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Comparative Mapping of the Barley *Ppd-H1* Photoperiod Response Gene Region, Which Lies Close to a Junction Between Two Rice Linkage Segments

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ABSTRACT

Comparative mapping of cereals has shown that chromosomes of barley, wheat, and maize can be described in terms of rice "linkage segments." However, little is known about marker order in the junctions between linkage blocks or whether this will impair comparative analysis of major genes that lie in such regions. We used genetic and physical mapping to investigate the relationship between the distal part of rice chromosome 7L, which contains the *Hd2* heading date gene, and the region of barley chromosome 2HS containing the *Ppd-H1* photoperiod response gene, which lies near the junction between rice 7 and rice 4 linkage segments. RFLP markers were mapped in maize to identify regions that might contain *Hd2* or *Ppd-H1* orthologs. Rice provided useful markers for the *Ppd-H1* region but comparative mapping was complicated by loss of colinearity and sequence duplications that predated the divergence of rice, maize, and barley. The sequences of cDNA markers were used to search for homologs in the Arabidopsis genome. Homologous sequences were found for 13 out of 16 markers but they were dispersed in Arabidopsis and did not identify any candidate equivalent region. The implications of the results for comparative trait mapping in junction regions are discussed.

COMPARATIVE mapping of cereals using restriction fragment length polymorphism (RFLP) markers has shown considerable colinearity of marker order (recently reviewed by Gale and Devos 1998; Devos and Gale 2000; Paterson *et al.* 2000). This has enabled the genomes of barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and maize (*Zea mays*) to be described in terms of chromosome sections of rice (*Oryza sativa*; Ahn and Tanksley 1993; Kurata *et al.* 1994; Moore *et al.* 1995; Van Deynze *et al.* 1995). Here we use the term "rice linkage segment" (RLS) following the terminology of Moore *et al.* (1995). The group 2 chromosomes of barley and wheat, which are highly colinear, can be represented as the insertion of rice chromosome 7 (RLS7) into rice chromosome 4 (RLS4a and RLS4b). Although this overall structure seems clear, much less is known about the organization of the junction regions between linkage segments apart from the observation that telomere and centromere regions are frequently involved.

The *Ppd-H1* photoperiod response gene plays a major role in regulating flowering time in barley, and its position on chromosome 2H suggests that it is homeologous to the wheat (*T. aestivum*) *Ppd* gene series (Laurie *et al.* 1995; Börner *et al.* 1998). Previous RFLP mapping has shown that the *Ppd* genes of barley and wheat probably lie in the vicinity of the RLS7/RLS4a junction (Van Deynze *et al.* 1995). More recently, Laurie (1997) showed that *Ppd-H1* is located between cDNA markers that map to the most distal part of rice chromosome 7L and that are therefore expected to be immediately adjacent to the junction. This region of rice chromosome 7 contains the *Hd2* heading date gene shown by Yamamoto *et al.* (1998) to be tightly linked to four markers (*Xrgc728*, *Xrgr411*, *Xrgs1979*, and *Xrgs2267*), which are among those analyzed in the present work.

We are interested in isolating *Ppd-H1* and although this could presumably be achieved by a direct map-based approach, as used for barley disease resistance genes (Büschges *et al.* 1997; Shirasu *et al.* 1999), we were also interested in exploring comparative approaches that utilize the small genome size of rice and in investigating the relationship between the *Ppd-H1* and *Hd2* regions. The primary aim was to determine if colinearity was disrupted by the proximity of the linkage segment junction. A secondary objective was to analyze RFLP probes from the distal region of rice chromosome 7 that detected two or more loci in rice and barley. We investigated whether the region as a whole might be duplicated and, if so, whether duplicated segments were

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associated with flowering time variation. RFLP markers were also mapped in maize to identify candidate regions that might contain counterparts of *Hd2* or *Ppd-H1*. The majority of RFLP markers that were used were wholly or partially sequenced and this information was used to determine if the *Hd2/Ppd-H1* region had a recognizable counterpart in Arabidopsis. If so, this might provide candidate genes for *Ppd-H1* and *Hd2*.

MATERIALS AND METHODS

Plant material: Wheat (*Triticum aestivum* L.) "Chinese Spring"/barley "Betzes" addition lines (Islam *et al.* 1981) were used to assign clones to barley chromosomes. The following populations were used for genetic mapping: rice: "Nipponbare" × "Kasalath" F_2 (Kurata *et al.* 1994; Harushima *et al.* 1998) and "IR20" × "63-83" F_2 (Quarrie *et al.* 1997); barley: "Igri" × "Triumph" doubled haploid lines (Laurie *et al.* 1995) and "Captain" × *H. spontaneum* F_2 (Wang *et al.* 1992; Laurie *et al.* 1993); and maize: "Tx232" × "Cm37" and "Tx303" × "CO159" recombinant inbred lines (RIL; Burr and Burr 1991; http://burr.bio.bnl.gov/acemaz.html and http://www.agron.missouri.edu/images/).

Clone libraries: Sources of rice RFLP probes were described in Kurata *et al.* (1994) and Harushima *et al.* (1998). Other RFLP probes were from John Innes Centre libraries (PSR and PSB clones) or from Cornell University libraries (BCD, CDO, and WG clones). Construction and analysis of the rice Nipponbare yeast artificial chromosome (YAC) library and methods for YAC DNA preparation and isolation of YAC end clones by PCR were as described in Umehara *et al.* (1995). Nipponbare YAC sizes were from the Rice Genome Program website (http://bank.dna.affrc.go.jp/). Southern hybridization to genomic DNA of rice and to digested YAC or bacterial artificial chromosome (BAC) inserts used the Amersham enhanced chemiluminescence direct labeling method (Kurata *et al.* 1994) or ^{32}P labeling as described by Laurie *et al.* (1993). An IR20 rice YAC library (John Innes Centre, Norwich, UK) was also used. The Nipponbare and IR20 YAC libraries each represented about seven genome equivalents. Subcloning from rice YAC DNA was by ligation of a partial Sau3A digest into Bluescript vector (Stratagene, La Jolla, CA).

Rice BAC clones were from "IR-BB21" (Wang *et al.* 1995), "Lemont," and "Teqing" libraries (Zhang *et al.* 1996), representing 10 genome equivalents in total. Plasmid DNA from BAC clones was prepared by alkaline lysis and end clones were isolated by the method of Mozo (http://www/mpimp-golm.mpg.de/101/mpi_mp_map/bac.html) except that the primers used to amplify the T7 end were 5′-CCTCTTCGCTATTAC GCCAG-3′ and 5′-GCCCTTCCCAACAGTTGCG-3′. A Nipponbare cosmid library (Rice Genome Program, Tsukuba, Japan) and an IR20 phage library (John Innes Centre) were also used.

Nomenclature: In the text and figures, loci detected by Southern blot hybridization are italicized and prefixed by *X* and a three-letter lab designator (*e.g.*, *Xrgc213* and *Xpsr109*) and the corresponding clones by capital letters (*e.g.*, C213 and PSR109) following the nomenclature of McIntosh *et al.* (1998).

Database analyses: Sequences of rice clones and putative functions were obtained from the Japanese Ministry of Agriculture, Forestry and Fisheries DNA Bank website (http://bank.dna.affrc.go.jp/). Further sequence analyses were carried out using BLAST programs provided at the United States National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence database accession numbers and putative functions for clones used in this article are given in Table 1.

RESULTS

Comparative mapping of the *Ppd-H1* region of barley with rice and maize: Previously, rice clones C213 and C924 were shown to detect loci distal and proximal to the barley *Ppd-H1* locus, respectively (Laurie 1997). These flanking markers formed the starting point for the current analysis. Markers from the *Xrgc924*–*Xrgc213* interval in rice were tested in barley but the only probes suitable for comparative mapping were R411 and a closely linked YAC end clone (Y2938R, Table 1). Both were located between *Ppd-H1* and *Xrgc924* and although the order of the mapped markers was conserved between rice and barley, the genetic length of the *Xrgc213*–*Xrgc924* interval was considerably greater in the latter (1.2 cM *vs.* 13.5 cM). However, *Xrgr411* and *Xrgy2938R* cosegregated in both species (Figure 1).

Maize is generally considered to have undergone tetraploidization at some stage in its evolution (Helentjaris *et al.* 1988; Moore *et al.* 1995; Gaut and Doebley 1997) and sequences from the relevant region of rice chromosome 7 were therefore expected to be located on two maize chromosomes. R411, Y2938R, and C924 detected closely linked loci on the long arm of maize chromosome 7 where they were tightly linked to *Xnpi45b*. This region shared a number of duplicated loci with maize chromosome 2L, including a second locus detected by R411 (Figure 1). Y2938R detected two strongly hybridizing bands in maize but only one, on 7L, was polymorphic. These results were consistent with previous observations on the relationship between maize and rice chromosomes by Ahn and Tanksley (1993). C213, however, did not map in the same region. C213 provides the most distal marker on rice chromosome 7 (Harushima *et al.* 1998) and the closest RFLP marker distal to *Ppd-H1* in barley (Figure 1). In maize it detected a single band, which mapped to the proximal region of chromosome 6L. C924 detected a locus at the same 6L location but no other markers suggested a link to maize chromosome arms 2L or 7L.

The barley cDNA BCD221 detected 6–12 bands of varying intensity on Southern blots of barley, depending on the restriction enzyme used. BCD221 and two rice clones (R518 and C74) gave identical hybridization patterns. Partial sequences from these clones identified them as elongation factor 1α (EF-1α), corresponding to a full-length barley gene described by Nielsen *et al.* (1997) who mapped EF-1α sequences on barley chromosomes 2HS, 4H, 5HL, and 6H. The 2HS copy of BCD221 was located between *Ppd-H1* and *Xrgr411a* (Figure 1), making it of particular interest for comparative mapping. In rice, three bands of similar intensity were detected with BCD221 or R518. One band was polymorphic in the Nipponbare × Kasalath and IR20 × 63-83 crosses and in both cases a locus on rice chromosome

TABLE 1

Clones used for comparative mapping of the *Ppd-H1* region with database accession numbers, putative functions, and locations of homologous sequences in the Arabidopsis genome

RFLP clone (sequence accession)	Putative identification (sequence accession)	TBLASTN score (bits)	TBLASTN e value	Arabidopsis genomic location
R3156 (D25101)	*Sb* protein serine/threonine kinase cSNFL2 (Y12465)	171	2e-41	
	At CBL-interacting protein kinase 3 (CIPK3; AF286051)	160	3e-38	Chromosome 2 T20P8 (AC005623) [10.4]
	Several other related *At* sequences	116	5e-25	Chromosome 5 K15122 (AB016870) [18.7]
		114	2e-24	Chromosome 1 F28N24 (AC021043) [10.1]
		114	1e-24	Chromosome 4 F21A21 (AL035526) [13.8]
C924 (D22689)	*At* peptidyl-prolyl *cis-trans* isomerase (ATZ86095)	154	2e-36	Chromosome 5 F7K24 (AF296837) [6.5]
R1862 (D24417)	*At* ATP-dependent RNA helicase (O22899)	211	1e-53	Chromosome 2 T8I13 (AC002337) [17.1]
R1638 (D24287)	Full-length rice gene from Chromosome 10 BAC OSJNBb0064P21 (*mlo*-like protein)			
	At mlo-like protein (O22752; sequence O22815 is similar)	77	1e-15	Chromosome 2 BAC F5J6 (AC002329) [6.8]
R143 (D23782)	Full-length rice gene.osnas1 nicotianamine synthase 1 (AB021746)			
	At nicotianamine synthase (AB021936)	271	2e-72	Chromosome 1 T12M4 (AC003114) [2.8]
	Two other related *At* sequences	258	2e-68	Chromosome 5 MUG13 (AB005245) [1.5]
		257	5e-68	Chromosome 5 MDA7 (AB011476) [22.6]
C586 (D22623)	*Vu* glycine-rich protein (X87948); similar homologies in several other species	58	1e-05	Ambiguous
S1828 (AU070526)	*Hs* NADH:ubiquinone oxidoreductase PGIV subunit (AF044953)	66	3e-10	
	At unknown protein on BAC F24P17	175	2e-43	Chromosome 3 F24P17 (ATAC011623) [1.9]
R216 (D38992)	No significant homology			Unknown
R1158 (AU031732)	*St* spliceosomal protein (U2B) mRNA (M72892)	122	2e-27	
	At U2B from BACs T27E13 and T9D9	121	4e-29	Chromosome 2 T27E13, T9D9 (AC002338) [10.4]
S2267 (D40349)	No significant homology			Unknown
R411 (DC23852)	Full-length rice gene; 26S proteasome subunit 4 (D17789)			
	At 26S proteasome AAA-ATPase subunit RPT2a (AF123391)	756	0.0	Chromosome 4 F19B15 (AL078470) [16.2]
				Chromosome 2 T2G17 (AC006081) [7.9]
S941 (D39533)	*At* subunit RPT3 (AF123392)	381	e-105	Chromosome 5 MCK7 (AB019228) [23.2]
Results as for R411	*At* subunit RTP5 (AF123394)	332	1e-90	Chromosome 3 F22F7 (AC009606) [1.5]
				Chromosome 1 F7G19 (AC000106) [2.8]
	At subunit RPT6 (AF123395)	328	4e-89	Chromosome 5 F28I16 (AF296836) [7.1]
	At subunit RPT4 (AF123393)	320	8e-87	Chromosome 5 MBD2 (AB008264) [17.4]
				Chromosome 1 F27F5 (AC007915) [16.6]
	At subunit RPT1 (AF123390)	308	3e-83	Chromosome 1 T18A20 (AC009324) [19.6]
Y2938R (AZ301088)	*At* root EST. Putative P-loop-containing protein (AV541112)	189	6e-67	Chromosome 5 F7A7 (AL161946) [0.6]

(*continued*)

TABLE 1

(Continued)

RFLP clone (sequence accession)	Putative identification (sequence accession)	TBLASTN (score bits)	TBLASTN e value	Arabidopsis genomic location
C728 (D15501)	Full-length rice gene; *Os* L-ascorbate peroxidase (AB053297)			
	At ascorbate peroxidase *APX1* (X59600)	391	e-107	Chromosome 1 F24B9 (AC007583) [2.6]
	At ascorbate peroxidase *APX2* (X98275)	308	e-104	Chromosome 3 F11F8 (AC012394) [2.6]
S1979 (D40188)	*At* peroxidase *prxr5* (X98317)	68	8e-11	Chromosome 2 T1J8 to F3G5 (AC006260) [13.4]
C213 (C97957)	*At* 2-oxoglutarate dehydrogenase E1 subunit (AJ223802)	55	1e-14	Chromosome 5 F6H11 (AL021684) [25.4]
	Similar *At* gene on BAC T22E16	53	4e-11	Chromosome 3 T22E16 (AL132975) [21.3]
BCD221/R518 (AU031683)	Full-length rice gene; *Os* elongation factor 1α (DC63580)			
	Hv EF-1α (HVEF1ALFA)			
	At EF-1α (X16430); three copies on BAC T6D22	849	0.0	Chromosome 1 T6D22 (AC026875) [2.7]
		555	e-157	Chromosome 5 MUF9 (AB011483) [23.5]
		211	5e-54	Chromosome F1504 (AC007887) [13.2]

Species abbreviations: *At*, *Arabidopsis thaliana*; *Hs*, *Homo sapiens*; *Hv*, *Hordeum vulgare*; *Os*, *Oryza sativa*; *Sb*, *Sorghum bicolor*; *St*, *Solanum tuberosum*; *Vu*, *Vigna unguiculata*. Numbers in brackets in the Arabidopsis genomic location column are approximate physical positions (in megabases) from the top of the respective chromosomes. The sizes of chromosomes 1–5 are estimated to be 29.2, 17.5, 23.5, 22, and 26.2 Mb, respectively. Physical map locations and chromosome sizes are from The *Arabidopsis* Information Resource (TAIR) website (http://www.arabidopsis.org/).

3 was detected (Harushima *et al.* 1998 and Quarrie *et al.* 1997, respectively). To determine the location of the nonpolymorphic bands, BCD221 was used to screen the IR20 rice YAC library. Two clones were detected, both of which showed the same three bands that were detected on genomic Southern blots when digested with the appropriate restriction enzyme. This showed that all copies of the sequence in rice were at the chromosome 3 location, that barley had more genomic copies of BCD221 than did rice, and that the 2HS locus had no counterpart on rice chromosome 7. Thus, the gene content of the *Xpsr924-Ppd-H1-Xrgc213* region of barley cannot be fully determined by analysis of rice chromosome 7.

BCD221 detected ∼12 strongly hybridizing bands in maize, depending on the enzyme used, and at least six loci have been mapped (Chao *et al.* 1994; Davis *et al.* 1999; this article) using BCD221 itself, C74 (a rice EF-1α clone), or umc116 (maize EF-1α; Keith *et al.* 1993). One copy was mapped on chromosome 2L in the UMC maize population (Davis *et al.* 1999), and this location was similar to that of *Xrgr411b*. However, the copy mapped on chromosome 7L in the RIL populations was not closely linked to *Xrgr411a*, *Xrgc924a*, or *Xrgy2938R* (Figure 1). This may mean that the 2L and 7L copies of BCD221 are duplicate loci and that they are not the equivalent of the barley 2HS locus, but this conclusion must be treated with caution because not all the copies of BCD221 could be mapped. At least three loci were detected on maize 6L by BCD221 but these were not tightly linked to *Xrgc213* (Figure 1).

Three markers distal to *Xrgc213* are included on the barley map (Figure 1). PSR108 did not give a clear signal in rice but detected several bands in maize, one of which was mapped to chromosome 6L where it was linked to two *Xbcd221* loci. PSR666 and PSR109 detected loci in the centromeric region of maize chromosome 2L but were not polymorphic in rice. Nevertheless, these results were consistent with previous work showing that the homology of wheat and barley group 2 chromosomes to rice chromosome 7 ends close to the *Ppd-H1* region (Van Deynze *et al.* 1995; Laurie 1997), that more distal regions of 2HS are homeologous to rice 4 (Moore *et al.* 1995; Van Deynze *et al.* 1995), and that this part of rice 4 is homeologous to the centromere region and short arm of maize 2 (Ahn and Tanksley 1993).

Development of a YAC and BAC contig of rice chromosome 7: To investigate the relationship between rice chromosome 7 and the *Ppd-H1* region of barley chromosome 2H, we used R411 as the starting point for a contig of rice YAC and BAC clones (Figure 2). RFLP probes were hybridized to large insert libraries and clone order was verified by hybridizing additional probes and by isolating and hybridizing end clones. The contig contained three sequences mapped proximal to *Ppd-H1* in barley (C924, R411, and Y2938R) and C213. Cloned amplified fragment length polymorphism fragments

Figure 1.—Comparative maps of the *Ppd-H1* region of barley chromosome 2HS in relation to rice chromosome 7 and maize chromosomes 2 and 7. For clarity, barley 2HS is shown inverted. Markers from the *Ppd-H1* region are shown in boldface type. Maps with marker positions indicated by short solid lines are from (a) rice: Nipponbare × Kasalath (Harushima *et al.* 1998 plus additional data from this article); (b) barley: Igri × Triumph (Laurie *et al.* 1995 plus additional data from this article); (c) maize: Cm37 × Tx232 and Co159 × Tx303 (http://www.agron.missouri.edu/images/ plus additional data from this article). Loci mapped in other crosses (*e.g.*, *Xrgc74c* on maize 2L) are not connected to chromosomes and are positioned approximately using common markers. Additional markers in rice are from Causse *et al.* (1994), in barley from Laurie *et al.* (1993) plus additional data from this article, and in maize from Davis *et al.* (1999). Figures in brackets after a probe name indicate other chromosomes of rice (R) or maize (M) to which the probe maps. Open ovals show the approximate positions of the centromeres.

closely linked to *Ppd-H1* (Decousset *et al.* 2000) were also tested but they hybridized poorly to rice DNA and were not useful for comparative analysis.

Duplicate loci in rice revealed by RFLP mapping and analysis of the rice chromosome 7 contig: RFLP mapping in the rice Nipponbare × Kasalath cross showed that the rice cDNA clones R411, S1636, and S941 each detected two loci, one on chromosome 7 and one on chromosome 3. R411 and S1636 gave indistinguishable hybridization patterns on Southern blots, with strong hybridization to bands of the chromosome 7 locus and weak hybridization to bands of the chromosome 3 locus. S941 detected the same bands but with a reversed pattern of hybridization strengths. This, together with expressed sequence tag (EST) sequence comparisons, suggested that R411 and S1636 were cloned transcripts of a gene on chromosome 7 while S941 derived from a closely related gene on chromosome 3 (predicted coding regions and 3′ untranslated regions of R411 and S941 had 86 and 40% nucleotide identity, respectively). Hybridization of R411 to restriction digested YAC DNA showed that the band mapped to chromosome 7 detected two YACs (Y1273 and Y2938) while the band mapped to chromosome 3 detected five YACs previously assigned to chromosome 3 by hybridization to other mapped bands (Figure 2).

Hybridization of C586 to YAC DNA showed that the band mapped on chromosome 7 in the Nipponbare × Kasalath cross was present in one of the YACs detected by R411 (Y2938) and that an additional nonpolymorphic band was present in four chromosome 3 YACs previously assigned to the region immediately adjacent to the *Xrgr411b* locus (Figure 2). The remaining RFLP markers from the region of chromosome 7 shown in Figure 2 did not detect bands in the chromosome 3 YACs.

Two chromosome 3 cDNAs mapped in the Nipponbare × Kasalath cross (R3156 and S1828) detected bands when hybridized to DNA of the chromosome 7 YACs. R3156 hybridized to Y3607 (chromosome 3) and to Y4504 and Y2938 (chromosome 7). For S1828, the mapped band was present on the chromosome 3 YACs Y4932, Y3608, Y4421, and Y5002. An additional faint band was found on the chromosome 7 YACs Y1273 and Y2938 and BAC 14L15. The nonpolymorphic band of R3156 and of S1828 assigned to chromosome 7 and the

Figure 2.—Genetic and physical maps of the distal region of rice chromosome 7 and of a segment of rice chromosome 3. YAC and BAC clones show the positions of RFLP probes (solid square), genetically mapped YAC/BAC end fragments (open circle), and their hybridization to other YAC/BAC clones (solid circle); YAC/BAC end fragments that could not be genetically mapped are shown as open ovals and their hybridization to other YAC/BAC clones by solid ovals; one rice microsatellite is shown (solid diamond). For clarity, the YACs are not drawn to scale. Brackets show probes that could not be ordered from genetic or physical mapping data.

band of C586 assigned to chromosome 3 were detected on at least two YACs, making it unlikely that the results were due to chimerism.

The genetic map locations and patterns of hybridization to YACs and BACs showed that the four duplicated sequences were in the same order on both chromosomes, suggesting that a chromosome segment was duplicated during the evolution of the rice genome. However, there was good evidence that the respective regions were no longer duplicated in their entirety. Mapping and cross-hybridization of end clones from the chromosome 7 YACs showed that they formed a contig spanning the *Xrgr2576S* to *Xrgs1979* interval (Figure 2). Therefore, the failure of additional chromosome 3 probes from the *Xrgr3156–Xrgr411b* interval to detect sequences on chromosome 7 was not due to gaps in the chromosome 7 contig. Hybridizations using chromosome 3 RFLP markers and YAC end clones showed that the chromosome 3 YACs did not form a single contig. However, this was not the reason for the failure of the chromosome 7 probes L831, C924, and S2267 to detect chromosome 3 sequences because for these probes all bands detected by Southern hybridization using genomic DNA could be accounted for by hybridization to the chromosome 7 YACs.

The four duplicated clones were located within a region of no more than 400 kb on chromosome 7 because they hybridized to a single YAC of this size. The size

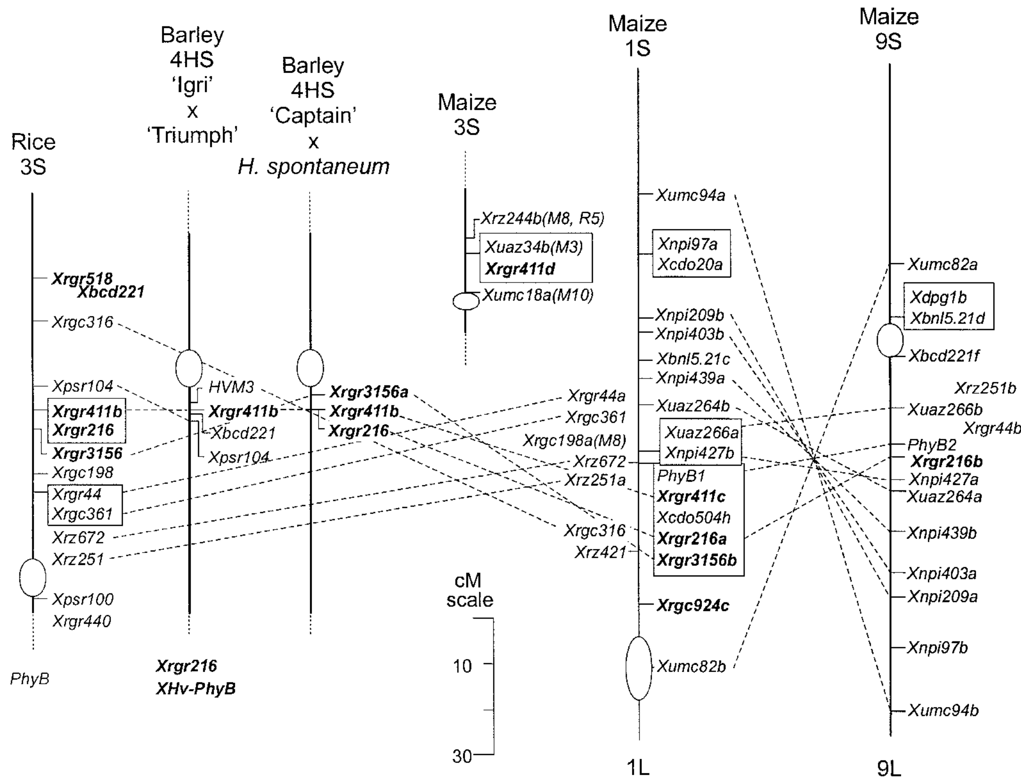

Figure 3.—Comparative maps of rice chromosome 3, barley chromosome 4H, and maize chromosomes 1 and 9. Markers from the rice 3 and rice 7 regions in Figure 2 are shown in boldface type. Data sources are the same as in Figure 1.

of the equivalent chromosome 3 region remains to be determined but it must be >810 kb, which is the combined size of the nonoverlapping YACs Y2565 and Y5002. This may be a considerable underestimate because these YACs do not contain the sequences detected by R3156 and R411/S941. The genetic distance between the duplicated loci was 0.9 cM on chromosome 7 compared to 3.1 cM on chromosome 3, showing that the chromosome 3 region was physically and genetically larger.

Comparative mapping using probes detecting duplicate sequences in rice: Probes detecting loci on rice chromosomes 3 and 7 (Figure 2) were used to analyze barley and maize. In barley, R411 detected a second locus on barley chromosome 4H (Figure 3) in addition to the 2HS locus (Figure 1) . In maize, hybridization of R411 to the parents of the two maize RIL populations detected three strongly hybridizing bands, all of which could be mapped. Two were the 2L and 7L copies (Figure 1) and the third mapped on 1S close to the *PhyB1* locus, a region previously shown to be colinear with rice chromosome 3 (Ahn and Tanksley 1993) and consistent with the location of the rice *Xrgr411b* locus (Figure 3). No copy of R411 was present on maize chromosome 9 although the long arm shares other duplicated loci with 1S. R411 detected a weakly hybridizing band in one of the RIL populations that was mapped to maize chromosome 3, a region not known to be related to any of the others where copies of R411 were detected.

R3156 detected one strongly hybridizing band in maize and several weaker bands. The strongly hybridizing band gave a polymorphism that cosegregated with *Xrgr411c* on chromosome 1S in both RIL populations (Figure 3). A second polymorphism was mapped to chromosome 7L where it cosegregated with *Xrgr411a* (Figure 1). Thus, R3156 behaved in a similar way to R411. In barley, R3156 detected one strongly hybridizing band and one to three weaker bands depending on the restriction enzyme used. The most strongly hybridizing band was assigned to 4H using wheat/barley addition lines and cosegregated with the 4H copy of R411 in the Captain × *H. spontaneum* cross (Figure 3). The weakly hybridizing bands were not polymorphic but at least one could be assigned to chromosome 2H using wheat/barley addition lines. C586 and S1828 gave poor hybridization with barley and maize DNA and were not suitable for comparative mapping.

Clones from the *Xrgr3156–Xrgr411* intervals (Figure 2) that were not duplicated in rice were also tested. Of these only R216 was suitable for comparative mapping and polymorphic. R216 detected a single copy sequence in rice chromosome 3 (Figure 2) and a single band in barley that cosegregated with *Xrgr411b* on chromosome 4H (Figure 3). R216 detected two bands in maize, both of which were polymorphic in the Tx232 × Cm37 RIL population. One mapped on chromosome 1, cosegregating with *Xrgr411c*, and the other mapped on chromosome 9 close to *PhyB2* (Figure 3). PHYB behaves similarly to R216. It is present only on maize chromosomes 1 and 9 and was present as a single copy sequence

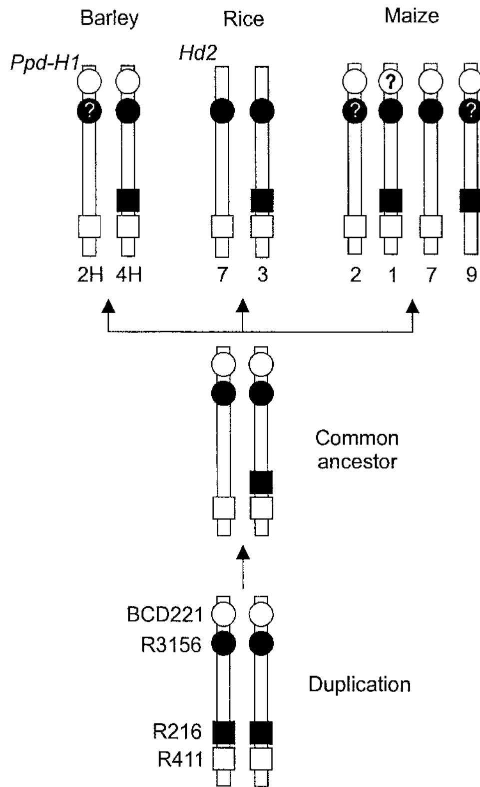

Figure 4.—Model for the evolution of the duplicated segments in barley, rice, and maize.

on barley chromosome 4H [determined by hybridization of a PHYB specific sequence (GenBank accession no. U08169) to wheat/barley addition lines]. BCD221 (EF-1α) detected copies linked to *Xrgr411a* (2H) and *Xrgr411b* (4H) in barley (Figures 1 and 3), but detected neither region in rice. In maize several copies were mapped on the relevant chromosomes but their relationship was less clear.

Two YAC end clones (Y1273L and Y2938R) from the rice chromosome 7 contig were suitable for comparative mapping. Y1273L detected several bands, and Y2938R one band, on Southern blots of rice genomic DNA. Both probes detected two loci in barley. For Y1273L, only one band was polymorphic and this mapped to the centromere region of chromosome 5H. For Y2938R, both bands could be mapped, detecting loci on 2HS and 7HS. Y1273L hybridized poorly to maize while Y2938R detected two bands in maize, one of which was polymorphic and shown to be on chromosome 7L (Figure 1). Thus, although R411 and Y2938R were closely linked, they showed different patterns of duplication in the three species tested.

These results suggest that a region spanning at least *Xrgr411* to *Xbcd221* was duplicated in the common ancestor of barley, rice, and maize and had evolved part of its present structure before these species diverged (Figure 4). For example, R216 would have been deleted from one linkage segment, or inserted into the other, before divergence. After the barley, rice, and maize lineages separated, BCD221 sequences were deleted from rice 3 and 7 but retained in barley 2H and 4H. The duplication of R411 was retained in rice, barley, and maize but one copy was subsequently deleted from maize 9, possibly after tetraploidization. If this model is correct, additional sequences such as Y2938R should have been within the original duplication. The absence of Y2938R from rice 3 and from barley 4H but its presence on 7HS suggests either additional deletion and duplication events or translocation of the 4H copy. It would be interesting to investigate the behavior of the duplicated sequences in other monocot lineages.

Duplicated sequences in Arabidopsis: Candidate Arabidopsis homologs of sequences from the relevant regions of rice chromosomes 3 and 7 were identified by BLAST analyses using the predicted peptide sequences of the rice RFLP markers or, where available, the full-length sequence of the rice gene (Table 1). Where matches were found, the nucleotide sequence of the Arabidopsis gene was used to identify its genomic location. The full-length peptide corresponding to the R411 EST sequence was highly homologous to Arabidopsis 26S proteasome AAA-ATPase subunit RPT2 (predicted peptides were 93% identical and 96% similar). The R411 sequence, or its Arabidopsis equivalent, detected two highly conserved copies in the Arabidopsis genome, one on chromosome 2 (BAC T2G17) and the other on chromosome 4 (BAC F19B15). However, analysis of other sequences linked to R411 in rice (Figure 2), plus BCD221 (EF-1α), showed no association with RTP2 sequences in Arabidopsis (Table 1).

The BAC sequences containing the R411 homologs were within the extensive duplication of Arabidopsis chromosomes 2 and 4 described by Lin *et al.* (1999) and Mayer *et al.* (1999) and might result from a separate duplication event. Therefore, more diverged Arabidopsis R411-like sequences were also analyzed (RPT1 and RTP3–RPT6, Table 1). The only significant clustering was the association of sequences homologous to C728, EF-1α, RTP5, and R143 in a region of chromosome 1 spanning ~600 kb. However, if marker order was conserved with the predicted ancestral region in cereals, R143 would be predicted to be between R411 and Ef-1α, which was not observed. Thus, no clear equivalent to the *Hd2* or *Ppd-H1* regions that would provide candidate genes for further analysis was identified. Consequently, it was not possible to determine if the duplication observed in cereals had an equivalent in Arabidopsis. The lack of extensive fine-scale colinearity observed in this study is consistent with two recent comparisons of rice and Arabidopsis (Devos *et al.* 1999; van Dodeweerd *et al.* 1999).

DISCUSSION

Comparative mapping of the *Ppd-H1* region: Although the *Ppd-H1* gene of barley lies close to a junction between linkage segments RLS7 and RLS4a, the order

of markers derived from rice was not altered when they were mapped in barley. Duplicated sequences closely linked to *Ppd-H1* complicated the comparison, but their analysis in barley, rice, and maize suggested that they were the remnants of an ancient duplication and were not generated by the formation of the 2HS chromosome. The results from rice 7 and rice 3 contrast with the well-conserved duplicated segments on chromosomes 11 and 12 (Nagamura *et al.* 1995; Wu *et al.* 1998) and suggest that if a duplication occurred it is likely to have been considerably earlier in evolution. The results in rice, barley, and maize are consistent with the hypothesis that a duplication had occurred and had assumed at least part of its present form before these species diverged some 50–80 million years ago (Wolfe *et al.* 1989; Bennetzen and Freeling 1997).

If the model for the duplication of markers in the *Ppd-H1* region shown in Figure 4 is correct, it is important to consider whether the duplication extended beyond BCD221 to include the closely linked *Ppd-H1* gene in barley or *Hd2* in rice. No markers distal to *Ppd-H1* followed the same pattern of duplication and there is no evidence in the literature for a major flowering time gene in the relevant regions of barley 4H or any of the other Triticeae group 4 chromosomes. This suggests that *Ppd-H1* was not duplicated, although deletion from the 4H location is possible. Similarly, the only major flowering time gene in the relevant regions of rice or maize is *Hd2* on rice chromosome 7.

Strategies for isolating *Ppd-H1*: Comparative analysis provided valuable additional markers for the *Ppd-H1* region but there were significant complications in using rice to target the barley gene. Whether this is a general conclusion for rice-to-barley comparisons remains to be seen, but for the *Ppd-H1* region it will be desirable to develop a physical map of the region in barley and to sequence a minimum region defined by flanking markers to identify genes that can be assayed for their effects on flowering. Despite the complications described above, rice genomic sequence is likely to provide significant benefits in the development of new markers for mapping and library screening. Rice genes identified from genomic sequencing could be used directly as probes or to identify homologous Triticeae ESTs. This is preferable to the use of random subclones of YAC DNA, which we found unrewarding.

As the relationship between the *Ppd-H1* and *Hd2* regions is clarified it will be possible to assess the relationship between the two genes in more detail. Recent work using larger mapping populations suggests that *Hd2* is distal to *Xrgc213* in rice (M. Yano, unpublished data), making it less likely to be a direct counterpart of *Ppd-H1*, but it will be interesting to see whether any equivalent of *Ppd-H1* is present in rice or any equivalent of *Hd2* is present in barley.

Analyzing the genomic sequences of Arabidopsis did not identify any region that could be equivalent to the *Hd2* or *Ppd-H1* regions of rice and barley, respectively, and therefore failed to provide candidate genes for further analysis. Thus, while homology at the gene level between Arabidopsis and cereals will be of considerable value, as shown by Peng *et al.* (1999), map-based comparisons seem unlikely to be useful in this case.

More generally, further analysis of the genetic and physical maps of rice and of the rice genomic sequence will provide evidence of the extent to which duplication has occurred in the evolution of cereal genomes and will be useful for assessing the importance of segment duplication in the evolution of gene families. These data, in turn, will help determine if segment duplication has been significant in the evolution of agronomically important characters in crop species. Combined with expression data, this should provide insights into the selective advantage of duplication and the ways in which duplications evolve.

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