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A comparative map of wild rice (*Zizania palustris* L. 2n=2*x*=30)

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Abstract We present the first genetic map of wild rice (*Zizania palustris* L., 2n=2*x*=30), a native aquatic grain of northern North America. This map is composed principally of previously mapped RFLP (restriction fragment length polymorphism) genetic markers from rice (*Oryza sativa* 2n=2*x*=24). The map is important as a foundation for genetic and crop improvement studies as well as a reference for genome organization comparisons among species of Gramineae. A comparative mapping approach with rice is especially useful because wild rice is grouped in the same subfamily, Oryzoideae, and no other mapping comparison has yet been made within the subfamily. As rice is the reference point for mapping and gene cloning in cereals, establishing a consensus map within the subfamily identifies conserved and unique regions. The genomes of wild rice and rice differ in total DNA content (wild rice has twice that of rice) and the number of chromosome pairs (wild rice=15 versus rice=12). The wild rice linkage map reported herein consists of 121 RFLP markers on 16 linkage groups spanning 1805 cM. Two linkage groups consist of only two markers. Colinear markers were found representing all rice linkage groups except #12. The majority of rice loci mapped to colinearly arranged arrays in wild rice (92 of 118). Features of the map include duplication of portions of three rice linkage groups and three possible translocations. The map gives basic information on the composition of the wild rice genome and provides tools to assist in the domestication of this important food source.

Key words RFLPs · Synteny · *Oryza* · Oat · Map

Introduction

Wild rice (*Zizania palustris* var interior L.), the native aquatic grain of North America, is an annual diploid with a chromosome number of 2n=2*x*=30 (see Elliott 1980). Wild rice genetics and breeding are in their infancy. Wild rice is a highly heterogeneous and heterozygous crop (Elliott 1980). Progress in breeding and genetics has been difficult due to seed storage problems, inbreeding depression, outcrossing propensity, and intense labor requirements. Wild rice is a crop in transition from a wild to a domesticated form having been harvested by the Ojibway, Menomini, and Cree Native American tribes for centuries in the upper midwest (Oelke et al. 1982). Wild rice is a crop that is currently cultivated in the United States primarily in Minnesota and California under a paddy crop-management system (Oelke et al. 1997). Cultivated wild rice contributed $21 million dollars to the 1997 United States agricultural economy (Oelke personal communication) and consumption worldwide has steadily risen. Much remains to be learned about the genetics and breeding of wild rice.

The construction of genetic linkage maps of Gramineae species is being facilitated by the widespread colinearity of markers among the grass genomes (Bennetzen and Freeling 1993). Comparative maps within the Gramineae include rice vs maize (Ahn and Tanksley 1993), barley (Saghai Maroof et al. 1996), wheat (Van Deynze et al. 1995a), oat (Van Deynze et al. 1995b), and millet (Devos et al. 1998). These maps have indicated a high degree of conservation for the presence and linear order of markers. Thus, markers exhibiting colinear relationships among these comparisons should have a high likelihood of exhibiting colinearity between rice and wild rice. Rice is becoming the ”pivotal genome” for mapping

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and gene cloning among all cereals: the high basic chromosome number allows for alignments and comparisons (Moore et al. 1995), the small genome size allows for efficient physical mapping and sequencing (Izawa and Shimamoto 1996; Briggs and Helentjaris 1997), and the availability of high-density maps provides many candidate markers for reference points (Causse et al. 1994; Harushima et al. 1998). Colinearity is especially expected between wild rice and rice, since they are taxonomically grouped in the same subfamily, Oryzoideae, and tribe, Oryzeae (Gould and Shaw 1983; Duvall et al. 1993). Genome rearrangements as detected by comparative mapping with rice are becoming useful tools to understand grass evolution (Kellogg 1997). Since rice is a reference point for comparative mapping, comparisons of wild rice to *Oryza* sp. would be especially useful to understand unique and more-ancestral genome organization in the Oryzoideae. A comparative map will be especially useful for genetic research and marker-assisted breeding as genome conservation of expressed genes has already been demonstrated (Ahn and Tanksley 1993) and QTLs for traits associated with domestication have been mapped to consensus regions among Gramineae species (Paterson et al. 1995). A comparative map framework will facilitate mapping trait loci in rice and other grass species in wild rice. Wild rice is particularly poised to reap commercial benefits because it is just beginning to be domesticated and is the most closely related genus of agronomic value to rice.

Materials and Methods

Plant materials and population generation

The mapping population was developed from a cross of a single plant of the cultivated variety Johnson to that of a single plant from a natural lake population, Dora Lake, Minn. The mapping population includes 172 F_2 individuals derived from the self pollination of a single F_1 plant. DNA was isolated from individuals in the mapping population according to a modification of the CTAB procedure (Murray and Thompson 1980). Restriction digestion, gel electrophoresis, and Southern blotting were performed according to standard protocols (UMC RFLP Manual, Maize Genetics Cooperative 1989; Sambrook et al. 1992).

Detection of informative probes and linkage analysis

Probes used in the construction of rice maps are of rice, oat, barley and maize origin, and are predominantly cDNAs (McCouch et al. 1988; Coe 1993; Causse et al. 1994; Kurata et al. 1994; Harushima et al. 1998). Sets of probes used in the construction of rice maps are designated as follows: ”Anchor” and ”Core” Sets from Cornell University, Ithaca, New York=rice cDNA (RZ), oat cDNA (CDO), barley cDNA (BCD), and rice genomic probes (RG); ”Landmarker Sets 1 and 2” from the Rice Genome Project at the National Institute of Agrobiological Resources, Tsukuba, Japan, included rice cDNA (C) and rice genomic probes (G). The anchor probes received from Cornell University have been specifically chosen for strong hybridization to DNA of different Gramineae genera (maize, rice, wheat, barley, sorghum, and sugar cane). A small number of maize cDNA (UMC; Coe 1993) and wild rice genomic probes (PAWG; Grombacher et al. 1996) were also used in map construction. Various plasmids for these clones were transformed into DH5α competent cells, grown in nutrient broth, purified, and digested with appropriate restriction enzymes. Digests of plasmids or PCR amplifications were electrophoresed into low-melting-point agarose, the inserts excised, and radio-labelled via random hexamer priming (Feinberg and Vogelstein 1984). Probes were characterized according to signal strength, detection of discrete bands, and polymorphism. Probes were hybridized in 5×SSC, 10% Dextran sulfate, 10 mM Tris pH 8.0, 10 mM EDTA, 0.1% SDS at 65°C >14 h and subsequently washed in 30-min stages of 2×SCC room temperature, 2×SCC 65°C, 0.5×SCC room temperature, and 0.5×SCC 65°C. Polymorphisms were assessed among a random sample of 6–8 F_2 individuals. Exact parents for all probes were not screened due to the large amount of DNA required for RFLPs and the severe inbreeding depression that retards line development. F_2 individuals were screened with four enzymes, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII, for all probes evaluated. The number of enzymes was increased to eight by the addition of *Bam*H1, *Kpn*1, *Xba*I and *Xho*I for those probes in which polymorphism was not detected with the initial four enzymes.

Probes detecting RFLPs among parents and shown to segregate in a subset of the Johnson × Dora Lake F_2 mapping population were used to evaluate the entire 172-individual mapping population. The individuals were scored for the three classes of genotypes (homozygous Johnson parent, homozygous Dora Lake parent, and heterozygous). Assignments of allele parentage were based on five related individuals from the same seed lot since DNA from the exact parents was not available. Distortion of segregation ratios was tested with the computer program LINKAGE-1 (Suiter et al. 1983).

Detection of linkage and recombination distances was achieved using the multipoint linkage analysis software MAPMAKER 3.0 (Lincoln et al. 1992). Mapmaker 3.0 was employed using the commands group (LOD 3.0, REC 0.4) and order. Markers not clearly ordered were placed by ripple, compare, and try commands. Linkages determined by MAPMAKER 3.0 were confirmed with the two-point analyses of LINKAGE-1. Linkage distances were compared to rice maps to assess regions of colinearity [*O. sativa* × *O. longistaminata*, Causse et al. 1994, and *O. sativ*a subspecies *japonica* (Nipponbarre) × *indica* (Kasalath), Kurata et al. 1994]. Markers in wild rice that were present together in a linkage group and in the same sequence as in rice were deemed colinear if they were in groups of three or more.

Results and discussion

DNA and probe cross-hybridization

Total wild rice DNA was used as a probe in Southern hybridization to different Gramineae genera: rice, oat, barley, wheat, and maize (Fig. 1). Hybridization of total wild rice DNA to the different Gramineae genera occurred strongly with rice but relatively little to oat, barley, wheat, or maize. Thus, some repetitive sequences appear conserved among wild rice and rice species but showed relatively little conservation to the more distantly related grass species. The dramatically greater hybridization of wild rice DNA to rice reflects the taxonomic classification, as genera in the Oryzoideae appear more closely related to each other than to those genera in either the Pooideae (oat, barley, wheat) or the Panicoideae (maize) grass subfamilies.

Heterologous cDNA probes are generally useful as markers in wild rice. The majority of cDNA probes from the various species detected restriction fragments in wild rice. Of 326 different cDNA probes screened to detect segregation in the mapping population, 248 (76%) gave strong hybridization patterns to discrete RFLP fragments and 156 (48%) exhibited polymorphism using four enzymes. Of the heterologous probes evaluated (Table 1), rice cDNA probes exhibited the highest frequency of signal detection (92%), while oat cDNA probes exhibited the highest frequency of polymorphism (75%). For those probes not detecting polymorphism, we screened with an additional four enzymes. Only 23% of the probes that were polymorphic for a particular enzyme were polymorphic for all four of the enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hind*III). Thus, polymorphism was generally probe/enzyme specific, indicating point mutations as the basis of the polymorphism. A cross-hybridizing signal was detected at a slightly greater frequency with rice cDNAs (92%) than with oat cDNAs (85%) or barley cDNAs (75%), perhaps reflecting the greater relatedness between these species. Rice cDNAs from the two sources, Anchor Set and Core Sets (Cornell University) and from the Rice Genome Project (Tsukuba, Japan), gave similar frequencies of polymorphism.

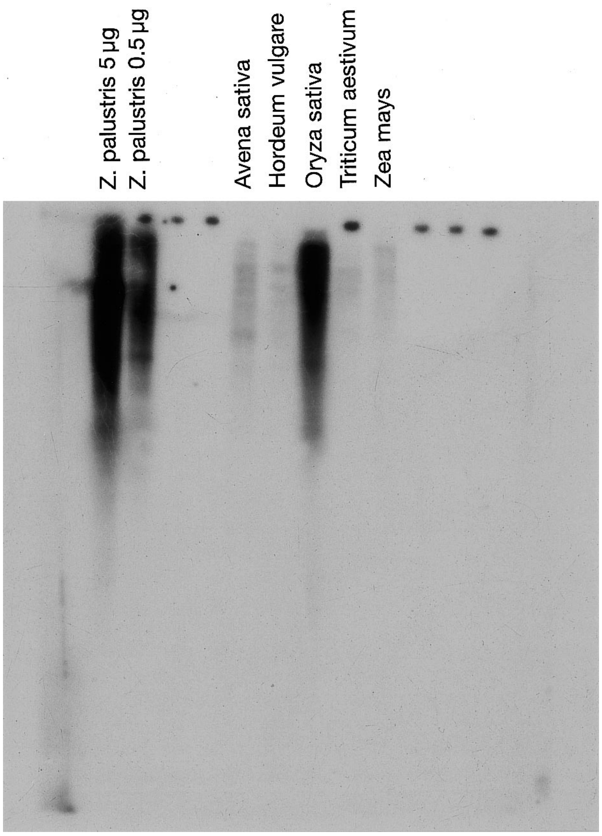

Fig. 1 Southern hybridization of total wild rice DNA to Gramineae species. All genomic DNA samples were digested with *Hind*III. *Lane* samples: *1* λ/*Hind*III; *2 Z. palustris*, 5 µg; *3 Z. palustris*, 0.5 µg; *4* blank; *5* blank; *6 Avena sativa*, 5 µg; *7 Hordeum vulgare*, 5 µg; *8 O. sativa*, 5 µg; *9 Triticum aestivum*, 5 µg; *10 Zea mays*, 5 µg

Genomic probes from rice are less useful than cDNAs for detecting markers in wild rice. Of 56 *Pst*I genomic probes screened from both Core and Landmarker Sets, only 32 (57%) detected a strong signal after a >5-day exposure. Of the genomic probes that provided a signal, only ten (31%) detected discrete banding patterns, while the majority hybridized as non-discrete smears. A given rice genomic sequence is most likely sufficiently divergent to not hybridize efficiently under moderately high stringency.

We initially suspected that the wild rice genome may have undergone widespread duplication or polyploidization on the basis of a greater number of RFLP fragments and an estimated DNA content double that of rice. Most probes hybridized to a greater number of fragments in wild rice than rice. The average number of RFLP fragments per probe detected in F_2 individuals of wild rice (4.9±1.9) was higher than that found in a maize inbred (A188; 2.3±1.3) or a rice variety (ssp. *japonica*, var Nipponbarre; 1.9±1.0). Wild rice individuals evaluated were open-pollinated and rice and maize lines were inbred, but the number of fragments observed in wild rice is greater than expected by heterozygosity alone. The DNA content of wild rice is estimated to be twice that of rice using microspectrophotometry (Bennett et al. 1982). Estimates of the genome size via flow cytometry places rice at 430 Mbp/haploid cell (Arumunagathan and Earle 1991) indicating the genome size of wild rice is approximately 860 Mbp per haploid cell. Part of the greater DNA content may be due to the greater repetitive DNA content, as cytological observations indicate extensive regions of pericentromeric heterochromatin (Grombacher et al. 1996). In situ hybridization experiments in wild rice have indicated that one pair of chromosomes possesses an NOR and a different chromosome has a 5S rDNA locus (unpublished results). This result is consistent with that reported in *O. sativa* ssp. *japonica* (Fukui

Table 1 Probe evaluation for signal detection, frequency of polymorphism (6–8 randomly chosen individuals of the mapping population, digested with four enzymes), and number of loci mapped

Type of probe	Number	Detected signal	Distinct band(s)	Non-distinct bands	Polymorphism (four enzymes)	Mapped
cDNA						
Rice	232	213(92%)	181(85%)	32(15%)	114(63%)	83
Oat	67	56(84%)	47(85%)	9(14%)	35(75%)	24
Barley	23	18(78%)	14(77%)	4(22%)	7(50%)	6
Maize	3	3(100%)	3(100%)	0	1(33%)	1
Genomic						
Rice	56	32(57%)	10(31%)	22(68%)	4(40%)	5
Wild Rice	4	4(100%)	8(100%)	0	2(50%)	2

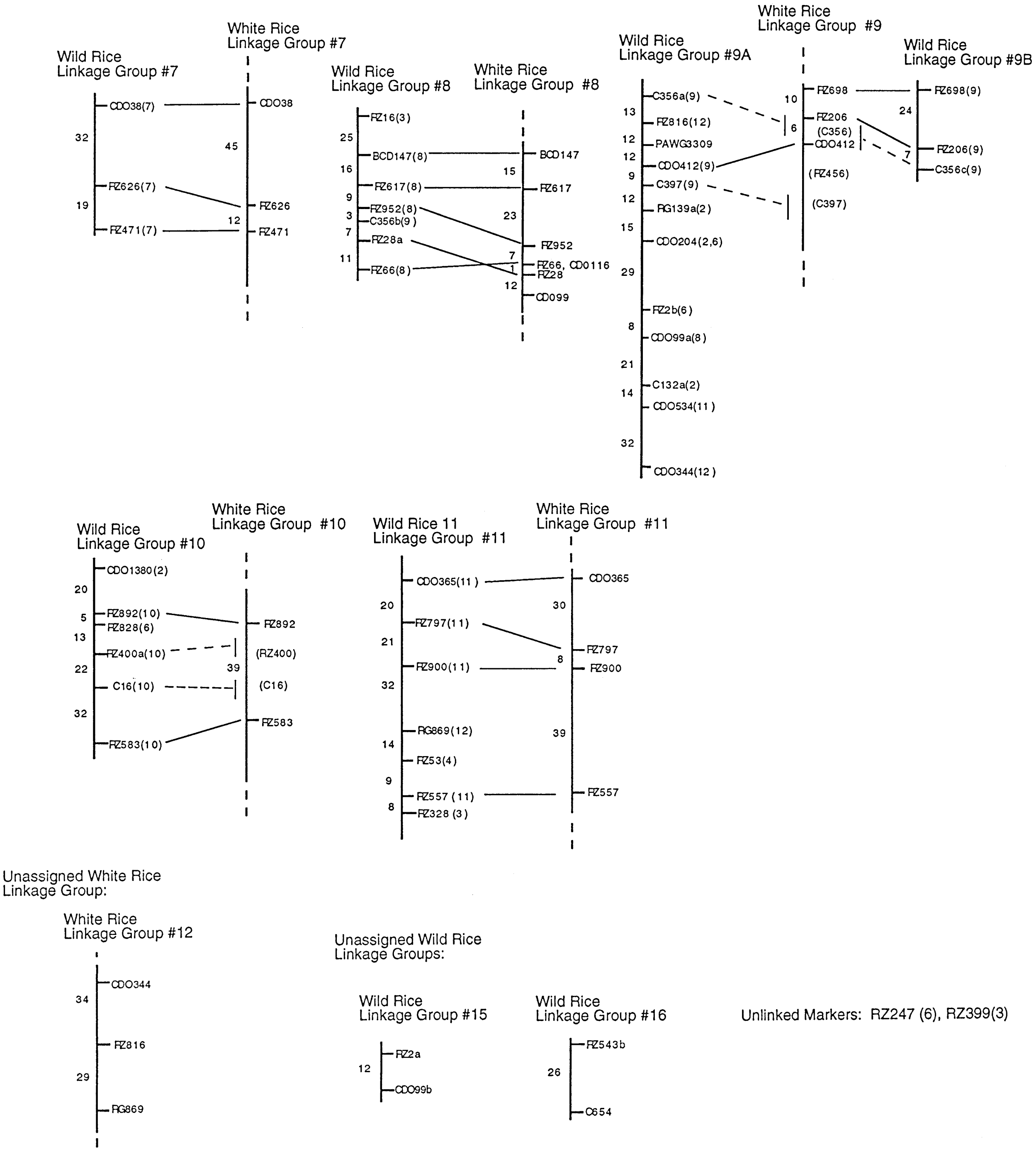

Fig. 2 Comparative maps of wild rice and rice. *Vertical lines* indicate linkage groups of wild rice and rice. RFLP markers are placed to the right and linkage distances to the left. *Numbers in parentheses* after markers on a wild rice linkage group denote the rice chromosome to which markers have been mapped. All markers assigned to a "Rice Linkage Group" in the figure have been mapped to the respective linkage group in a prior mapping study [*O. sativa* × *O. longistaminata*, Causse et al. 1994; *O. sativa* subspecies *japonica* (Nipponbarre) × *indica* (Kasalath), Kurata et al. 1994]. Genetic distances for selected marker loci are reproduced from the rice RFLP *O. sativa* × *O. longistaminata* map (Causse et al. 1994). Other markers mapped in rice but for which the precise location is uncertain within the *O. sativa* × *O. longistaminata* map are placed in *parentheses* on the rice linkage groups. *Horizontal bars* connect corresponding marker loci on respective linkage groups, *dashed horizontal lines* indicate that a marker has been mapped to a colinear linkage group, but relative placement in the *O. sativa* × *O. longistaminata* map is uncertain (Causse et al. 1994). Multiple loci that were detected by a single probe are designated with *suffix letters* (i.e., probe C356, detected three independently segregating loci C356a, C356b, and C356c)

et al. 1994). Thus, we found no cytological evidence to indicate a polyploidization event.

The comparative map

Markers were predominantly codominant and fit expected segregation ratios. Codominant loci fit expected 1:2:1 segregation ratios in 105 out of 116 cases. Dominant loci fit 3:1 expected segregation ratios in 8 out of 10 cases. Our wild rice map is composed of 83 rice cDNA (RZ, C, and R), 24 oat cDNA (CDO), six barley cDNA (BCD), one maize cDNA (UMC), five rice genomic (G and RG), and two wild rice genomic (PAWG) markers. The wild rice genetic linkage RFLP map consists of 121 markers on 16 linkage groups spanning a genetic distance of 1805 cM (Fig. 2). Marker density is an average of one per 15 cM among loci with a maximum distance of 35 cM. Linkage groups are composed of as few as two loci and as many as 18 loci. Two markers, RZ247 and RZ399, remain unlinked. Thus, the map remains unsaturated as there are two unlinked markers, and one more linkage group than the number of chromosome pairs observed.

Greater recombination was found throughout the wild rice map than has been reported in homoeologous regions in rice. Reported total genetic distances in rice maps range from 1389 to 1484 M (McCouch et al. 1988; Causse et al. 1994; Kurata et al. 1994). Pairwise comparison of colinear intervals between wild rice and rice indicates that 82% of the time the genetic distance in wild rice is greater than, or equal to, that of rice. Of those intervals that are greater, the average increase in recombination is 2.1-times (SD 4.1-times) over rice.

We used probes detecting markers previously mapped in rice to efficiently construct and utilize our wild rice map to determine genome organization. Colinear linkage groups were assigned by detecting homosequential ordered loci on the basis of previous reported linkages in rice (Causse et al. 1994; Kurata et al. 1994). In two cases we observed inverted orders of loci. In both of these cases the reported rice linkage distance was less than 10 cM. A consensus homoeologous linkage group emerged for 14 of the 16 wild rice linkage groups. All but three of the markers (pAWG853, pAWG3309, and UMC305) have been previously mapped in rice. UMC305 has been mapped to maize chromosome #10, which is largely colinear with rice linkage group #4. Eleven of the twelve rice linkage groups were represented as homoeologous linkage groups in wild rice. The majority of loci mapped to the respective homoeologous linkage groups in wild rice (77 of 117). Homoeologous linkage groups were composed of as few as 27% (#9A) and as much as 100% (#1A, #7) of colinear loci. The per cent of the genome conserved between rice and wild rice, calculated on the basis of total adjacent colinear segments versus the total map length, is 82%.

Colinear markers were found representing all rice linkage groups except rice linkage group #12. This linkage group also has been the most difficult to establish colinearity among maize (Ahn and Tanksley 1993; Ahn et al. 1993), wheat (Van Deynze et al. 1995a), oat (Van Deynze et al. 1995b), and foxtail millet (Devos et al. 1998). Failure to establish colinearity in this study may be due to the lack of polymorphic markers. We obtained segregation data for only three markers from rice linkage group #12. These markers map to wild rice linkage groups #9 and #11. RG869, for example, maps to linkage group #12 in rice, but linkage group #11 in wild rice. The association of RG869 to linkage group #11 may be expected since linkage groups #11 and #12 have been found to be involved in translocations in rice (Moore et al. 1995). An explanation for the association of RG869 and CDO344 to linkage group #9 is not obvious, but the marker CDO344 was found mapped to the homoeologous segment of rice linkage group #9 in wheat (Van Deynze et al. 1995a).

Duplicate loci, linkages, and linkage groups

Some probe/enzyme combinations identified duplicate segregating loci. Ten probes detected duplicate loci and one probe (C356) detected loci in triplicate for a total of 23 mapped loci. We also attempted to map duplicate loci with probes that were polymorphic for a single locus with different enzyme combinations. We mapped an additional three duplicate loci in this manner. Duplicated loci were interspersed among all loci and appeared randomly distributed within 12 of the 16 linkage groups.

The comparative mapping effort may be confounded by duplicate loci. A number of loci did not map to homoeologous linkage groups (39 of 116). This observation may be due to locus duplication. Because single probe/enzyme combinations were generally used to map loci, the total number of duplicate loci is underestimated since monomorphic bands go undetected as duplicate loci. Duplicated linked pairs of loci were detected in two cases. Probes BCD450 and RZ912 detected duplicated linked pairs of loci on linkage groups #3 and #5, and probes RZ2 and CDO99 detected duplicated linked pairs of loci on linkage groups #9A and #15. BCD450 and RZ912 have been observed to be linked in rice (9.6 cM) but RZ2 and CDO99 segregate independently in rice (Causse et al. 1994).

Two conserved colinear linkage groups were found for a single rice chromosome in three cases, i.e., linkage groups #1, #4 and #9. These linkage groups consist of loci derived from the same rice linkage group, but segregate independently from each other. These conserved colinear linkage groups are probably composed of duplicate loci, but also could be composed of colinear markers dispersed from the same rice linkage group. For linkage groups #1 and #9, duplicate loci detected with a single probe mapped to conserved colinear linkage groups. No duplicate loci were demonstrated for linkage group #4. While duplicated linkage groups #4B and #9A contain a substantial proportion of nonhomologous loci, duplicated linkage groups #1A and #1B are composed of 100% and 76% colinear loci, respectively.

Rearrangements

Evidence for possible duplication and translocation with regard to rice include colinear linked loci deriving from three different rice linkage groups. Segments corresponding to rice linkage groups #3 and #8 are observed linked to opposite ends of wild rice linkage group #5. The genetic distance for the rearranged segment from rice linkage group #3 spans 38 cM on wild rice linkage group #5 whereas the homoeologous segment spans 49 cM in rice. The genetic distance for a rearranged segment from linkage group #8 spans 3 cM versus 1 cM in rice. Evidence for translocation is also observed from the presence of marker loci on linkage group #9 that are on rice linkage group #2. This segment spans 15 cM compared to 38 cM in rice. Interestingly, all three regions of possible translocation and rearrangement are composed of at least one duplicate locus that mapped to duplicated regions of the wild rice genome. Inter-specific comparisons among maps of *Oryza* AA and CC genomes have found a largely homosequential ordering of all linkage groups except for rearranged and inverted sequences among loci for chromosome #1 (Jena et al. 1994).

Relationship among other Gramineae maps

The wild rice map provides an opportunity to evaluate the unique and general attributes of genome organization among species in the subfamily Oryzoideae. No characteristic rearrangements similar to those of cereals in other subfamilies were evident, reinforcing the taxonomic classification alignment of the species near *Oryza*. Rearrangements of Panicoideae (maize, sorghum, and sugar cane) and Pooideae species (wheat, oat, and barley) relative to rice include rearrangements of linkage group #7. Among the Panicoideae and the Pooideae similar segments of linkage group #7 are rearranged, but to different homoeologous rice segments. Of taxonomic interest is whether the apparent ancient breakage of linkage group #7 is evident in the Oryzoideae. While we have only three markers on wild rice linkage group #7, markers are found linked in wild rice that span the two segments found rearranged in the Pooideae (wheat) and Panicoideae (maize). Thus, we can provide support for an ancestral genome organization common to rice and wild rice. However, more markers are necessary to draw a conclusion regarding the syntenic homoeology of linkage group #7.

Conclusion

We report herein the first genetic linkage map of wild rice spanning 1805 cM and 16 linkage groups. Our map is still unsaturated as one more linkage group, compared to chromosome pairs is observed and two unlinked markers remain. Because only two markers are unlinked and only one extra linkage group is found, we anticipate that we are approaching complete map coverage. The map provides a foundation for future genetic mapping projects. Since there have been few genetic studies in wild rice, our understanding of genome organization and the inheritance of important traits will be strengthened tremendously with a linkage map.

We undertook the comparative mapping effort to describe each of the 15 pairs of wild rice chromosomes in the context of rice. Fourteen of the wild rice linkage groups were described on the basis of colinearity to 11 of the 12 rice linkage groups. The three extra chromosomes in wild rice appear due to be complete or partial duplications of rice chromosomes #1, #4, and #9. Duplications may have occurred through nondisjunction of particular chromosomes or by unreduced gamete production followed by hybridization and chromosome loss.

Comparative mapping illustrates both similarities and differences between the genomes of wild rice and rice. Evidence for rearrangements is found on linkage groups 5 and 9. All of these rearrangements are associated with duplicated loci. With an increased map density, we suspect that we may find more rearrangements. Seven of the fourteen homoeologous wild rice linkage groups have noncolinear markers on the ends of the linkage groups and more markers may indicate that some of these regions are associated with translocations. Wild rice also appears from cytogenetic observations to have a greater amount of pericentromeric heterochromatin. The duplication of particular chromosomes, duplicated and translocated segments, and the greater amount of repetitive DNA may collectively account for the two-fold increase of the DNA content of wild rice relative to rice.

Comparing the genome organization of wild rice relative to rice has far reaching implications. We anticipate that a comparative map will have great value for genetic and breeding studies as genome relatedness may extend beyond the colinear order of DNA markers to the conservation of order of expressed orthologous genes (Ahn and Tanksley 1993). Wild rice is a crop just undergoing domestication. Genes controlling domestication traits (seed size, shattering, day length insensitivity) have been found to be conserved in colinear regions among grass species as diverse as rice, wheat, and maize (Paterson et al. 1995). Many genes deleterious to the successful cultivation of wild rice (e.g., shattering, dormancy, *Bipolaris oryza* susceptibility) are still present in cultivated wild rice germplasm. A comparative map framework will allow mapped and cloned orthologous genes of rice to be immediately mapped in wild rice providing streamlined access to marker loci for assisted breeding efforts. Orthologous genes will most likely have similar regulation, genetic mechanisms, and biochemical pathway homologs such that trait-manipulation strategies may be extrapolated to wild rice. This becomes particularly valuable for alternative crops such as wild rice in which genetic mapping projects are just underway and research is still on a relatively small scale.

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