Progress in Genetic Mapping of Wild Rice

Wayne Kennard, Ronald Phillips, and Raymie Porter

Introduction

A genetic map of wild rice (*Zizania palustris*, 2n=2x=30) has been constructed to aid in the understanding of genome organization and to determine the heritability of economically important traits. RFLP (restriction fragment length polymorphism) genetic markers have been used to construct the map. The construction of our genetic linkage map has been facilitated by the discovery of widespread colinearity of markers among the grass genomes (Bennetzen and Freeling 1993, Ahn and Tanksley 1993). The map is composed primarily of markers from white rice (*Oryza sativa* 2n=2x=24) and leads to genome organization comparisons among the two species. This approach is being used because the two species are taxonomically grouped in the subtribe Oryzeae, high density maps of white rice exist, and white rice is becoming the reference point for mapping and gene cloning in cereals. The genomes differ in total DNA content (wild rice = 2.0 pg/cell versus white rice = 0.84 pg/cell) and chromosome pairs (wild rice = 15 versus white rice = 12). The mapping project provides basic information on the comparative composition of the wild rice genome and tools to assist in the breeding of shattering and other traits. We extend our comparative mapping to the analysis of shattering, since genes controlling shattering have been mapped to colinear linkage groups among maize, sorghum, and rice (Paterson et. al. 1995). Thus, we have undertaken a comparative mapping strategy to determine whether the same reported genes among other cereals control shattering in wild rice.

Materials and Methods

The mapping population was developed from a cross of a single nonshattering plant (of the cultivated variety Johnson) and that of a shattering plant (from a natural population, Dora Lake, MN). The mapping population includes 172 F2 individuals derived from the self pollination of a single F1 plant. DNA was isolated from individuals in the mapping population according to McCouch et al. (1988). DNA isolation, restriction digestion, gel electrophoresis, Southern blotting, hybrizations and washes (0.5XSSC, 65°C) were performed according to standard protocols (Sambrook et al. 1989). Probes used in the construction of white rice maps and maize maps were used in this study. These probes are of rice, oat, barley, and maize origin, predominantly cDNAs that have been previously mapped (Coe et al. 1993, Causse et al. 1994; Kurata et al. 1994; McCouch et al. 1988). Detection of linkage and recombination distances was

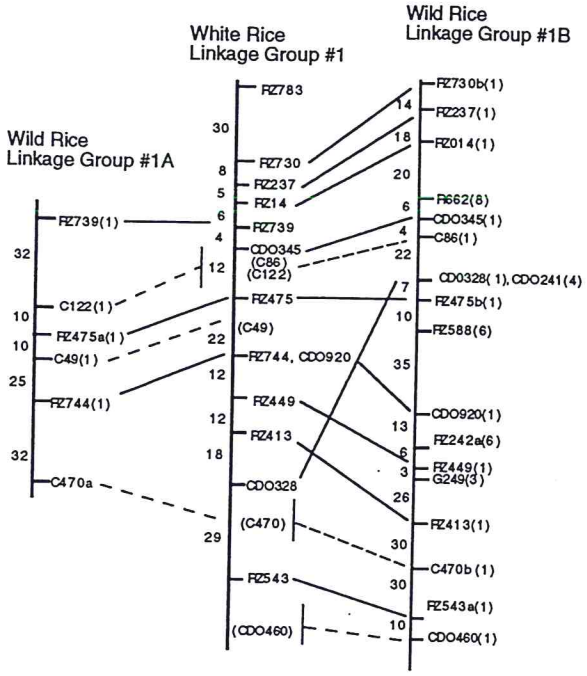
achieved using the mutipoint linkage analysis software MAPMAKER 3.0 (Lincoln et al. 1992).

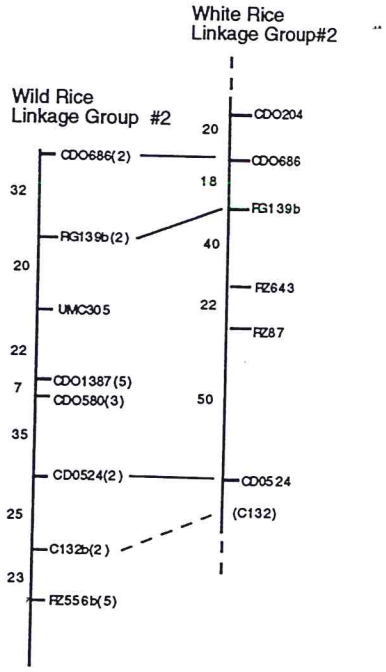
F2 individuals of the mapping population were subjectively scored for shattering versus nonshattering on a +/- basis for male floret and seed retention. F2 individuals were self-pollinated and provided 80 F3 families that were similarly scored for shattering in both greenhouse and a replicated field trial. Tests of marker associations to the trait were performed by single-factor ANOVA among the genotypic class means (Proc GLM, SAS Institute).

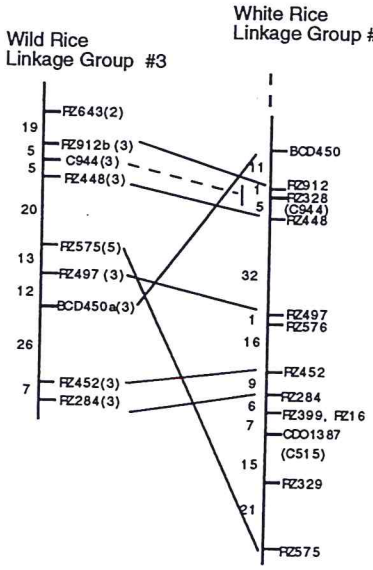
Results and Discussion

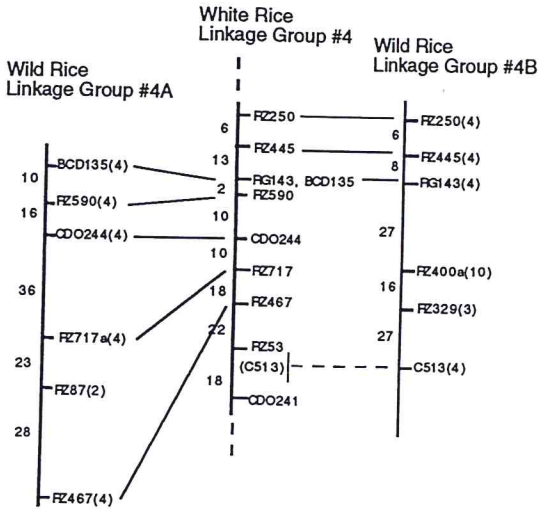
The majority of cDNA probes detected restriction fragments in wild rice. Of 325 different cDNA probes screened for segregation in the mapping population, 245 (75%) gave strong hybridization patterns to discrete RFLP fragments and 157 (48%) exhibited polymorphism using four enzymes. Conversley, of 56 genomic probes screened only 10 (17%) detected discrete bands. The average number of RFLP fragments detected in F2 individuals of wild rice (3.5 +/- 1.7) was higher than that found in a maize inbred (A188; 2.3 +/- 1.3) or a rice variety (ssp. japonica, var Nipponbarre; 1.8 +/-1.1).

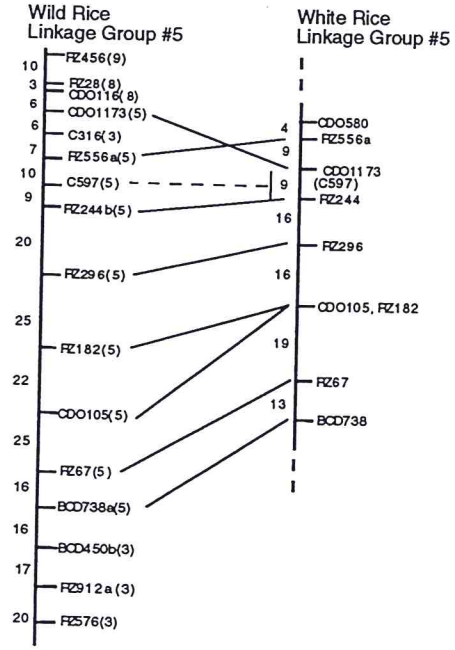
Currently our RFLP map consists of 113 markers on 15 linkage groups spanning a genetic distance 1754 cM (Fig. 1). Segregating RFLPs in this F2 population generally fit (P>0.05) 1:2:1 (98/105) or 3:1 (6/8) segregation ratios. Two markers remain unlinked. Colinear markers were found representing all white rice linkage groups except #12. The majority of white rice loci mapped to colinearly arranged linkages in wild rice (79 of 110). The map illustrates similarities and differences among wild rice and white rice. Conservation of the order of white rice markers is usually, but not always, maintained in wild rice. For 11 of the 12 white rice linkage groups, colinearity is observed in wild rice, allowing assignment of related linkage groups. RFLP markers indicate a great degree of genetic conservation and will allow future mapping efforts to build upon these apparently related linkage groups. Part of the greater DNA content of wild rice to white can be attributed to the duplication of chromosomes as recognized by duplicated linkage groups. White rice linkage groups #1 and #9 are at least partially duplicated in wild rice. White rice linkage group #4 shows evidence of either chromosome breakage and/or duplication in wild rice. Other duplicated linkages include a translocation of part of linkage group #3 to linkage group #5 and a duplication of a

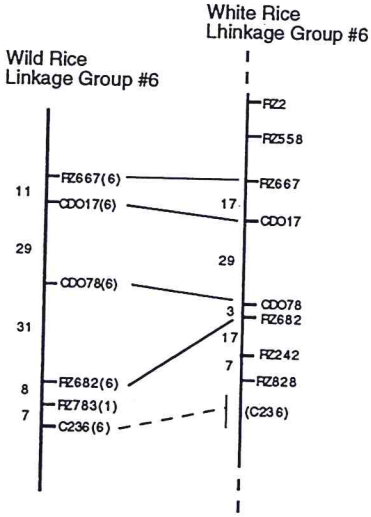

White Rice
Linkage Group #1
Wild Rice
Linkage Group #1B
Wild Rice
Linkage Group #1A
RZ783
RZ730
RZ237
RZ14
RZ739
CDO345
(C86)
(C122)
RZ475
(C49)
RZ744, CDO920
RZ449
RZ413
CDO328
(C470)
RZ543
(CDO460)
RZ739(1)
C122(1)
RZ475a(1)
C49(1)
RZ744(1)
C470a
RZ730b(1)
RZ237(1)
RZ014(1)
R662(8)
CDO345(1)
C86(1)
CD0328(1), CDO241(4)
RZ475b(1)
RZ588(6)
CDO920(1)
RZ242a(6)
RZ449(1)
G249(3)
RZ413(1)
C470b(1)
RZ543a(1)
CDO460(1)


White Rice
Linkage Group#2
Wild Rice
Linkage Group #2
CDO686(2)
RG139b(2)
UMC305
CDO1387(5)
CDO580(3)
CD0524(2)
C132b(2)
RZ556b(5)
CDO204
CDO686
RG139b
RZ643
RZ87
(C132)
CD0524


White Rice
Linkage Group #3
Wild Rice
Linkage Group #3
RZ643(2)
RZ912b(3)
C944(3)
RZ448(3)
RZ575(5)
RZ497(3)
BCD450a(3)
RZ452(3)
RZ284(3)
BCD450
RZ912
RZ328
(C944)
RZ448
RZ497
RZ576
RZ452
RZ284
RZ399, RZ16
CDO1387
(C515)
RZ329
RZ575


White Rice
Linkage Group #4
Wild Rice
Linkage Group #4A
Wild Rice
Linkage Group #4B
BCD135(4)
RZ590(4)
CDO244(4)
RZ717a(4)
RZ87(2)
RZ467(4)
RZ250
RZ445
RG143, BCD135
RZ590
CDO244
RZ717
RZ467
RZ53
(C513)
CDO241
RZ250(4)
RZ445(4)
RG143(4)
RZ400a(10)
RZ329(3)
C513(4)


Wild Rice
Linkage Group #5
White Rice
Linkage Group #5
RZ456(9)
RZ28(8)
CDO116(8)
CDO1173(5)
C316(3)
RZ556a(5)
C597(5)
RZ244b(5)
RZ296(5)
RZ182(5)
CDO105(5)
RZ67(5)
BCD738a(5)
BCD450b(3)
RZ912a(3)
RZ576(3)
CDO580
RZ556a
CDO1173
(C597)
RZ244
RZ296
CDO105, RZ182
RZ67
BCD738


White Rice
Lhinkage Group #6
Wild Rice
Linkage Group #6
RZ667(6)
CDO17(6)
CDO78(6)
RZ682(6)
RZ783(1)
C236(6)
RZ2
RZ558
RZ667
CDO17
CDO78
RZ682
RZ242
RZ828
(C236)

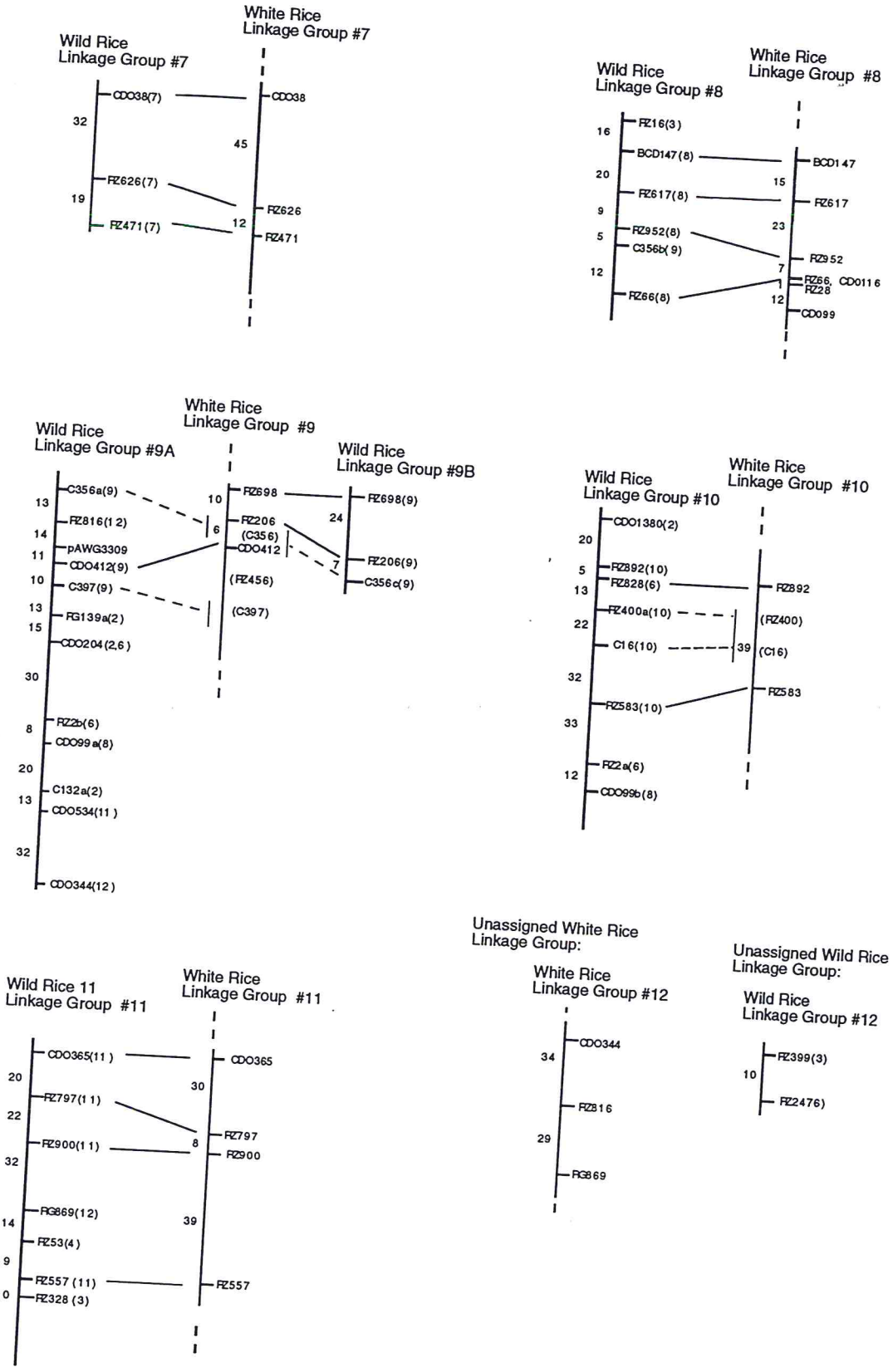


Figure 1. Comparative map of wild rice. Vertical lines indicate linkage groups of wild rice and white rice. RFLP markers are placed to the right and linkage distances to the left. Numbers in parentheses after markers of wild rice linkage group denote the white rice chromosome to which markers have been mapped. Selected marker loci mapped in this study are reproduced form the white rice RFLP map*Oryza sativa* x *O. longsistaminata* ; Causse et al., 1993). Other markers mapped in white rice but which precise location is uncertain within *O. sativa* x *O. longsistaminata* map are placed in parentheses on white rice linkage groups. Horizontal bars connect corresponding marker loci on respective linkage groups, dashed horizontal lines indicate marker has been mapped to colinear the linkage group, but relative placement is uncertain within *Oryza sativa* x *O. longsistaminata* map.

pair of linked loci on #9A and #10. The comparative map and duplication events help us understand wild rice genome organization in the context of white rice and other cereals.

The F2 population segregated for seed shattering versus nonshattering and segregated 123:60. Scores among F2 individuals for seed shattering were highly correlated to F2 male floret shattering (r=0.86). Correlations were also significant (P<0.001) but lower with F2 seed shattering to F3 family evaluations [field F3 seed shattering (r=0.61), field F3 male floret shattering (r=0.59), greenhouse F3 seed shattering (r=0.40), greenhouse F3 male floret shattering (r=0.36)]. These lower correlations of shattering are likely due to segregation among heterozygous shattering alleles, lower numbers of F3 families than F2 individuals, and small sample size within an F3 family.

Table 1. Per-cent variation described (% r^2) with markers to male floret and seed shattering traits as measured on F2 individuals and means of F3 families.

		% r^2 Shattering of Male Florets			% r^2 Shattering of Seed		
Linkage Group	Probe	F2 individuals	F3 mean (Field)[b]	F3 mean (Greenhouse)[c]	F2 individuals	F3 mean (Field)	F3 mean (Greenhouse)
2	CDO686	14.0	20.0	ns[d]	11.9	15.6	ns
2	RG139a	23.6	31.3	16.5	26.4	25.7	8.0
2	UMC305	39.5	34.2	16.8	37.7	37.7	31.5
2	CDO580	10.8	11.4	16.7	12.1	12.6	13.6
2	CDO1387	8.4	13.7	ns	11.9	15.4	ns
4	BCD135	4.4	ns	12.1	ns	ns	ns
4	RZ590	4.4	ns	ns	ns	ns	s
4	CD0244	6.8	ns	10.0	ns	ns	11.6
4	RZ87	7.7	14.9	10.7	6.7	16.3	ns
4	RZ467	ns	ns	ns	7.4	ns	ns
6	CDO78	ns	9.3	ns	ns	9.7	ns
8	BCD147	ns	20.7	ns	ns	21.8	ns
8	RZ617	ns	ns	16.7	ns	ns	ns
12	RZ247	ns	17.8	ns	ns	17.8	ns

[a] number of F2 individuals = 172

[b] number of F3 families in replicated field trial = 63

[c] number of F3 families in greenhouse = 79

[d] ns = non significant association

We are using the map to detect genes controlling the trait. The picture is emerging that one locus has a relatively large effect and one or more other loci have lesser effects in the control of the shattering trait. A major locus, detected by markers on linkage group 2, describes as much as 37.7% of variation for seed shattering in the F2 (Table 1). A marker on linkage group 4A describes as much 9.3 % of the variation for seed shattering in the F2. Other markers that were found associated to shattering on the basis of F3 means but not in F2 include markers on linkage groups #6, #8, and # 12. Comparison to previous reports indicate shattering loci have been mapped to some of the same genomic regions as detected in this study (i.e., white rice linkage groups #2, and #4). Whether these are similar "homoeologous" genes in both species remians to be determined. We are continuing to add markers to our wild rice map to ensure genome saturation and more fully describe the inheritance of the shattering trait.

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