49 respectively. The K2-Vomela X Dora segregations fit a 3:1 ratio ($P > 0.05$) and these populations likely contain one segregating shattering gene. The Johnson X Dora does not fit a 3:1 segregation ratio ($P < 0.05$). This population is now being emphasized for mapping as it likely harbors more than one shattering gene.

We are in the process of restriction-fragment-length-polymorphism (RFLP) map construction with probes used as markers in white rice (*Oryzeae sativa*). By comparing wild rice to white rice we can build upon an extensive knowledge base and more quickly approach the resolution of existing high density maps of white rice (Causse et a1.,1994; Kurata et al., 1994; McCouch et al.,1988). It has been demonstrated that many genomic regions among grass genomes are conserved for RFLP markers and genes conditioning similar traits (Ahn and Tanksely 1993; Paterson et al. 1995). Sets of probes currently being used are of rice, oat, and barley origin that have been previously mapped in white rice (Kurata et al. 1994, National Institute of Agrobiological Resources, Tskuba, Japan; Causse et al. 1994, Cornell University). RFLP

evaluation consists of radioactively labelling probe DNA, and subsequent hybridization to wild rice DNA. The hybridization is based on the complementary base-pairing nature of double-stranded DNA. The wild rice DNA has been cut with restriction enzymes, which allow us to detect DNA variation. Restriction enzymes cut DNA at specific sites which may be unique (polymorphic) among individuals. The probe allows us to target small fragments of DNA which can be visualized as bands on gels. Thus a useful probe will hybridize to wild rice DNA, detect discrete bands, and detect size variation (polymorphism). The majority of our mapped white rice probes hybridize to wild rice DNA. cDNA probes (DNA encoding genes) have been found to be generally more useful in wild rice while genomic probes (DNA providing chromosome structure) have not (Table1).

Table 1. Probe hybridization to *Z. palustris* mapping population.

Type of probe	Number	Detected signal	Distinct band(s)	Non-distinct bands	Polymorphism (four enzymes)
cDNA					
Rice	94	87(92%)	80(85%)	7(9%)	51(63%)
Oat	57	43(75%)	37(65%)	6(14%)	28(75%)
Barley	8	6(75%)	4(50%)	2(33%)	2(50%)
Genomic					
Rice	26	15(57%)	8(31%)	7(47%)	3(38%)

In general, the number of restriction fragments hybridizing in wild rice is greater than white rice. It has been reported that wild rice has twice the genome size of white rice (2.2 pg/cell as opposed to 1.0 pg/cell, Bennett et al. 1982). The number of restriction fragments detected in wild rice by single copy rice probes is approximately two times that of white rice (2.2+/- 0.8, adjusting for heterozygous allelic fragments). The greater amount of total DNA and greater number of restriction fragments may reflect partial or global duplication events of the genome of wild rice chromosome with respect to white rice.

Construction of the wild rice map is an ongoing process. The polymorphic probes detected have been used to initiate wild rice map construction. A polymorphic probe can be used to obtain segregation data. Once segregation data is obtained, probes become markers (or loci) to which relative locations among other markers can be determined. These relative positions are illustrated by groups of co-segregating loci or linkage groups. Thus far we have obtained genetic segregation data for 64 loci. Expected 1:2:1 segregation ratios are found with 57 of 64 loci. Expected ratios indicate the Johnson X Dora population is useful for genetic analysis. Linkage analysis using computer

software LINKAGE-1 (Suiter et al., 1983) with 64 loci generated 13 linkage groups with 49 loci(Fig.1). Fifteen markers remain unlinked. Since we have fewer linkage groups than chromosome pairs as well as unlinked markers, we do not have a saturated map. Obtaining a saturated map is simply a matter of time and obtaining more markers. Continued effort will be made to increase map saturation as this is directly related to its value as a breeding tool. Of the linkage groups we have constructed, we have found colinear regions with white rice. This is an anticipated result based on comparative maps among other grass genomes. Other linkages are not colinear. Noncolinearity of wild rice and white rice markers may be due to rearrangements of wild rice with respect white rice chromosomes. Alternatively, the duplicated nature of wild rice with respect to white rice may complicate our ability to establish colinear relationships. Even though establishment of colinear linkages may be complicated, laying the comparative mapping foundation is a priority, as it will allow us the most efficient use the highly resolved white rice genetic map in wild rice.

Detection of genes for shattering resistance.

With our partial map we are tempted to use the markers therein to test associations to the shattering trait. We scored F2 individuals as shattering or nonshattering approximately 30 days after pollination (Elliot and Perlinger 1977). Marker genotypes were tested against shattering versus nonshattering phenotypes in single-factor analysis of variance tests using SAS statistical software (SAS Institute, Cary NC). Three (CD01387, CD0244, and RZ590) of the 64 markers tested were significantly ($P< 0.05$) associated to the trait (Fig.1). Of these three markers, two (CD0244 and RZ590) are linked and likely detect the same gene while the other (CD01387) is segregating independently and likely detects a different gene. Thus two independent regions have been found that appear linked to genes controlling shattering. These markers combine to explain 19.5% of the variation for the trait. The unaccounted variation for the trait is likely attributable to incomplete marker saturation. That is, the association may be diminished by genetic recombination between the marker and the shattering gene, or there may be one or more other shattering gene(s) that have as yet gone undetected. Another reason for undescribed variation may be that environmental factors may confound the genetic analysis. We will

evaluate F3 progenies derived from mapping F2 individuals in a replicated paddy trial to reduce possible confounding environmental variation.

Genes controlling traits, as well as RFLPs, are candidates for comparative mapping analysis. Two shattering genes in white rice have been mapped, *sh-1* and *Sh-3*. Interestingly, the dominant *Sh-3* gene in white rice maps in the region of chromosome four in which the markers CD0244 and RZ590 are located. Thus, the shattering gene in wild rice may be a similar (homeologous) gene in white rice. The use of the white rice map will help us increase marker saturation in this region.

We will begin to use the map and markers to develop a nonshattering population. Our initial strategy will be to identify lines from the mapping population itself that are fixed for nonshattering alleles. To do this we need to increase map saturation to identify markers flanking all nonshattering genes. We also need to propagate the mapping population via selfing until we can reliably identify all fixed nonshattering lines. Toward this end we will self F3 lines to generate F4 lines this spring. As fixed nonshattering lines become identified we will perform crosses among them (to avoid inbreeding depression). Markers associated to nonshattering genes may also be used in development of other nonshattering populations as genetic locations of genes should be maintained. RFLPs detected by the same probe or another tightly linked marker would be used for selection of homozygous nonshattering individuals.

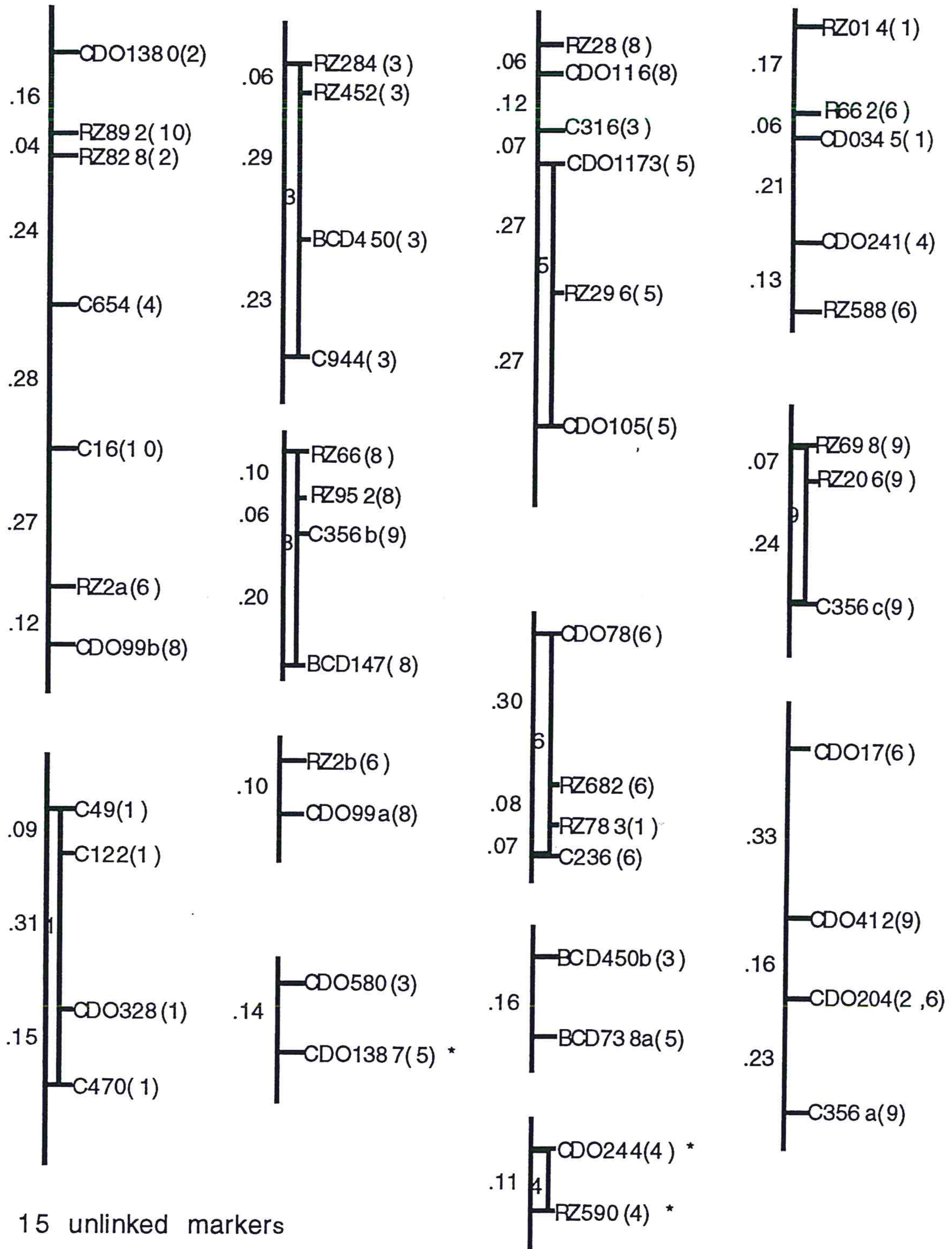

Fig1. Wild rice RFLP map. Vertical lines indicate linkage groups. Marker designations are on the right with the chromosome to which the marker was mapped in white rice in parenthesis(). Adjacent boxes indicate regions of colinearity with white rice. Recombination distance are on the left. * indicate markers were significantly (*P*< 0.05) associated to the trait, shattering versus nonshattering.

Detection of markers linked to genes controlling nondormancy.

We are using a different approach to identify linked markers to genes controlling nondormancy. In this study, we are using probabilities associated with donor parent allele elimination through recurrent backcrossing to detect markers linked to genes controlling nondormancy (e.g., it is unlikely that donor parent germplasm remains in backcross lines except that which is linked to genes under selection; Kaeppler et al., 1993). We evaluated three different backcross families generated by crossing nondormant *Z. aquatica* to dormant *Z. palustris* with repeated backcrossing to *Z. palustris* and selection for nondormancy for 4 to 5 generations. We evaluated 30 probes and unique DNA fragments were readily observed between *Z. aquatica* and *Z. palustris* (16 of 30 probes). Evaluation of backcrosses indicated *Z. aquatica* DNA introgression in one of the three families (P = 0.093 by chance) and one probe (RZ698) indicated *Z. aquatica* DNA introgression in two of the three families (P = 0.003 by chance). There is a strong likelihood this latter marker is linked to a gene controlling nondormancy. We will test other markers that are linked to this probe on the basis of the recently determined linkages in wild rice.

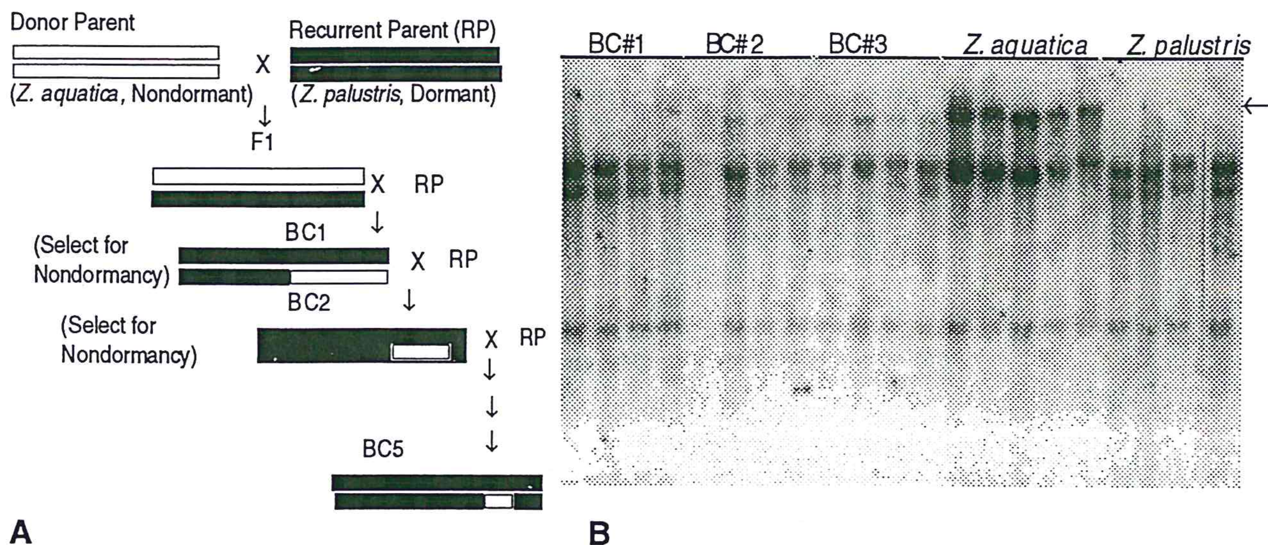

Fig 2. A.) Backcrossing scheme for introgression of nondormant genes. B.)Hybridization of rice cDNA probe RZ698 to nondormant backcross lines of wild rice. Note the unique *Z. aquatica* restriction fragment (absent in *Z. palustris*) but present in 2 of 3 backcross lines.

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