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Genetic dissection of seed shattering, agronomic, and color traits in American wildrice (*Zizania palustris* var. interior L.) with a comparative map

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Abstract A comparative map of American wildrice (*Zizania palustris* var. interior L.) was used to identify loci controlling seed shattering, plant height, maturity, tiller number, plant habit, panicle length seed length, and color traits. Two to six significant quantitative-trait-loci (QTLs, $P < 0.05$) were detected for each trait evaluated, representing the first trait-mapping in wildrice. The chosen population was designed to emphasize the mapping of loci controlling the shattering trait, which is the most important trait in the management of this newly domesticated species. Three loci were detected that controlled the discretely categorized variation between shattering and non-shattering plants. Seed-shattering loci were detected and validated among the F_2 and F_3 generations. A multiple regression model with these three loci described 49.6% of the additive genetic variation. A genetic model with the same three loci including dominance and locus interactions predicted the shattering versus non-shattering phenotype at a success rate of 87%. The comparative map was based on mapped RFLP markers used in white rice (*Oryza sativa* L.) and other grass species. Anchor loci provided a reference point for the identification of potential orthologous genes on the basis of white rice mutant loci and consensus grass species QTLs. Candidate orthologous loci were identified among all traits evaluated. The study underscores the benefits of extending trait analysis through comparative mapping, as well as challenges of QTL analysis in a newly domesticated species.

Keywords Comparative map · QTL · Rice · Shattering · Wildrice

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Introduction

American wildrice (*Zizania palustris* var. interior L.), the native aquatic grain of the United States, is an annual diploid with a chromosome number of $2n = 2x = 30$ (Elliott 1980). Wildrice is a crop in transition from the wild to the domesticated form. Native American tribes have harvested wildrice for centuries. Only recently has it undergone paddy cultivation (Oelke et al. 1982; Grombacher et al. 1997). Presently, 11,000 hectares of wildrice are under cultivation in Minnesota, Wisconsin and California (E.A. Oelke, University of Minnesota, personal communication). One predominant characteristic of domesticated cereals is the absence of seed dispersal mechanisms (shattering) (DeWet and Oelke 1978). A major step toward domestication of wildrice occurred in 1972 when the wildrice breeding project at the University of Minnesota began focusing on the recently discovered non-shattering trait. However, seed shattering is still the major component of yield loss as shattering plants tend to accumulate from dispersed seed in paddies under continuous cultivation. Understanding the inheritance of the shattering trait is necessary to improve yield, as losses have been estimated from 26% (Schertz and Boedicker 1977) to more than 40% (Porter et al. 1994).

Simple inheritance conditioned by one or two dominant genes has been proposed to describe the inheritance of seed shattering (Woods and Clark 1976; Elliott and Perlinger 1977). Observations of 3:1 and 9:7 F_2 segregation ratios provided evidence for a two-complementary dominant-gene system to control shattering (Elliott and Perlinger 1977). The model consists of A_ B_genotypes that condition shattering phenotypes, and aa B_, A_bb and aa bb, genotypes that condition non-shattering phe-

notypes. In this model, if one of the two loci in the recessive state conditions non-shattering, shattering alleles will often be present in non-shattering plants (i.e., at the other locus). As a result, progeny from crosses between non-shattering plants with complementary shattering genes will produce shattering plants. This implies that a grower should maintain a genetically fixed non-shattering population since a shift toward shattering is inherent to the current crop management process. With open-pollination and natural re-seeding, the presence of genes for shattering will enable a non-shattering variety to revert to shattering. This has been observed in growers' paddies (Porter and Schumer 1991). While a genetic model has been proposed, the biochemical and cellular basis for shattering is not fully understood. Differences between shattering and non-shattering plants have been described in relation to abscission layer densities, sizes of sclerenchyma and parenchyma cells, levels of hydrolytic enzymes and lignification (Hanten et al. 1980; Jin et al. 1993; Oelke and Jin 1994).

Other traits important to cultivation and agronomic performance of wildrice include height, maturity, seed-dormancy, tiller synchrony and panicle traits (seed size, number and panicle length). Early maturing and relatively shorter varieties have been developed to reduce losses due to late season storms and early frost (Oelke et al. 1982). Maturity has a high narrow-sense heritabilty (0.47–0.88; Foster and Rutger 1980; Palm 1984; Hutomo 1986), and has been shown to be significantly correlated to height (r = 0.66; Foster and Rutger 1980) but not to yield (Hutomo 1986). Breeding efforts have also been devoted to increasing tiller number and synchronizing maturity among tillers (Everett and Stucker 1983; Hayes and Stucker 1987). While heritability estimates have been made on agronomic traits, more precise studies to determine individual gene effects have not been performed due to the absence of genetic stocks in wildrice.

We constructed a comparative genetic map with markers previously mapped in rice (*Oryza sativa* L; Kennard et al. 2000) to determine the number, location and effects of economically important trait loci in relation to mapped rice mutant loci and QTLs. Rice markers were used because of the degree of colinearity of markers among grass species, and the utility of rice as a reference point for alignment and reference of other cereal genomes (Bennetzen and Freeling 1993; Izawa and Shimamoto 1996). The wildrice comparative map reinforced the close phylogenetic relationship of the rice and wildrice genomes (Kennard et al. 2000). Sixteen linkage groups were constructed representing the 15 pairs of wildrice chromosomes. Colinear linkage groups were identified for 11 of the 12 rice linkage groups, and three linkage groups were found duplicated from rice linkage groups. Comparative mapping with rice is expected to be especially useful to wildrice since both species are taxonomically grouped in the subtribe Oryzoideae (Gould and Shaw 1983). Rice is also appropriate for comparison due to genome conservation of discretely inherited genes expressed across genera (e.g., genes for loss of ligules and waxy endosperm, Ahn and Tanksley 1993) as well as QTLs associated with domestication (Paterson et al. 1995b). Markers linked to shattering genes in rice (Khush and Kinoshita 1991) as well as quantitative-trait-loci (QTLs) for shattering (Paterson et al. 1995b; Cai and Morishima 2000) are expected to be useful for identifying shattering loci in wildrice. Wildrice is particularly poised to reap the benefits of comparative trait mapping because it is a newly domesticated crop, the most closely related genus of agronomic value to rice, and codominant markers are well suited to identify favorable recessive alleles conditioning the non-shattering trait.

Materials and methods

The linkage map

The comparative map of wildrice and rice was previously described (Kennard et al. 2000). A comparative map of wildrice was constructed with cDNA probes of rice, oat, and maize which have been previously mapped in rice (McCouch et al. 1988; Causse et al. 1994; Kurata et al. 1994). The current linkage map was constructed with MAPMAKER/3.0 (Lincoln et al. 1992) and consists of 116 RFLP markers on 16 linkage groups (wildrice has 15 chromosomes) spanning 1,690 cM. The majority of rice markers mapped to colinearly arranged linkages in wildrice (79 of 110 markers) representing all white rice linkage groups except 12. The map provides a foundation for mapping of other markers and trait loci in the context of rice.

Population development

Potential mapping populations were developed from crosses of single non-inbred non-shattering plants (cultivated varieties Johnson and K2) to single non-inbred shattering plants (from a natural lake population, Dora Lake, Minn.). The chosen F_2 mapping population included 172 F_2 individuals derived from the self-pollination of a single F_1 plant of the Johnson × Dora Lake cross. F_2 individuals were self-pollinated to derive F_{2-3} Families. Due to severe inbreeding depression only 82 F_3 families were recovered. Candidate F_2 populations generated from single crosses of K2 × Dora Lake consisted of 154 and 180 individuals respectively and were used for F_2 shattering trait-scoring only.

Trait scoring

Evaluation of shattering, heading date, height and tiller number was performed on parental, F_1, F_2 and F_3 generations of the Johnson × Dora Lake population. Parental lines (from the same open seed lot as the individual parents of the cross), F_1 individuals, and F_3 lines (two reps) were evaluated in a rice paddy at the University of Minnesota North Central Research and Outreach Center, Grand Rapids, Minn. (F_1 individuals and parental lines in 1995, F_3 families and parental lines in 1997). F_2 individuals and F_3 families (one rep) were evaluated in the greenhouse at the University of Minnesota; St. Paul, Minn. (F_2 individuals and parental lines in 1996; and F_3 families and parental lines in 1997). F_3 families were evaluated in both paddy (two replications with three to five individuals per plot) and greenhouse (one replication with three to five plants per pot). Data for greenhouse F_3 evaluations is reported for shattering related traits only, where an additional independent analysis is compared to the paddy evaluation. Traits measured only on the greenhouse-grown F_2 individuals included stem color and flower color. Traits measured only on the paddy grown F_3 families included seed number, plant habit, seed length and panicle length. F_2 individuals from other candidate mapping populations of K2 ×

Table 1 Means (standard deviations) and ranges for parents, F_1, F_2 and F_3 families derived from the mapping population (Johnson × Dora Lake)[a]

Trait	Johnson mean (n = 5)	Dora Lake mean (n = 5)	F_1	F_2 mean (n = 172)	Range	F_3 mean (n = 68)	Range
Seed shattering[b]	1.0 (0.0)	0.0 (0.0)	0	0.31 (0.46)	0–1	0.44 (0.43)	0–1
Heading date (days)[c]	92.3 (6.5)	75.8 (10.4)	110	88.6 (9.42)	68–118	89.7	77–102
Plant height (cm)[d]	247.5 (10.9)	157.4 (11.3)	265	197.6 (41.5)	56–310	127.8(29.4)	25–179
Tiller number[e]	15.4 (4.5)	11.6 (3.3)	16	12.5 (4.15)	4–24	7.7	2–17
Stem color (1–5)[f]	1.8 (1.0)	3.4 (1.2)	2	2.3	1–5	–	–
Flower color (1–5)[g]	1.5 (1.2)	3.7 (1.4)	3	2.5	1–4	–	–
Plant habit[h]	3.6 (0.54)	1.8 (0.45)	–	–	–	2.25 (0.64)	1–5
Seed length (mm)[i]	12.3 (0.9)	10.4 (1.3)	–	–	–	11.1 (1.4)	7.0–14.0
Seed number[j]	110 (26)	89 (34)	–	–	–	91.5 (29.3)	29–165
Panicle length (cm)[k]	22.4 (3.5)	12.1 (3.1)	–	–	–	18.7 (2.4)	9.7–25.5

[a] Parents and F_1 and F_3 evaluations performed in the paddy at the University of Minnesota North Central Research and Outreach Center, Grand Rapids, Minn. F_2 evaluations performed in the greenhouse at the University of Minnesota, St. Paul, Minn.

[b] 1 = non-shattering = <10% seed released upon maturation; 0 = shattering = ≥90% seed released upon maturation

[c] Heading date measured as the days after planting that the female spike emerged 1 cm or more from the culm or boot

[d] Plant height measured as the height from the submerged crown of the plant to the apex of the tallest female spike after full elongation

[e] Tiller number scored as the number of flowering tillers at harvest

[f] Stem color scored subjectively on a 1–5 scale (1 = green stem with visual absence of anthocyanin, to 5 = completely purple stem)

[g] Flower color scored subjectively on a 1–5 scale (1 = white flower with visual absence of anthocyanin, to 5 = completely purple flower)

[h] Plant habit scored subjectively on a 1–5 scale (where 1= tillers at a 45° angle or lower, to 5 = no more than 10° from the vertical) 97 days after planting

[i] Seed length measured as the length of the mature seed without the hull upon harvest

[j] Seed number measured as the number of seed bearing pedicels from the female portion of the spikelet at maturity

[k] Panicle length was scored as the length of the primary stem among seed-bearing racemes on the female portion of the panicle at maturity

Dora Lake were evaluated only for shattering traits in paddies at Grand Rapids, Minn., in 1996.

Seed shattering versus seed non-shattering was scored as a discretely inherited trait on the basis of the number of seed abscised vs those retained on the main culm upon maturation (e.g.), shattering = ≥90% seed released upon maturation and non-shattering = <10% seed released). Measurements also were made with a tensile strength force gauge (Everett and Stucker 1983). The device measures the greatest amount of resistance in the process of removing grasped seed from the plant (kg/pull). Male floret retention, highly correlated with seed shattering, was scored 3 days after anthesis. A plant was categorized as shattering if ≥90% of the male florets were released, or non-shattering if >90% male florets were retained. Measurements of seed shattering and staminate flower loss were made on F_2 individuals (n = 172) and on F_3 family means (n = 79; three F_3 families did not provide seed to evaluate for shattering).

Heading date was measured as the days after planting that the female spike emerged 1 cm or more from the culm or boot. Plant height was measured as the height from the submerged crown of the plant to the apex of the tallest female spike after full elongation. Stem color was scored subjectively on a 1–5 scale (1 = green stem with visual absence of anthocyanin, to 5 = completely purple stem). Flower color was scored subjectively on newly opened male flowers on a 1–5 scale (1 = white flower with visual absence of anthocyanin, to 5 = completely purple flower). Tiller number was scored as the number of flowering tillers at harvest. Plant habit or tiller erectness was scored subjectively on a 1–5 scale (where 1 = plant with the majority of tillers lower than an approximately 45° angle to the surface of the water, to 5 = where all tillers are no more than 10° from the vertical) 97 days after planting. Panicle length was scored as the length of the primary stem along seed-bearing racemes on the female portion of the panicle at maturity. Seed number was measured as the number of seed-bearing pedicels from the female portion of the spikelet at maturity. Seed length was measured as the length of the mature seed without the hull upon harvest. For panicle length and seed number, F_3 family means were derived from five plants per plot with two panicles per plant. For seed length, F_3 family means were derived from five plants per plot with two panicles per plant and two seeds per panicle (Table 1).

Statistical analysis

Summary statistics and correlations were calculated with PROC UNIVARIATE and PROC CORR with SAS software [SAS Institute Inc (1990)]. Marker-trait associations for shattering were detected on a genotypic class mean basis with F_2 genotypes. Analyses were performed on both the F_2 mapping population individuals and on derived F_3 family means. QTLs were detected by single-factor ANOVA with individual markers using PROC GLM as well as with intervals using PLABQTL (Utz and Melchinger 1997). Gene action assignments of 'additive', 'partial dominance', 'dominance' and 'over-dominance' were based on the magnitude of genotypic class means. Gene action was calculated on the basis of genotypic class means – [Aa – (AA + aa)/2)]/(AA + aa)/2); where <0.5 = additive, >0.5–0.75 = partial dominant, >0.75–1.25 = dominant, and >1.25 = overdominant.

Multiple regression models for seed shattering and male floret retention were constructed with both SAS and PLABQTL. Multilocus models were constructed with a multiple regression "backwards elimination" procedure using PROC GLM (Kennard et al. 1994; SAS Institute, Cary, North Carolina). PLABQTL (Utz and Melchinger 1997) composite interval mapping in a stepwise regression was used to confirm results with the "backwards elimination" procedure.

Phenotypically similar mutant loci or grass consensus QTLs were listed as possible orthologues if detected in the same colinear 50-cM region detecting the wildrice QTLs. Assignments of potential orthologous loci were made on the basis of integrated RFLP and trait loci maps, where common RFLP markers were used in this study (Causse et al. 1994; Paterson 1995b; Laurie 1997; Yoshimura et al. 1997). ln some cases, mutant loci were identified as possible orthologues after extrapolation using common marker loci with integrated RFLP and trait locus maps (Khush and Kinoshita 1991; Kurata et al. 1994; Matsuo et al. 1997).

Table 2 Significant ($P < 0.05$) correlation coefficients (r) of the shattering trait across F_2 and F_3 generations among greenhouse and field environments

Shattering trait	Seed shattering F_2 greenhouse	Flower shattering F_3 greenhouse	Seed shattering F_3 greenhouse	Flower shattering F_3 field	Seed shattering F_3 field
Flower shattering F_2 greenhouse	0.87 ($n = 162$)	0.49 ($n = 77$)	0.45 ($n = 76$)	0.61 ($n = 74$)	0.58 ($n = 72$)
Seed shattering F_2 greenhouse		0.36 ($n = 76$)	0.40 ($n = 72$)	0.59 ($n = 76$)	0.61 ($n = 67$)
Flower shattering F_3 greenhouse			0.92 ($n = 74$)	0.93 ($n = 73$)	0.89 ($n = 67$)
Seed shattering F_3 greenhouse				0.88 ($n = 66$)	0.87 ($n = 65$)
Flower shattering F_3 field					0.96 ($n = 67$)

Results and discussion

Trait evaluation and segregation ratios for seed shattering

Seed shattering was evaluated by both direct observation and with a tensile-force gauge. Nearly all individuals (96.4%) in the F_2 mapping population were readily classified as shattering or non-shattering (e.g.), shattering = ≥90% seed released upon maturation, and non-shattering = <10% seed released upon maturation). Measurements with a tensile strength-force gauge (Everett and Stucker 1983) reflected the same classification as with the percent seed retained. The mean of shattering plants was 0.0277 +/– 0.027 kilograms/pull and the mean of non-shattering plants was 0.103 +/– 0.038 kilograms/pull. The two methods provide an equally accurate classification of shattering and non-shattering plants.

All F_1 plants from crosses of shattering by non-shattering plants were discretely classified as shattering or non-shattering. The exact genotypes of the parents were unknown. An evaluation of 200 individuals of the open-pollinated seed lots from which parental individuals were obtained indicated that no shattering plants were present in this sample of the non-shattering cultivated variety of Johnson, while 8.5% of the non-shattering variety K2 were discretely classified as shattering. All of 200 individuals from the seed lot used to obtain the shattering parent (the wild lake population, Dora Lake) were classified as shattering.

The K2 × Dora Lake F_2 candidate mapping populations segregated as 134 shattering: 46 non-shattering, and 119 shattering: 35 non-shattering, while the Johnson × Dora Lake mapping population segregated 123 shattering: 60 non-shattering. Each K2 × Dora Lake population segregated in a 3:1 ratio ($P > 0.900$ and $P > 0.500$, respectively), while the Johnson × Dora Lake population did not fit either a 9:7 ($P <= 0.005$) or a 3:1 segregation ratio ($0.025 > P > 0.01$). Because our objective was to detect linked markers to as many shattering loci as possible, we chose to emphasize mapping in the Johnson × Dora Lake population with the 123:60 shattering: non-shattering ratio. In this population more than one locus was likely segregating for the shattering trait.

Measurements of shattering were based on seed and male-floret retention. Scores for seed shattering and male-floret retention were highly correlated among F_2 individuals ($r = 0.87$), among F_3 families in the field ($r = 0.96$), and among F_3 families in the greenhouse ($r = 0.92$) (Table 2). Correlations were also significant ($P < 0.001$) but lower with F_2 seed shattering to F_3 family evaluations [field F_3 seed shattering ($r = 0.61$), field F_3 male floret shattering ($r = 0.59$), greenhouse F_3 seed shattering ($r = 0.40$), greenhouse F_3 male floret shattering ($r = 0.36$)]. These lower correlations of shattering are most likely due to lower numbers of F_3 families than F_2 individuals, the small sample size within an F_3 family, and multiple segregating genes with dominant/recessive gene action. Evidence for dominant/recessive gene action comes from distinctions between shattering versus non-shattering inheritance. Non-shattering F_2 plants generated 88% (23/25) all non-shattering and 12% (3/25) segregating families. Shattering F_2 plants generated 56% (30/53) all shattering, 36% (19/53) segregating families and 8% (4/53) fixed non-shattering.

Marker analysis of seed shattering

Analyses of the seed-shattering trait and marker loci with single-factor ANOVA showed one locus with a relatively large effect and three loci with lesser effects conditioning shattering (Table 3; Fig. 1). A major locus for shattering was detected with marker loci on linkage group 2. The most highly significant association was detected with UMC305, which accounted for 37.7% of the seed-shattering variation in the F_2 generation. The highly significant association of UMC305 and other loci on linkage group 2 was confirmed in the F_3 generation in the greenhouse and field. Highly significant associations were also found for UMC305 ($R^2 = 39.5\%$) to the correlated male-floret retention trait. The association remained high ($R^2 > 30\%$) for F_3 seed shattering (field and greenhouse) and male-floret retention (field), but was lower for male-floret retention in the greenhouse (Table 3). The lower association of F_3 families in the greenhouse may have been due to environmental and developmental influences on male-floret retention. Associations of seed shattering to UMC305 flanking markers were highly significant: CDO686, RG139b, CDO580 and CDO1387 all were associated with seed shattering at $P < 0.0005$. Assuming the gene controlling shattering is located between the markers with the highest R^2 values, the locus is located in the 21-cM interval between

Table 3 R^2 and *P*-values for significant ANOVA tests of marker genotypes with seed shattering and male-floret retention. Results of F_3 confirmations with greenhouse and field are reported for significant ANOVA tests in the F_2 tests

Linkage group	Probe	%R^2 (*P*-value) seed shattering			%R^2 (*P*-value) male floret retention		
		F_2 Individuals (greenhouse)[a]	F_3 Families (field)[b]	F_3 Families (greenhouse)[c]	F_2 Individuals (greenhouse)[a]	F_3 Families (field)[b]	F_3 Families (greenhouse)[c]
2	CDO686	11.9 (0.0004)	15.6 (0.0067)	ns[d]	14.0 (0.0001)	20.0 (0.0011)	ns
2	RG139b	26.4 (0.0001)	25.7 (0.0003)	8.0 (0.0268)	23.6 (0.0001)	31.3 (0.0001)	16.5 (0.0058)
2	UMC305	37.7 (0.0001)	37.7 (0.0001)	31.5 (0.0001)	39.5 (0.0001)	34.2 (0.0001)	16.8 (0.0052)
2	CDO580	12.1 (0.0003)	12.6 (0.0144)	13.6 (0.0115)	10.8 (0.0001)	11.4 (0.0174)	16.7 (0.0052)
2	CDO1387	11.9 (0.0004)	15.4 (0.0054)	ns	8.4 (0.0041)	13.7 (0.0113)	ns
4	BCD135	ns	ns	15.2 (0.0098)	4.4 (0.0385)	ns	12.1 (0.0167)
4	RZ590	4.6 (0.0385)	ns	ns	5.6 (0.0244)	ns	ns
4	CD0244	5.2 (0.0318)	ns	11.6 (0.0168)	6.8 (0.0213)	ns	10.0 (0.0204)
4	RZ87	6.7 (0.0096)	16.3 (0.0036)	ns	7.7 (0.0039)	14.9 (0.0060)	10.7 (0.0192)
4	RZ467	7.4 (0.0063)	ns	ns	ns	ns	ns
9	RZ816	5.4 (0.0484)	ns	ns	ns	ns	ns

[a] Number of F_2 individuals = 172
[b] Number of F_3 families in replicated field trial = 63
[c] Number of F_3 families in greenhouse = 82
[d] ns = non-significant association at ($P < 0.05$)

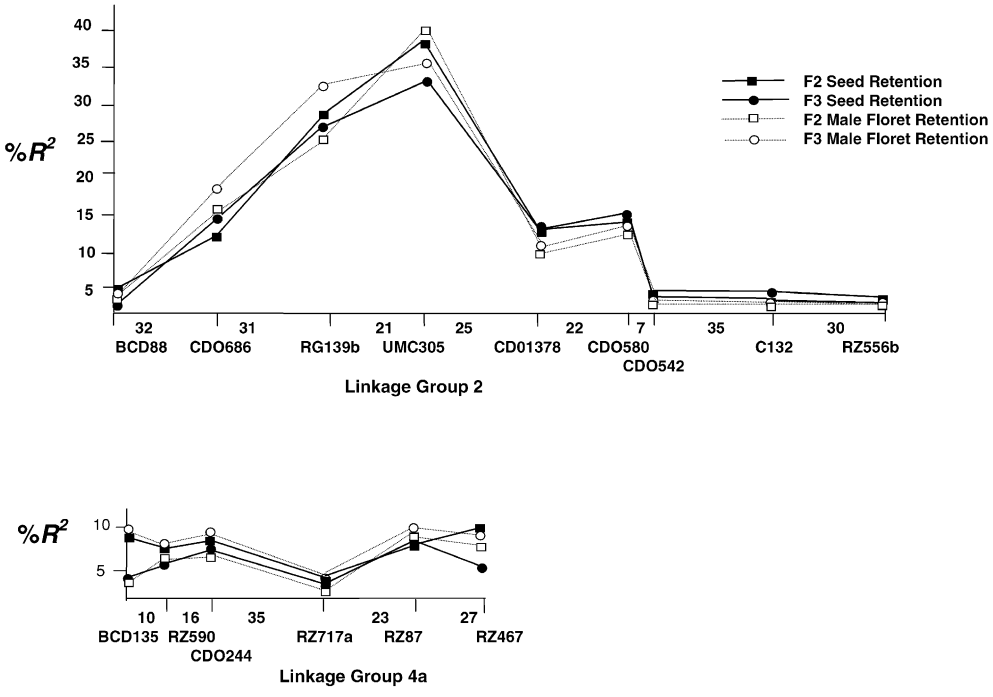

Fig. 1 Variation described by markers in regions important to shattering. R^2 values are reported for individual single-factor analysis of variance tests for seed shattering and male floret retention on chromosomes 2 and 4a across F_2 and F_3 generations

UMC305 and RG139b (Fig. 1). Placement of the peak LOD score (>18.0) between these two flanking markers was confirmed with interval mapping using PLABQTL and estimated to be in the 21-cM interval (15 cM from RG139b and 6 cM from UMC305; data not shown). Dominance/additive ratios for seed shattering (0.67) and male-floret retention (0.69) indicated the gene action for the major QTL to be dominant.

Markers detecting significant associations but describing smaller amounts of variation were found in three other regions. Marker loci CDO244 and RZ87 at unlinked locations on linkage group 4a described 5.2% and 6.7% of the variation, respectively, in the F_2 population. The association of CDO244 and RZ87 to seed shattering was confirmed in the F_3. Marker locus RZ816 on linkage group 9a described 5.4% of the variation for seed shattering in the F_2, but this association was not confirmed in the F_3. Gene action classifications for QTLs were dominant for CDO244, partial dominant for RZ87, and additive for RZ816 (Table 4).

Multigenic inheritance of seed shattering

Multi-locus models were constructed using marker loci that were significantly associated by single-factor analysis using a backwards elimination and PLABQTL. Multiple regression was only performed on the F_2 generation

Table 4 Most significantly associated markers to seed shattering, heading date, plant height, stem color, flower color, plant habit, seed number, seed length and panicle length. Peak LOD scores are derived from PLABQTL simple-interval mapping, and peak R^2 values are derived from single-factor ANOVA. Additive effects dominance effects, and gene-action assignments are derived from genotypic class means. F_3 validation tests of F_2 significant associations were performed on seed shattering, plant height, heading date and tiller number. Candidate orthologous loci are listed if a mutant or grass consensus QTL of similar function mapped to the same colinear 50-cM region in rice

Trait	Interval	Linkage group[a]	R^2 (P-value) ANOVA	Peak LOD score[b]	Add. effect[c]	Dom. effect[d]	Gene action [e]	Validation F_3 (R^2 %)[f]	Trait locus in rice colinear region[g]
Seed shattering (F_2 and F_3)	UMC305	2	39.3 (0.0001)	18.2	0.352	–0.266	Dom	37.7	Consensus QTL for shattering, Paterson et al. 1995b
	CDO244	4a	5.2 (0.0318)	1.8	0.147	–0.125	Dom	11.2	*Sh-3*, Shattering 3, Matsuo et al. 1997; Yoshimura et al. 1997 QTL for shattering, qSHT-4, Cai and Morishima 2000
	RZ87	4a	6.7 (0.0096)	2.6	0.179	–0.128	Part. Dom	16.3	–
	RZ816	9	5.4 (0.0484)	1.8	0.182	–0.053	Additive	ns	–
Heading date (F_2 and F_3)	RZ475a	1a	7.2 (0.0223)	2.3	2.87	–0.87	Additive	ns	–
	C470a	1b	9.6 (0.0110)	3.9	1.34	–3.79	Over Dom	12.5	–
	RG139b	2	8.2 (0.0193)	2.9	3.52	–0.10	Additive	ns	–
	RZ448	3	5.8 (0.0188)	2.0	1.70	4.36	Over Dom	ns	–
	RZ244b	5	8.9 (0.0169)	2.3	3.78	0.04	Additive	ns	
	CDO78	6	5.4 (0.0258)	1.8	3.00	–1.55	Additive	11.7	*Se-1*, photoperiodic sensitivity, Laurie 1997
Plant height (F_2 and F_3)	CDO328	1a	13.0 (0.0087)	2.1	–2.38	19.60	Over Dom.	12.1	*d-18*, hosetsu dwarf, Yoshimura et al. 1997
	RZ730b	1b	12.1 (0.0108)	3.3	20.46	2.98	Additive	44.8	*d-10*, kikeibanshinriki dwarf, Yoshimura et al. 1997 *Sd-1*, dee-geo-woo-gen dwarf, Matsuo et al. 1997
	RG139b	2	15.4 (0.0019)	4.3	14.08	5.14	Additive	ns	*d-5*, bunketsu-waito tillering dwarf, Yoshimura et al. 1997
	BCD135	4a	6.5 (0.0107)	2.8	13.75	–2.63	Additive	ns	*d-3*, bunketsu-waito tillering dwarf, Yoshimura et al. 1997
	RZ296	5	6.8 (0.0187)	2.9	13.86	–0.3.70	Additive	ns	–
	CDO1380	10	14.7 (0.0034)	3.5	14.62	5.66	Additive	ns	–
Tiller number (F_2 and F_3)	RZ237	1b	4.5 (0.0424)	2.8	–1.25	0.06	Additive	ns	*d-10*, kikeibanshinriki tillering dwarf, Matsuo et al. 1997
	RZ912a	5	13.1(0.0084)	3.2	2.01	0.32	Additive		
	RZ66	8	9.7 (0.0087)	3.0	1.86	0.53	Additive	8.9	
	RZ399	Unlink	10.8 (0.0056)	3.1	1.93	0.63	Additive	ns	
Stem color (F_2 only)	C122	1a	5.1 (0.0328)	1.8	–0.54	0.027	Additive	–	*A*, anthocyanin activator, Matsuo et al.1997; Yoshimura et al.1997
	CDO920	1b	17.5 (0.0014)	2.5	0.56	–0.31	Additive	–	*A*, anthocyanin activator, Matsuo et al. 1997; Yoshimura et al.1997
	RG139b	2	5.5 (0.0380)	3.2	–0.41	0.41	Dominant	–	–
	C597	5	6.8 (0.0086)	1.9	–0.32	–0.01	Additive	–	–
	RZ2b	9a	15.3 (0.0053)	2.4	–0.52	–0.42	Dominant	–	–
	RZ583	10	23.7 (0.0009)	2.7	–0.10	0.91	Over Dom.	–	–
Flower color (F_2 only)	RZ475b	1b	6.2 (0.0410)	2.1	–0.31	0.05	Additive	–	*A*, anthocyanin activator, Yoshimura et al. 1997
	RZ87	4a	8.7 (0.0306)	2.6	0.42	0.07	Additive	–	–
	RZ583	10	6.2 (0.0403)	2.2	–0.30	0.31	Part Dom	–	–
	RZ797	11	18.7 (0.0012)	4.3	–0.25	0.29	Dominant	–	–
	CDO99b	15	16.2 (0.0028)	3.9	–0.30	–0.35	Dominant	–	–
	RZ543b	16	15.2 (0.0034)	3.7	–0.49	0.01	Additive	–	–
Plant habit (F_3 only)	RZ448	3	19.2 (0.0407)	3.8	0.52	0.16	–	–	*dl*, drooping leaf, Matsuo et al. 1997; Yoshimura et al. 1997
	RZ576	5	25.5 (0.0301)	2.4	0.53	0.19			
	CDO365	11	14.4 (0.0031)	1.8	0.32	0.09	–	–	

Table 4 (continued)

Trait	Interval	Linkage group[a]	R^2 (*P*-value) ANOVA	Peak LOD score[b]	Add. effect[c]	Dom. effect[d]	Gene action [e]	Validation F_3 (R^2 %)[f]	Trait locus in rice colinear region[g]
Seed length (F_3 only)	RZ244b	5	9.8 (0.0407)	2.8	1.5	0.10	–	–	Consensus QTL for seed mass, Paterson et al. 1995b
	RZ206	9b	14.4 (0.0031)	3.7	1.1	–0.30	–	–	
Seed number (F_3 only)	RZ2b	9a	15.3 (0.0053)	4.5	–16.68	–9.23	–	–	*Dn-1*, dense panicle, Matsuo et al. 1997; Yoshimura et al. 1997
	RZ828	10	8.7 (0.0453)	2.8	9.85	–6.57	–	–	–
Panicle length (F_3 only)	CDO460	1b	13.2 (0.0118)	3.2	0.72	1.86	–	–	–
	RZ952	8	10.6 (0.0243)	2.7	–0.79	1.20	–	–	–
	RZ557	11	20.1 (0.0080)	5.2	0.92	1.47	–	–	*sp*, short panicle, Matsuo et al. 1997; Yoshimura et al.1997

[a] Linkage group to which marker has been mapped in this study
[b] Peak LOD score as detected with PLABQTL simple interval mapping
[c] Additive effect, (AA – aa)/2, as detected by single-factor ANOVA, +/– assignment relative to Johnson parent
[d] Dominance effect, Aa – (AA + aa/2), as detected by single-factor ANOVA, +/– assignment relative to Johnson paren
[e] Gene action calculated on the basis of genotypic class means [Aa – (AA + aa)/2)] / (AA + aa)/2]; where < 0.5 = additive, > 0.5 – < 0.75 = partial dominant, >0.75 – < 1.25 =dominant, and >1.25 = overdominant; – denotes gene action is not estimated because RFLP marker is dominant (i.e., scored presence and absence)
[f] R^2 reported only for significant ($P < 0.05$) single-factor ANOVA tests with F_3 families
[g] Previously reported locus of similar phenotype (grass consensus QTL or single-gene rice mutant); "–" denotes no previously reported gene or QTL in this region

because of the larger population sizes needed to acquire multiple combinations of genotypic classes and test a larger degree of freedom models. The backwards elimination multiple-regression model for seed shattering included marker loci UMC305 and RZ87 (*P*-value cut-off for all marker inclusion was 0.05, R^2 = 43.4%). The PLABQTL multiple regression for seed shattering included only the one interval flanked by markers RG139b and UMC305 (LOD cut-off for marker inclusion >2.5, R^2 = 46.8%). The backwards elimination multilocus model for male-floret retention included UMC305, CDO244 and RZ87 (R^2 = 47.9%). The multiple-regression model eliminated the marker RZ816 as non-significant in a model with UMC305, CDO244 and RZ87 markers. Support for RZ816 being spuriously associated to shattering comes from lack of confirmation in the F_3. Using PLABQTL multiple regression male-floret retention included two intervals flanked by markers RG139b and UMC305, and RZ590 and CDO244 (R^2 = 44.1%). Tests of epistatic interactions among CD0244 and RZ87 indicated significant interactions for both seed shattering and male-floret retention (P = 0.025 and P = 0.013, respectively). Inclusion of the significant interaction term increased the R^2 for multilocus models between 4.2 to 4.9% for both seed shattering (from 45.4% to 49.6%) and for male-floret retention (from 47.9% to 52.8%).

We constructed a multi-locus model employing dominant gene action because all detected shattering loci have strong dominance components on the basis of marker genotypic-class comparisons (see Table 4). We evaluated a model for the genetic control of shattering in the context of a previously described genetic model (Elliot and Perlinger 1977). We expected to recover a population in which two dominant loci condition non-shattering when either, or both, are in the recessive state. The mapping population segregated 126 shattering: 60 non-shattering which did not fit the 9:7 expectation for two loci; ($P < 0.005$) or the 3:1 expectation for a single locus; ($P < 0.025$)]. Marker analysis in this study indicated strong evidence for at least three loci with differing magnitudes of effect. Multi-locus models and confirmation analysis included a locus with a large magnitude of effect on linkage group 2 and two loci with lesser effects independently segregating on linkage group 4a. A three-gene dominant/recessive model consistent with our QTL results, and that builds upon previously developed two-locus models, is one in which shattering is conditioned by the dominant gene "A_", or dominant genes "B_" and "C_" in concert. In this model non-shattering is conditioned when either locus "A" is in the recessive state (i.e., aa __ __) or when "B" and "C" are in recessive states simultaneously (i.e., __bbcc), and all other combinations produce shattering (Table 5). Consistent with this model is the observed phenotypic segregation ratio of 126:60 that fits the expected 45:19 segregation ($0.50 < P < 0.25$), and lines are obtained in which the combination of recessive alleles at the two minor loci appear to condition non-shattering in the absence of recessive alleles at the major locus. Also consistent with this model is the dominant or partially dominant gene action classifications of all loci, multiple-regression models indicating the significance of all loci simultaneously, and significance of the interaction term among B and C loci. Most importantly, a model of the three most-tightly linked marker loci (where UMC305 = the major locus A, and CDO244 and RZ87 = the minor loci B and C) correctly predict phenotypes 87% of the time. This model builds upon the previous two-gene models derived from obser-

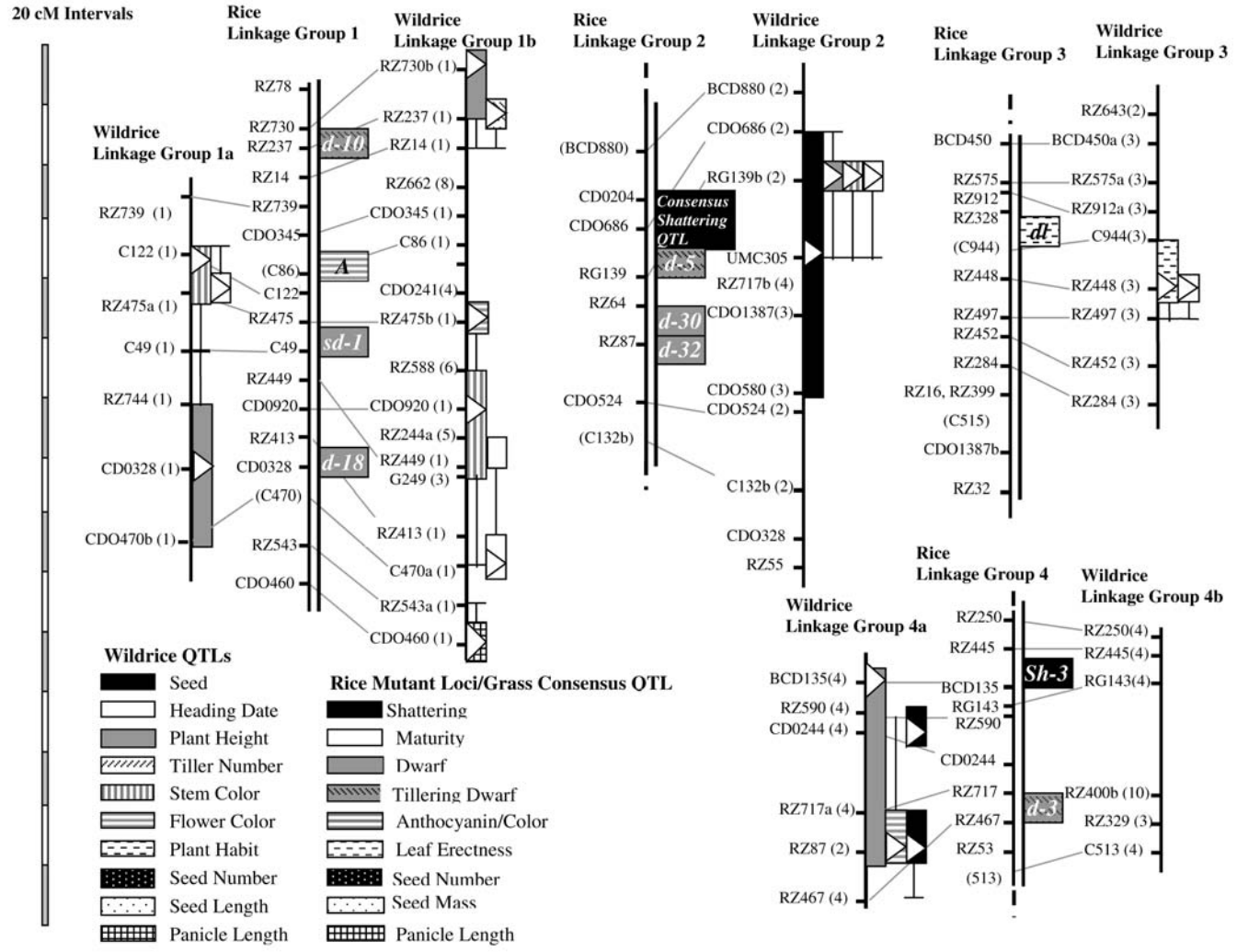

Fig. 2 Comparative trait maps of wildrice and white rice. Wildrice linkage groups are reproduced as in Kennard et al. (2000) and selected marker rice linkage groups are reproduced from Causse et al.(1994) (*Oryza sativa* × *O. longistaminata*). Wildrice RFLP linkage groups are indicated by *bold vertical lines* with markers names listed on the left followed by rice linkage-group assignment in parenthesis. The RFLP markers used in the wildrice map were combined from sets of previously mapped markers in rice and maize (Causse et al. 1994; Kurata et al. 1994; Coe 1993). Wildrice trait loci and white rice mutant loci are indicated through *pattern-coded-vertical bars*. Significant tests of marker associations ($P < 0.05$) are illustrated by *vertical bars* spanning the region of significance adjacent to the left wildrice linkage groups. Relative significance is indicated by a *white triangle* within the vertical bar (the most significant association in the region, open triangle); extended vertical bars around the triangle ($P < 0.01$; white triangle in shaded box), and single lines extending from the bar ($0.01 < P < 0.05$; white triangle in shaded box with extended line). The rice map is represented from selected markers from Causse et al. (*O. sativa* × *O. longistaminata* 1994) and supplemented with markers from Kurata et al. (1994) and Coe (1993). In cases where markers could not be confidently ordered in the white rice map, the markers were placed in parenthesis. Adjacent to the white rice map are approximate locations of selected rice mutants and consensus grass QTLs (Causse et al. 1994; Paterson et al. 1995b; Laurie 1997; Matsuo 1997; Yoshimura et al. 1997)

Table 5 Punnett-square for three-gene model of shattering "S" versus non-shattering "N", where non-shattering is conditioned by either the the homozygous recessive genotype "aa" or the combination of "bbcc" homozygous recessive genotypes

	ABC	ABc	AbC	aBC	aBc	abC	Abc	abc
ABC	AABBCC=S	AABBCc=S	AABbCC=S	AaBBCC=S	AaBBCc=S	AaBbCC=S	AABbCc=S	AaBbCc=S
ABc	AABBCc=S	AABBcc=S	AABbCc=S	AaBBCc=S	AaBBcc=S	AaBbCc=S	AABbcc=S	AaBbcc=S
AbC	AABbCC=S	AABbCc=S	AAbbCC=S	AaBbCC=S	AaBbCc=S	AabbCC=S	AAbbCc=S	AabbCc=S
aBC	AaBBCC=S	AaBBCc=S	AaBbCC=S	aaBBCC=N	aaBBCc=N	aaBbCC=N	AaBbCc=S	aaBbCc=N
aBc	AaBBCc=S	AaBBcc=S	AaBbCc=S	aaBBCc=N	aaBBcc=N	aaBbCc=N	AaBbcc=S	aaBbcc=N
abC	AaBbCC=S	AaBbCc=S	AabbCC=S	aaBbCC=N	aaBbCc=N	aabbCC=N	AabbCc=S	aabbCc=N
Abc	AABbCc=S	AABbcc=S	AAbbCc=S	AaBbCc=S	AaBbcc=S	AabbCc=S	AAbbcc=N	Aabbcc=N
abc	AaBbCc=S	AaBbcc=S	AabbCc=S	aaBbCc=N	aaBbcc=N	aabbCc=N	Aabbcc=N	aabbcc=N

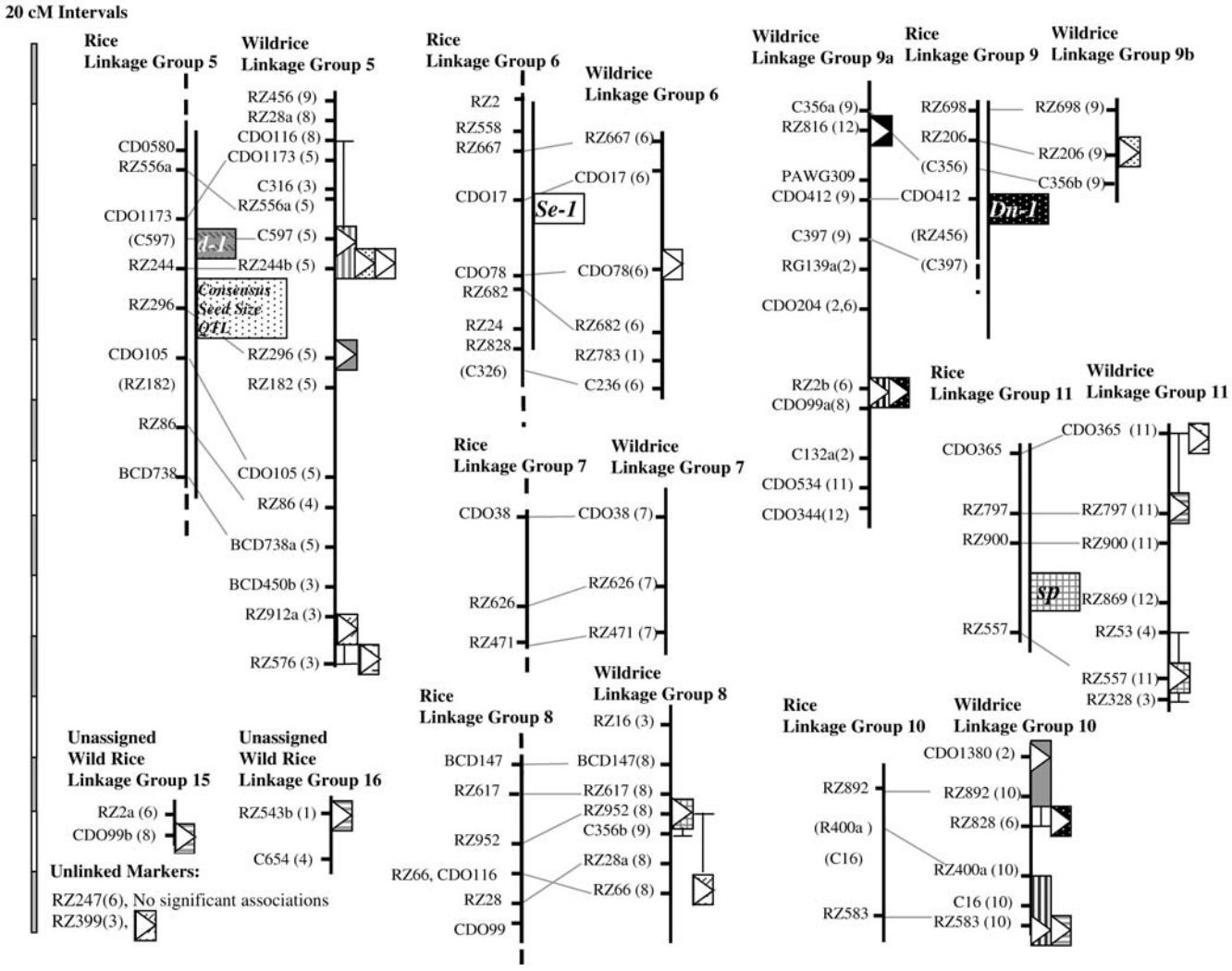

Fig. 2 (continued)

vations of 9:7 and 3:1 segregation ratios. A 9:7 ratio is attained if Aa is segregating, bb is fixed in the recessive state, and Cc segregating (or if Aa and Bb are segregating and cc is fixed). A 3:1 ratio is attained if Aa is segregating and either BB or CC is fixed in the dominant state (or if only Bb is segregating with AA and cc fixed, or if only Cc is segregating with AA and bb fixed). However, the proposed model falls short of always successfully predicting shattering versus non-shattering with a 13% failure rate. Observations inconsistent with the model include recovery of three segregating F_3 family plants derived from non-shattering F_2 plants (since all non-shattering individuals should remain fixed for non-shattering upon selfing). Further, correlations among greenhouse and field environments indicate environmental factors influencing the trait. However, the simple genetic model as described above may be realistic if non-shattering is conditioned by a loss-of-function process.

Comparative mapping of seed-shattering loci

Comparisons to previous reports on rice indicate seed-shattering loci have been mapped to some of the same genomic regions as detected in this study (i.e., rice linkage groups 2 and 4; Table 4, Fig. 2). QTLs have been shown to control seed shattering across genera for genomic regions colinear to rice linkage group 2 (Paterson et al. 1995b). A mutant locus has been found to control seed shattering on rice linkage group 4 (Khush and Kinoshita 1991). The mutant rice locus on chromosome 4 (*Sh-3*) has similar gene action as wildrice, with shattering being dominant to non-shattering. A study of shattering loci in a mapping population of cultivated × Asian common wild rice (*O. sativa* var. indica × *O. rufipogon*) indicated a QTL segregating in the same region as *Sh-3* (qSHT-4, flanking markers CDO244-RG214; Cai and Morishima 2000). A shattering locus has been mapped to chromosome 9 in white rice (Paterson et al. 1995a). However, the association on linkage group 9 (as detected with RZ816) in our study was not confirmed in the F_3.

Further, the shattering locus in rice is located in a non-homoeologous region 60-cM away.

Comparative mapping of height, maturity, color and panicle traits

The mapping population segregated for height, maturity, pigmentation and panicle characteristics, as well as shattering (Table 1). The female parent (the cultivated variety Johnson) of the mapping population is notable among cultivated varieties for greater height, later maturity, and less anthocyanin pigmentation than most wild-rice varieties. The male parent of the mapping population (from a natural population, Dora Lake) has characteristics of a wild population, being shorter and earlier to mature than cultivated varieties. While there is intra-population variation, the panicles, and seed-bearing portion of the panicles, and the seed itself of Johnson are longer than those of Dora Lake. The stems and flowers of plants from the Dora Lake population have a greater degree of anthocyanin pigmentation than the cultivated variety Johnson.

QTLs were detected for all other traits evaluated (Table 4, Fig. 2). Of the nine traits measured (excluding shattering related traits) a total of 1,044 (9 traits × 116 markers) single-factor ANOVA tests were performed. Of these tests, 68 (6.5%) were significant at the $P < 0.05$ level, only slightly more than expected by chance. However, more were detected than was expected by chance (3.0%) at the $P < 0.01$ level. Significant R^2 values ranged from 4.4 to 39.5% evaluated with F_2 individuals, versus 8.2 to 44.3% with F_3 families.

Wildrice undergoes severe inbreeding depression limiting the QTL analyses across generations. This is reflected in the reduced number of lines (172 to 82), and reduced the mean height (197.6 to 127.8 cm) from the F_2 to the F_3. In contrast to shattering, correlations were low and insignificant among F_2 and F_3 height and heading-date measurements (data not shown). This was primarily due to severe inbreeding depression as height was reduced to an average of 35% and population size was reduced by 54%. Further, F_2 plants that were later in maturity or shorter in height tended not to be as prolific or develop to maturity. This is reflected by correlations of successful F_3 progeny generation with F_2 measurements of heading date ($r = –0.21$, plants that never flowered or flowered very late and never set seed) and height ($r = 0.41$, many shorter dwarf plants remained vegetative).

A total of six QTLs were detected for heading date, all of which indicated that the Johnson parent provided the positive-effect allele for later maturity (Table 4, Fig. 2). Gene action was primarily additive, but two QTLs were classified as overdominant. Two QTLs for heading date were confirmed in the F_3. Shattering was not correlated to heading date, and only one common marker locus (RG139b) was found to be significantly associated with the two traits. QTLs in our study corresponded to loci in the same regions as those reported in rice (Li et al. 1995; Xiao et al. 1996; Yano and Sasaki 1997). We detected a QTL in the region in which a major locus, *Se-1*, controls flowering time in rice. Wildrice is a temperate long-day plant (Elliot 1980) and rice is a short-day plant. However there have been reports of maturity related effects detected in the homoeologous regions of *Se-1* with those of wheat and barley (Laurie 1997).

A total of six QTLs were detected for plant height with R^2 values ranging from 6.5 to 14.7% (Table 4, Fig. 2). For five of six QTLs for plant height, the Johnson parent provided the positive-effect allele for increased height. Five of the six QTLs were classified as additive, with one QTL overdominant. Two of the six QTLs for plant height were confirmed in the F_3. Lack of confirmation may have been due to the severe inbreeding depression reflected in both height reduction and lack of germination of F_3 progeny. Plant height was significantly positively correlated to seed shattering in both F_2 ($r = 0.19$) and F_3 ($r = 0.30$) generations. Common marker loci significantly associated to seed shattering and plant height include RG139b on linkage group 2, and RZ590 and CDO244 on linkage group 4a. For four of the six plant height QTLs, potential orthologous loci were identified (Yoshimura et al. 1997).

Four QTLs were detected for tiller number and three QTLs for plant habit (Table 4, Fig. 2). The R^2 values for tiller number ranged from 4.5 to 10.8%. Tillering was correlated among generations ($r = 0.29$), and one of the QTLs was confirmed in the F_3. Tiller number was significantly positively correlated to plant height in both F_2 ($r = 0.17$) and F_3 ($r = 0.32$). For one of the tiller number QTLs, a potential orthologous locus was identified (*d-10*, kikeibanshinriki tillering dwarf; Matsuo et al. 1997). The R^2 values for plant habit ranged from 9.6 to 25.5%. Plant habit was significantly positively correlated to tiller number in the F_3 ($r = 0.31$). A potential orthologous locus in rice was identified for one plant habit QTL (*dl*, drooping leaf; Matsuo et al. 1997; Yoshimura et al. 1997).

A total of six QTLs were detected for stem color and six QTLs for flower color (Table 4, Fig. 2). Heritability and chemical composition of anthocyanin pigments have been described (Gutek et al. 1980). R^2 values for markers associated with stem color ranged from 5.1 to 23.7% and for flower color from 6.2 to 18.7%. Stem color and flower color were not significantly correlated to any other trait but were correlated with each other ($r = 0.23$). Stem color and flower color were only evaluated in the F_2 generation. Five of the six QTLs for stem color and five of the six QTLs for flower color indicated that the Dora Lake parent contributed the allele for a greater amount of anthocyanin pigmentation. Common marker loci significantly associated to the two traits included only loci on linkage group 1b. These loci map to the homoeolgous rice region where the major gene *Anthocyanin activator* locus, *A*, is located (Causse et al. 1994; Yoshimura et al. 1997).

Two QTLs were found for seed number; two QTLs for seed length; and three QTLs for panicle length (Ta-

ble 4, Fig. 2). Seed number, seed length, and panicle length were only evaluated in the F_3, so gene action estimates are not reported. One of the two QTLs for seed number indicated that the Johnson parent provided the allele for an increased number of seed. Both QTLs for seed length indicated that the Johnson parent contributed the alleles for increased seed length. Two of the three QTLs for greater panicle length were contributed by Johnson parent alleles. The greatest effect for seed number was detected with RZ2b on linkage group 9a, where the Dora Lake allele provided an average increase of 16.7 seeds even though the Dora parent had fewer seeds than Johnson. Colinearity has not been demonstrated throughout linkage group 9a, but the locus, *dense panicle, Dn-1*, is found on linkage group 9 in rice (Causse et al. 1994; Matsuo et al. 1997; Yoshimura et al. 1997). The greatest effect for seed length was detected with RZ206 on chromosome 9. A consensus QTL for seed size among grass species has been mapped to the colinear region on rice chromosome 5 that includes RZ244. The greatest effect for panicle length was detected with RZ557 on linkage group 11. A strong colinear relationship has been established between rice and wildrice on linkage group 11, where the rice locus, *short panicle, sp,* is located (Matsuo et al. 1997, Yoshimura et al. 1997).

Conclusions

Major genes for shattering remain present in essentially all wildrice germplasms currently under cultivation in Minnesota and Wisconsin (Porter et al. 1994). Wildrice is open-pollinated and fallen seed provides the subsequent crop in Minnesota and Wisconsin paddies, so there is strong recurrent selection pressure for shattering alleles. The presence of shattering alleles in California germplasm is unknown, but yearly re-seeding after a dry fallow period provides no recurrent selection pressure as in the Midwest. For germplasm that contains shattering alleles, marker-based selection would be extremely efficient over test-crossing with recessive genes. In this study we have found markers linked to genes controlling non-shattering and such markers will enhance the chances that breeding crosses in the future will be fixed for non-shattering alleles. Based on previous studies, we expected to find two loci segregating for shattering. Genetic segregation ratios in those crosses from the recently discovered non-shattering germplasm in the 1970s provided 3:1 and 9:7 segregation ratios. In this study we found one major and two minor shattering loci from a cross of non-shattering germplasm maintained by the breeding program. It is possible that in the course of 20 years of breeding and selection, another non-shattering locus was fixed in the current non-shattering varieties. Alternatively, the third locus may have been fixed in the particular lines used in the initial shattering study (Elliot and Perlinger 1977), but might have been segregating in other plants of Johnson and related cultivars.

Genetic marker loci can effectively accelerate the elimination of deleterious genes and allow strategic selection for desirable recombinants in undomesticated or recently domesticated crops. Genetic improvement of wildrice stands to benefit greatly with the application of marker loci. However, in cases where genetic load can be reduced with marker-assisted selection, challenges are posed in population development when lethal and sublethal genes hamper line development. The severe inbreeding depression observed in this study reduced our ability to confirm QTLs across generations. Fewer F_3 lines than F_2 individuals were evaluated due to male sterility, probable female sterility, and lack of seed germination. Confirmation of height and maturity QTLs was further hampered due to significant correlations of shorter-and later-maturing plants with sterility. Of 19 QTLs evaluated across generations, seven were confirmed in the F_3. This study indicates it is necessary to have approximately twice the number of F_2 individuals as the required number of F_3 lines in a cross employing natural lake wildrice germplasm. However, the F_3 families offered a robust QTL validation test as inbreeding combined with changes in allele frequencies through strong natural selection offered a very different population structure than the F_2 generation.

We anticipate exploiting a great amount of genetic and agronomic information by using rice as the comparative genomic model. Wildrice is uniquely suited to gain from marker-assisted selection and comparative mapping. Colinearity among DNA markers and conservation among trait loci are expected to be greatest with the most-closely related grass genus under cultivation. Wildrice is on the "door step of cultivation" (Oelke et al. 1982) so the full potential of marker-assisted selection can be realized through the selective elimination of deleterious alleles. In this study, we have provided the first trait mapping of wildrice, and have provided many examples of possible orthologous rice loci. To be more certain of orthologous loci with rice, a higher-density wildrice map will be required. We have just begun to exploit rice genetic mapping information to improve the wildrice map, determine orthologous expressed sequences, and perform marker-assisted selection on wildrice using rice as a well-characterized reference point.

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