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Rapid reorganization of resistance gene homologues in cereal genomes

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ABSTRACT **We used conserved domains in the major class (nucleotide binding site plus leucine-rich repeat) of dicot resistance (*R*) genes to isolate related gene fragments via PCR from the monocot species rice and barley. Peptide sequence comparison of dicot *R* genes and monocot *R*-like genes revealed shared motifs but provided no evidence for a monocot-specific signature. Mapping of these genes in rice and barley showed linkage to genetically characterized *R* genes and revealed the existence of mixed clusters, each harboring at least two highly dissimilar *R*-like genes. Diversity was detected intraspecifically with wide variation in copy number between varieties of a particular species. Interspecific analyses of *R*-like genes frequently revealed nonsyntenic map locations between the cereal species rice, barley, and foxtail millet although tight collinear gene order is a hallmark of monocot genomes. Our data suggest a dramatic rearrangement of *R* gene loci between related species and implies a different mechanism for nucleotide binding site plus leucine-rich repeat gene evolution compared with the rest of the monocot genome.**

Plant resistance to particular pathogens involves specific recognition events (1). These resistance reactions are race-specific and triggered by corresponding resistance (*R*) genes in the host and avirulence (*Avr*) genes in the pathogen. Resistance mechanisms operate in both major classes of flowering plants, dicots and monocots. Several dicot and one monocot *R* gene to diverse pathogens have been isolated, revealing structural similarities of the deduced proteins (1).

One class comprises genes containing both a 5′ terminal nucleotide binding site (NBS) and 3′ terminal leucine-rich repeats (LRRs) of various length. The NBS-LRR type includes *Rps2*, *Rpm1*, *N*, *L6*, *M*, *Rpp5*, *Prf*, and *I2C-1* (reviewed in ref. 1). The second group comprises genes containing a kinase and/or a LRR domain. The genes *Cf-2*, *Cf-4*, *Cf-9*, *Xa-21*, and *Pto* all possess 5′ localized LRRs and/or a serine/threonine kinase domain (reviewed in refs. 1 and 2). Evidence supports that the highly variable LRRs in both classes of *R* genes might have a role in pathogen recognition, providing a structural backbone that has been modified over evolution in response to variation in *Avr* gene products (3–5).

Although the overall sequence homology among members of the NBS-LRR class is poor, short stretches of peptide sequences are well conserved (6–9). These conserved motifs, in and adjacent to the NBS domain, enabled a PCR-based approach that used degenerate primers to amplify *R*-like genes from the dicot species potato and soybean (10–12). For the kinase and/or LRR class, only one homologue has been reported in wheat (13). To evaluate the possible functions of these genes, their linkage to characterized resistance specificities was tested by restriction fragment length polymorphism (RFLP) mapping. Twelve homologues representing seven different classes in potato were isolated and revealed either linkage or cosegregation to a fungal and nematode resistance locus (10). Similar results were obtained by Kanazin *et al.* (11) and Yu *et al.* (12), who isolated nine and 11 different classes of NBS-LRR homologues, respectively, in soybean and detected correlations with characterized resistance loci. The wheat kinase/LRR-like homologue cosegregated genetically with the rust-resistance locus *Lr10* (13).

Monocot and dicot plants are believed to have diverged from each other 120–200 million years ago (14). All resistance genes, except *Xa-21* in rice (15), have been isolated so far from dicot plants. Isolation of genetically characterized resistance genes in the grasses has been hampered mainly by their large genome size. A fundamental feature of grass species is their tight conserved gene order (synteny) (16, 17), which may facilitate map-based gene isolation by using DNA marker information from syntenic intervals across monocot species.

Here we describe the isolation and characterization of NBS-LRR homologues via PCR from two monocot species, rice and barley, based on structurally conserved motifs in dicot NBS-LRR *R* genes. We have analyzed their sequence diversity and their linkage to genetically characterized *R* genes. The results from a comparative mapping in rice, barley, and foxtail millet indicates a rapid evolution of *R* genes in each species and suggests possible mechanisms to generate diversity in resistance loci.

METHODS

Plant Material. For PCR-based isolation of *R* gene candidates we used the rice cultivar Nipponbare and the barley cultivar Ingrid. Nipponbare belongs to the subspecies *Oryza sativa japonica* and represents one parent for the intraspecific cross used for rice RFLP mapping (see below) and was used to construct the rice yeast artificial chromosome library (18).

RFLP Probes, RFLP Mapping, and Southern Analysis. Sequences of all rice and barley NBS-LRR gene fragments and their precise map coordinates have been deposited in the GenBank. In addition, comparison of deduced amino acid sequences with characterized dicot NBS-LRR genes is provided in the Grain Gene Database (http://wheat.pw.usda.gov/ggpages/R-likes.html).

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Abbreviations: NBS, nucleotide binding site; LRR, leucine-rich repeat; RFLP, restriction fragment length polymorphism; RHC, *R* gene homologue cluster.
Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF032679 and AF032680–AF032703).
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For RFLP mapping of NBS-LRR probes in rice, 186 F_2 plants of the intraspecific cross Nipponbare × Kasalath were used (19). The genetic map locations of *R* gene candidates in barley were determined by RFLP analysis using doubled haploid populations of *Hordeum vulgare* cultivars Igri × Franka (20), Igri × Triumph (21), or Blenheim × Kym (22) and a F_2 population of Captain × *Hordeum spontaneum* (23, 24). Populations consisted of 71, 94, 99, and 120 individuals, respectively. Genetic mapping in foxtail millet was carried out in 127 F_2 plants from the interspecific cross *Setaria italica* accession B100 × *Setaria viridis* accession A10 (25).

For Southern analysis, genomic DNA of rice (4 μg), foxtail millet (5 μg), or barley (10 μg) were digested with restriction enzymes, size-fractionated, blotted, and hybridized as described (19, 20). Hybridization in rice was performed by using the ECL system (Amersham) and in barley and foxtail millet by use of randomly primed ^{32}P-labeled probes (26). Washes were performed at 65°C with 0.2× SSC (homologous probes) or 2× SSC (heterologous probes).

PCR Analysis. DNA was prepared as described earlier (27). PCRs were performed in a total volume of 25 μl with 0.5 units of *Taq* DNA polymerase (Boehringer) in 10 mM Tris·HCl, pH 8.3/1.5 mM $MgCl_2$/50 mM KCl/0.1 mM dNTPs/0.25 μM of each primer/20–100 ng of template DNA. Amplification of NBS-LRR genes was performed with primer combinations 1–8 according to the combination of the sense primer with each of the antisense primers (#1–8; Table 1). Annealing temperatures of 42–48°C were applied according to the individual GC content of each degenerate primer. Cycling conditions were: initial denaturation, 2 min, 30 sec at 93°C, followed by 35 cycles each with 15-sec denaturation at 93°C, 45 sec annealing at temperatures given above and 1 min, 20 sec elongation at 72°C. For reverse transcriptase-PCR total RNA was isolated by using the RNeasy Plant System (Qiagen) and first-strand cDNA was synthesized by using the SuperScript Preamplification System (GIBCO/BRL). One microliter of first-strand cDNA mixture was used for PCR amplification in a total volume of 25 μl.

Amplification Product Analysis. Amplified fragments showing the expected size of 450–550 bp and representing a heterogenous population of PCR products were individualized by cloning into the pGEM-T vector system (Promega) and electroporating DH10B electrocompetent cells (GIBCO/BRL) according to the supplier's instructions. One hundred clones of each PCR product population were picked randomly and grouped by restriction digests by using enzymes with 4-bp recognition sequences. One representative of each identified group was sequenced by using the Dye Terminator Cycle Sequencing Kit and the Applied Biosystems Prism 377 DNA sequencer (Perkin–Elmer).

Sequence Analysis. Sequence data were analyzed with the Genetics Computer Group, Wisconsin Program, version 8 (28), and GenBank and EMBL databases were searched by using the BLAST algorithm (29). Predicted amino acid sequences of rice and barley NBS-LRR gene fragments were aligned by using the CLUSTAL W program, version 1.60 (30). The phylogenetic tree was constructed by using the neighbor-joining method (31).

Subtraction of Repetitive Genomic DNA. Repetitive sequences from barley genomic DNA were subtracted according to Clarke *et al.* (32). Genomic DNA was sonicated, denatured, and reannealed by using a C_0t of 100–120. Double-stranded DNA (repetitive DNA) was removed by adsorption onto a hydroxyl apatite column. The remaining single-stranded (low/single copy) DNA was quantified by ethidium bromide staining. Aliquots of 5–10 ng were used for amplification with degenerate primers as described above.

Isolation of Flanking Sequences by Rapid Amplification of cDNA Ends (RACE). Flanking sequences of rice and barley NBS-LRR genes were isolated by RACE using the Marathon cDNA Amplification Kit (CLONTECH). Total RNA was isolated as described above, and double-stranded cDNA was generated. After ligation of adaptors, the library of adaptor-ligated double-stranded cDNA was used as a template for the amplification of 5′ and 3′ ends. Nested PCR was applied, by using primers complementary to the adaptors (AP1 and AP2), and two nested primers for each 5′ and 3′ ends, complementary to the NBS-LRR genes. PCR products were cloned and submitted to sequence analysis as described above.

RESULTS

Members of the major class of plant *R* genes, characterized by the presence of a NBS and various units of LRRs (1, 7, 9), were isolated from rice (*Oryza sativa* subspecies *japonica*) by using genomic DNA as template in PCRs with degenerate primers (see *Methods*). PCR products of the expected size (400–550 bp) were obtained, cloned, and sequenced. The 16 different rice NBS-LRR homologues (designated r1-r16) were isolated and assigned to 14 classes based on sequence comparison by CLUSTAL W analysis (30) (Fig. 1). In barley this protocol gave only unspecific PCR products most likely because of its large DNA genome (33). However, we isolated nine barley NBS-LRR homologues (designated b1-b9; Fig. 1 and *Methods*) by using either RNA and reverse transcriptase–PCRs (b2, b4, b5, b6, b7, and b8) or a subtraction procedure for genomic DNA involving hydroxyl apatite columns (32) (b1, b2, b3, b4, b5, and b9). Further 5′ and 3′ sequence was obtained by rapid amplification of cDNA ends for five rice (r1, r2, r10, r11, and r15) and five barley candidates (b1, b4, b5, b8, and b9), and each revealed additional conserved motifs as described for dicot NBS-LRR genes (9) but no monocot-specific signature (see *Methods*). These findings suggest that these PCR products may represent genuine homologues of *R* genes rather than genes merely containing a NBS domain. A comparison of rice versus barley NBS-LRR homologues by CLUSTAL W indicated only

Table 1. Oligonucleotides designed to conserved peptide motifs of dicot NBS-LRR *R* genes *N*, *Rps2*, and *L6*

Consensus motif	Primer designation								T_A, °C
P-loop		G	G	V/I	G	K	T	T	
	Sense	GGI	GGI	(A,G)TI	GGI	AAI	ACI	AC	48
GLPL(A/T)L		G	L	P	L	A/T	L		
	Antisense #1	IAG	IG(C,T)	IAG	IGG	IAG	ICC		48
	#2	IAG	IG(C,T)	IAG	IGG	IAA	ICC		46
	#3	IAG	IG(C,T)	IAA	IGG	IAG	ICC		46
	#4	IAG	IG(C,T)	IAA	IGG	IAA	ICC		46
	#5	IAA	IG(C,T)	IAG	IGG	IAG	ICC		46
	#6	IAA	IG(C,T)	IAG	IGG	IAA	ICC		44
	#7	IAA	IG(C,T)	IAA	IGG	IAG	ICC		44
	#8	IAA	IG(C,T)	IAA	IGG	IAA	ICC		42

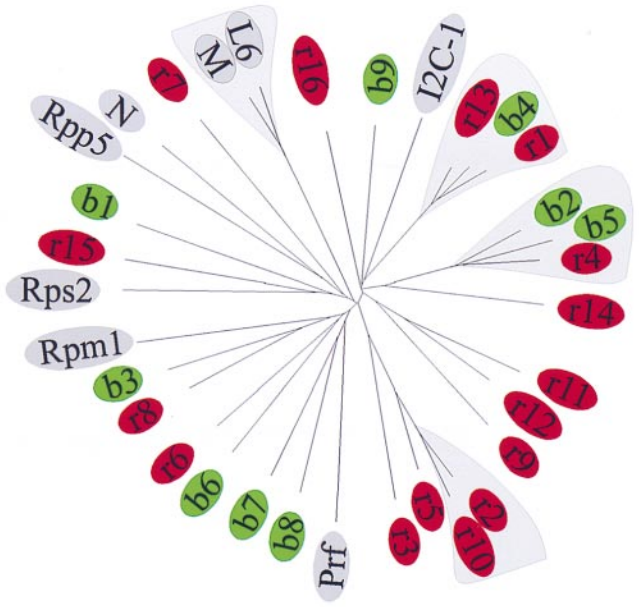

FIG. 1. Phylogenetic tree of monocot *R* gene homologues and characterized dicot NBS-LRR genes. Amino acid sequences of rice (red) and barley (green) NBS-LRR genes were aligned by using the CLUSTAL W program (30). The phylogenetic tree was constructed by using the neighbor-joining method of Saitou and Nei (31). For comparative purposes characterized dicot NBS-LRR genes *N* (tobacco), *M* and *L6* (flax), *RPS2*, *Rpm1*, and *Rpp5* (*Arabidopsis*), and *Prf* and *I2C-1* (tomato) have been included (1). Homologues or *R* genes sharing ≥80% DNA sequence similarity are marked by gray shaded circles.

two cases of high DNA sequence relatedness (b2, b5, r4 and r1, r13, b4; Fig. 1 gray shaded circles). It suggests that these family members either recently have diverged from each other or that their sequence conservation is the result of a similar functional selection acting in both species. Taken together, the CLUSTAL W analysis indicates that the barley and rice homologues represent a sample survey of 20 highly dissimilar monocot NBS-LRR genes.

Rice *R* gene homologues were mapped by RFLP analysis (loci designated *Os-rx*; *Methods*) and integrated in the high-resolution rice RFLP map (19). All 14 dissimilar NBS-LRR genes could be mapped, detecting a total of 18 different loci on nine of the 12 linkage groups. Most probes detected single loci with variable copy numbers whereas probes r4 and r6 detected two or four loci, respectively, on different chromosomes and these loci were named *Os-r4.x* or *Os-r6.x*, respectively. The latter observation may indicate different events in the parental rice lines (interchromosomal duplication or deletion). Interestingly, some highly divergent rice NBS-LRR probes detected genes that mapped to the same genetic locus. We designated them mixed *R* gene homologue clusters (RHCs): RHC-A (*Os-r2* and *Os-r6.4*), RHC-B (*Os-r11*, *Os-r12*, and *Os-r4.2*), and RHC-C (*Os-r15* and *Os-r16*). Expectedly, *Os-r2* in RHC-A also was detected by r10, a probe closely related to r2 (Fig. 1). The more distantly related probe r5 (Fig. 1) detected *Os-r5* at a distance of 14 centiMorgan proximal to RHC-A, suggesting that *Os-r5* might have arisen from *Os-r2* by an intrachromosomal duplication.

We discovered that several NBS-LRR genes were linked to characterized *R* genes (Table 2). It is striking that a large number of rice NBS-LRR genes map on rice chromosome 11, which is known to harbor a large collection of characterized resistance specificities (34). Probe r4, detecting locus *Os-r4.1*, cross-hybridizes with a rice yeast artificial chromosome (Y5212) at this chromosomal site. This yeast artificial chromosome harbors the *Xa-1* gene (35), indicating not only genetic but also tight physical linkage of *Os-r4.1* to a functional resistance specificity.

Table 2. Linkage of *R*-like gene loci to characterized resistance loci

Species	*R*-like gene loci	Linkage to resistance loci
Rice		
	RHC-A (*Os-r2*, *Os-r6.4*, *Os-b8*)	*Pi-1(t)*[46,49], *Xa-3*[47], *Xa-4*[47], *Pi-k*[48], *Pi-f*[49]: *R* gene cluster(chr.11)
	RHC-B (*Os-r4.2*, *Os-r11*, *Os-r12*)	*Pi-7(t)*[50](chr.11)
	Os-r6.2, *Os-r5*	*Xa-10*[51](chr.11)
	Os-r6.3	*Pi-11(t)*[22](chr.8)
	Os-r4.1	*Xa-1*[23](chr.4)*
Barley		
	Hv-b6.1	*Mla-6*[20](chr.1H)†
	Hv-b7	*Ml-k*[52](chr.1H)
	Hv-r1	*Ml-nn*[52](chr.1H)
	Hv-b3.2	*ym-11*(chr.4H)
	Hv-b7.2, *Hv-b2.3*	*Rph11*[53](chr.3H)
	Hv-b9	*Rpg1*[25](chr.7H)‡

R gene homologue loci were correlated to described *R* loci by comparison with flanking RFLP or morphological markers. Superscript numbers give references for each resistance trait.
*r4 probe identifies a rice yeast artificial chromosome clone containing *Xa-1*; see text.
†Cosegregation was found in 71 double haploid progeny segregating for *Mla-6*.
‡*Hv-b9* maps in the same genetic interval as *Rpg1*.

We used segregants from four crosses to locate *R* gene homologue loci in barley, *Hordeum vulgarum* (designated *Hv-bx*); seven probes were mapped and detected 14 loci on seven linkage groups. *Hv-b6.1* cosegregated with the powdery mildew (*Erysiphe graminis*) resistance specificity *Mla-6* (20) (Table 2). *Hv-b9* comprises several cosegregating copies mapping to the short arm of chromosome 7H and coincides with the map position of rust resistance *Rpg1* (36).

We observed that the copy numbers of some NBS-LRR genes varied considerably between the mapping parents. For example, twice the number of hybridizing fragments was

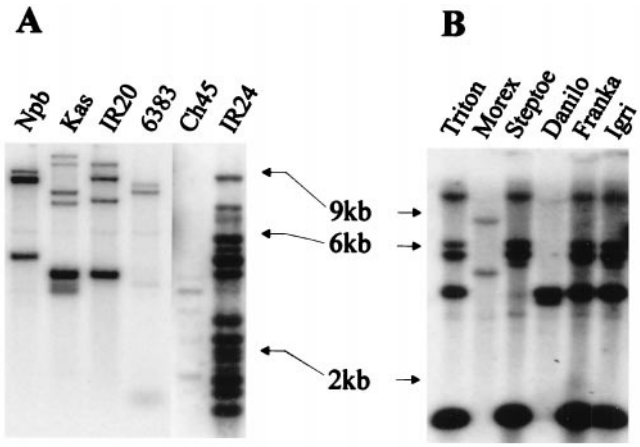

FIG. 2. Copy number variation of NBS-LRR genes in cultivars of rice and barley. Genomic Southern analysis for (*A*) the rice NBS-LRR probe r2 in six rice cultivars: Npb, Nipponbare; Kas, Kasalath; IR20; 6383; Ch45, Chugoku 45; and IR24. The number of bands range from two minor cross-hybridizing bands in Ch45 to 15 major bands in IR24. Analysis of segregants from the cross Ch45 × IR24 revealed that all NBS-LRR copies detected by r2 in Ch45 map to a single locus. (*B*) The barley probe b9 shows the diversity in copy number in six barley cultivars. Note that only two weakly cross-hybridizing signals are detected in cv. Franka but five prominent signals in cv. Igri.

detected for rice probes r2, r5, and r10 in rice accession Kasalath in comparison to Nipponbare (Fig. 2*A*). A survey of six rice accessions with the r2 probe revealed an extreme range from absence to very high copy numbers (compare accessions Chugoku 45 and IR 24). Variations in copy numbers also were observed between six barley cultivars probed with b9 (Fig. 2*B*).

We analyzed whether the intraspecific copy number variation of *R* gene homologues affected collinear gene order among related species, a hallmark of monocot genomes (16, 17). A comparative mapping of the isolated homologues was carried out in the monocot grass species rice, barley, and foxtail millet (Fig. 3). Unexpectedly, probes mostly gave weak signal intensities or no polymorphism when we attempted to map barley NBS-LRR genes in rice or rice genes in barley. Only two rice NBS-LRR probes, r1 and r6, detected strongly cross-hybridizing fragments, which were located on barley chromosome 1HS (*Hv-r1* and *Hv-r6*). Interestingly, the barley probe b8, which in rice detected locus *Os-b8*, represents an additional member of RHC-A (Fig. 3). Both *Os-b8* and *Hv-r6* mapped to nonsyntenic chromosomal intervals (Fig. 3). Three additional barley probes, b2/b5 and b4, exhibit the same fragment pattern as rice probes r4 and r1 in rice genomic Southerns, which is likely because of their DNA sequence similarity of ≥80% (Fig. 1).

Six rice and four barley *R* gene homologues could be located on the foxtail millet map (loci designated *Si-rx* and *Si-bx*) by RFLP analysis. Surprisingly, of 17 identified loci only five were found at syntenic map locations (highlighted by bold spokes in Fig. 3). Lack of synteny was more common than conservation and was revealed by rice probes r1, r2, and r9 or by barley probes b6, b7, and b9 (loci *Si-r1.2*, *Si-r2.2*, *Si-r2.3*, *Si-r2.4*, *Si-r9.1*, *Si-r9.2* and *Si-b6.1*, *Si-b6.2*, *Si-b7, Si-b9*). We identified in foxtail millet again two *R* gene homologue clusters comprising highly divergent genes (RHC-D: *Si-r2.3*, *Si-b1.1*, and *Si-b6.1*; RHC-E: *Si-r1.2* and *Si-b6.2*). Interestingly, the cross-hybridizing copies of b6 (*Si-b6.1* and *Si-b6.2*) each were found juxtaposed to divergent *R* gene homologues in RHC-D and RHC-E; similarly, r2 copies (*Os-r2* and *Si-r2.3*) each were found juxtaposed to divergent *R* gene homologues in the rice cluster RHC-A and the foxtail millet cluster RHC-D (dotted lines in periphery of Fig. 3).

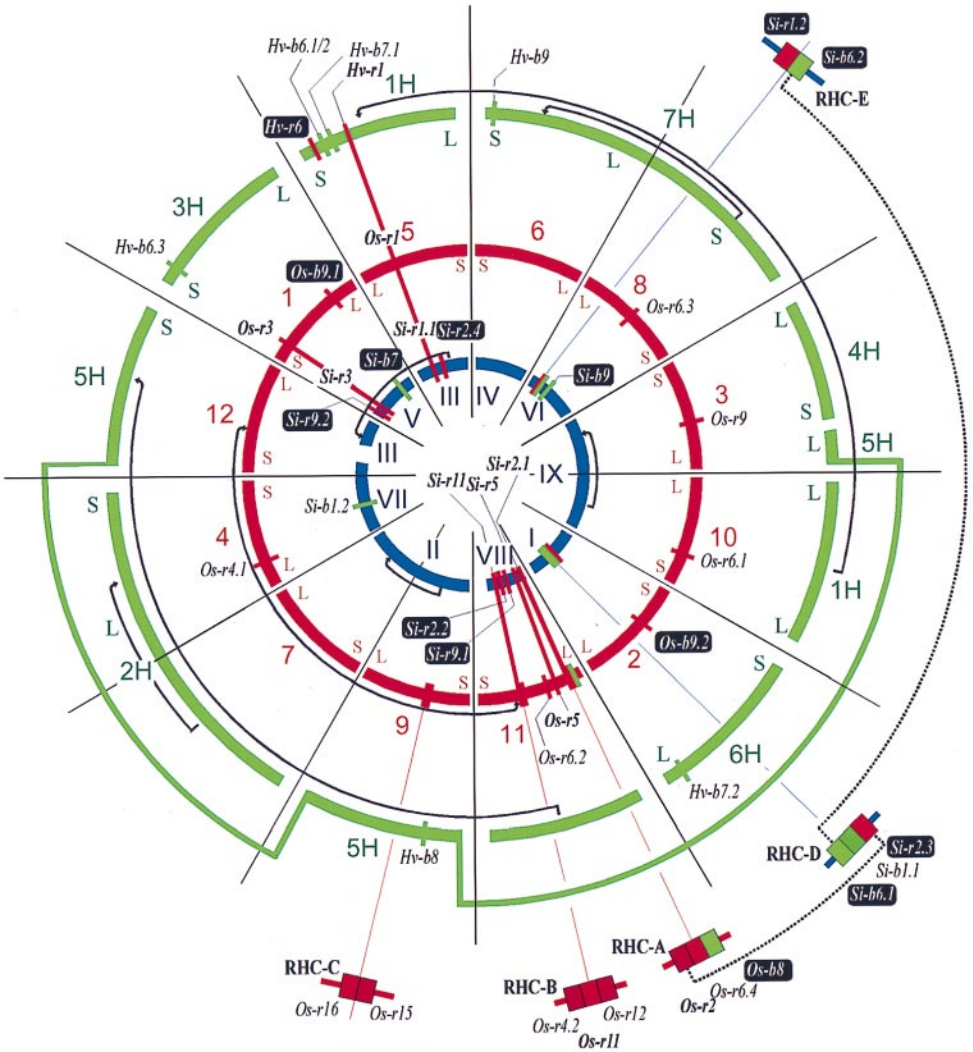

FIG. 3. Comparative mapping of *R* gene homologues in the monocot species rice, barley, and foxtail millet. A circle diagram according to Moore *et al*. (16, 17) was chosen to visualize syntenic relationships that align the genomes of barley (green), rice (red), and foxtail millet (blue). Map locations of NBS-LRR genes that could be mapped in at least two of the three tested species or are present in RHCs are given. Syntenic map positions are marked by bold red spokes and nonsyntenic *R* gene homologue loci are boxed in black. Clusters containing at least two highly divergent NBS-LRR genes in rice and foxtail millet (RHC-A to RHC-D) are highlighted in the periphery. Note that the rice NBS-LRR probe r2 detects loci in rice (*Os-r2*) and foxtail millet (*Si-r2.3*) that are organized in RHC-A and RHC-D (indicated by dotted black line). The barley NBS-LRR probe b6 detects two loci (*Si-b6.1* and *Si-b6.2*) in foxtail millet that are also organized in different mixed clusters (RHC-D and RHC-E; indicated by a dotted black line). Positions of chromosome insertions are indicated by solid black lines; barley chromosomes are numbered 1H to 7H, rice chromosomes 1 to 12, and foxtail millet chromosomes I to IX. S and L denote the short and long arm of each chromosome.

DISCUSSION

Isolation, sequence, and mapping of *R* gene homologues from two grass species have provided surprising insights into both *R* gene evolution and genome organization. We interpret our data in the context of the close evolutionary relationship of grass species and the rigorous collinear gene order of their genomes (16, 17). We interpret them also on the basis that rice, barley, and foxtail millet represent three separate grass tribes (*Pooideae*, *Bambusoideae*, and *Panicoideae*). Four lines of evidence strongly suggest a rapid rearrangement of NBS-LRR genes in the genomes analyzed: (*i*) the occurrence of intraspecific copy number variation both in rice and barley, (*ii*) the existence of mixed *R* gene homologue clusters comprising highly dissimilar genes, (*iii*) the absence of interspecific cross-hybridization signals for several NBS-LRR probes, and (*iv*) the frequent nonsyntenic map locations for NBS-LRR loci detected by probes that displayed interspecific cross-hybridization signals. These observations raise the question as to whether a specific mechanism acts to generate diversity of NBS-LRR genes, involving frequent ectopic recombination events (37–39) to both inter- and intrachromosomal sites and consequently leading to lack of syntenic map positions.

Clustering of *R* genes at a single locus is a well-documented phenomenon and has been explained by an initial tandem duplication event followed by unequal crossing-overs (40–42). Support of this model has been obtained for the rice r2 probe (Fig. 2*A*). The 15 major bands detected by this probe in rice accession IR24 and the two weakly cross-hybridizing bands in Ch45 were shown to segregate each as a single locus in a F_2 population derived from the cross IR24 × Ch45 (data not shown). Thus, the complex *Os-r2* locus in IR24 is a good example of a locus-dependent and intraspecific copy number expansion of a monocot NBS-LRR gene. A precedent for an intraspecific insertion/deletion event of a functional NBS-LRR gene has been described in *Arabidopsis thaliana* (43). *Rpm1* confers resistance to the bacterium *Pseudomonas syringae*, is present in ecotype Columbia but absent in at least six other tested naturally occurring accessions.

Absence of syntenic copies of NBS-LRR genes could be explained by a rapid sequence divergence, rendering them undetectable in our cross-hybridization experiments involving rice, barley, and foxtail millet. Alternatively, they represent species-specific ectopic recombination events of *R* gene homologue loci in the three *Poaceae* tribe genomes tested, similar to *Rpm1* in *A. thaliana*. To rule out the former possibility we would need contiguous genomic DNA sequences from syntenic intervals of the three species containing *R* gene homologues. Recent data from attempts to isolate the barley rust resistance *Rpg1* on chromosome 7H support lack of *R* gene homologous sequences in the syntenic rice interval on chromosome 6 (36) (A. Kilian, personal communication). Although synteny between rice and barley was shown with DNA markers flanking *Rpg1* genetically and physically (36), the subsequent contiguous DNA sequence of a 60-kb rice bacterial artificial chromosome did not reveal any homologous sequences to characterized resistance genes. Thus, either *Rpg1* encodes a novel type of resistance gene or, more likely, the syntenic interval in rice lacks a *Rpg1* homologue.

The unexpected observation of mixed RHCs comprising highly dissimilar genes both in rice and foxtail millet poses questions to their evolution (Fig. 3; periphery). One possibility is sequence divergence of individual copies subsequent to an initial copy number expansion as has been observed for the above described *Os-r2* locus. However, this possibility does not easily explain why we find within different RHCs related *R* gene homologues that are juxtaposed to different NBS-LRR genes (dotted lines in periphery of Fig. 3). If ectopic recombination is a frequent event for the diversification of *R* genes, then the observed juxtapositions of dissimilar *R* gene homologues in the RHCs might be the result of ectopic recombination events between *R* gene clusters or single *R* genes. However, the apparent rapid genomic reorganization of NBS-LRR genes across the three *Poaceae* tribes is not necessarily driven by a specific mechanism for *R* genes but may involve other genes. It is feasible that *R* gene function is adapted to frequent rearrangements and copy number variations. In contrast, ectopic recombination events involving other genes could have deleterious consequences and therefore collinearity of the monocot genomes generally is maintained.

Ectopic recombination is well studied in *Drosophila* and involves meiotic and mitotic exchange between ectopically paired interspersed repeat sequences (37, 44). Copies of the retrotransposon *roo* were involved in all of the interchromosomal exchanges (38). Given that retrotransposons can comprise up to 50% of a complex grass genome (45) and therefore provide abundant targets for ectopically paired repeat sequences, ectopic recombination must be under strict negative control to retain tight collinearity of gene order in the grass genomes.

At present, rapid sequence divergence and ectopic recombination are equally possible mechanisms to explain the lack of intraspecific syntenic relationships detected with our set of *R*-like gene probes. Regardless of whether the former or latter (or both) mechanism drives the evolution of monocot NBS-LRR genes, the data shown here provides strong evidence that this class of genes diversifies more rapidly than the rest of the tested monocot genomes.

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1. Baker, B., Zambryski, P., Staskawicz, B. & Dinesh-Kumar, S. P. (1997) *Science* **276,** 726–733.
2. Thomas, C. M., Jones, D. A., Parniske, M., Harrison, K., Balint-Kurti, P. J., Hatzixanthis, K. & Jones, J. D. G. (1997) *Plant Cell* **9,** 1–17.
3. Kobe, B. & Deisenhofer, J. (1994) *Trends Biochem. Sci.* **19,** 415–421.
4. Dixon, M. S., Jones, D. A., Keddie, J. S., Thomas, C. M., Harrison, K. & Jones, J. D. G. (1996) *Cell* **84,** 451–459.
5. Parker, J. E., Coleman, M. J., Szabo, V., Frost, L. N., Schmidt, R., van der Biezen, E., Moores, T., Dean, C., Daniels, M. J. & Jones, J. D. G (1997) *Plant Cell* **9,** 879–894.
6. Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. & Jones, J. D. G. (1995) *Science* **268,** 661–667.
7. Dangl, J. F. (1995) *Cell* **80,** 363–366.
8. Jones, D. A. & Jones, J. D. G (1997) *Adv. Bot. Res. Adv. Plant Pathol.* **24,** 89–167.
9. Hammond-Kosack, K. E. & Jones, J. D. G. (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48,** 575–607.
10. Leister, D., Ballvora, A., Salamini, F. & Gebhardt, C. (1996) *Nat. Genet.* **14,** 421–429.
11. Kanazin, V., Marek, L. F. & Shoemaker, R. C. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11746–11750.
12. Yu, Y. G., Buss, G. R. & Saghai Maroof, M. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11751–11756.
13. Feuillet, C., Schachermayr, G. & Keller, B. (1997) *Plant J.* **11,** 45–52.
14. Martin, W., Gierl, A. & Saedler, H. (1989) *Nature (London)* **339,** 46–48.
15. Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., Zhu, L.-H., Fauquet, C. & Ronald, P. (1995) *Science* **270,** 1804–1806.
16. Moore, G., Devos, K. M., Wang, Z. & Gale, M. D. (1995) *Curr. Biol.* **5,** 737–739.
17. Devos, K. M. & Gale, M. D. (1997) *Plant Mol. Biol.* **35,** 3–15.

18. Umehara, Y., Akiko, I., Hiroshi, T., Yuhji, Y., Yoshiaki, N., Shoko, S., Yoshiaki, O., Tatsuhito, F., Nori, K. & Yuzo, M. (1995) *Mol. Breeding* **1,** 79–89.
19. Kurata, N., Nagamura, Y., Yamamoto, K., Harushima, Y., Sue, N., *et al.* (1994) *Nat. Genet.* **8,** 365–372.
20. Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G. & Herrmann, R. G. (1991) *Theor. Appl. Genet.* **83,** 250–256.
21. Laurie, D. A., Pratchett, N., Bezant, J. H. & Snape, J. W. (1995) *Genome* **38,** 575–585.
22. Bezant, J. H., Laurie, D. A., Pratchett, N., Chojecki, J. & Kearsey, M. J. (1996) *Heredity* **77,** 64–73.
23. Devos, K. M. & Gale, M. D. (1993) *Theor. Appl. Genet.* **85,** 649–652.
24. Dunford, R. P., Kurata, N., Laurie, D. A., Money, T. A., Minobe, Y. & Moore, G. (1995) *Nucleic Acids Res.* **23,** 2724–2728.
25. Devos, K. M., Wang, Z. M., Beales, J., Sasaki, T. & Gale, M. D. (1997) *Nucleic Acids Res.*, in press.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
27. Edwards, K., Johnstone, C. & Thompson, C. (1991) *Nucleic Acids Res.* **19,** 1349.
28. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
29. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
30. Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680.
31. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4,** 406–425.
32. Clarke, B., Stancombe, P., Money, T., Foote, T. & Moore, G. (1992) *Nucleic Acids Res.* **20,** 1289–1292.
33. Bennett, M. D. & Smith, J. B. (1991) *Philos. Trans. R. Soc. London B* **334,** 309–345.
34. Causse, M. A., Fulton, T. M., Cho, Y. G., Ahn, S. N., Chunwongse, J., Wu, K., Xiao, J., Wu, Z., Ronald, P. C., Harrington, S. E., Second, G., McCouch, S. R. & Tanksley, S. (1994) *Genetics* **138,** 1251–1274.
35. Yoshimura, S., Umehara, Y., Kurata, N., Nagamura, Y., Sasaki, T., Minobe, Y. & Iwata, N. (1996) *Theor. Appl. Genet.* **93,** 117–122.
36. Kilian, A., Kudrna, D. A., Kleinhofs, A., Yano, M., Kurata, N., Steffenson, B. & Sasaki, T. (1995) *Nucleic Acids Res.* **23,** 2729–2733.
37. Lim, J. K. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9153–9157.
38. Montgomery, E. A., Huang, S. M., Langley, C. H. & Judd, B. H. (1991) *Genetics* **129,** 1085–1098.
39. Meuth, M., Berg, D. E. & Howe, M. M., eds. (1989) *Mobile DNA* (Am. Soc. Microbiol., Washington, DC), pp. 833–860.
40. Sheperd, K. W. & Mayo, G. M. E. (1972) *Science* **175,** 375–380.
41. Holub, E. B. (1997) in *The Gene-for-Gene Relationship in Plant-Parasite Interactions*, eds. Crute, I. R., Holub, E. B. & Burdon, J. J. (British Society for Plant Pathology, CAB International, Oxon, U.K.), pp. 5–26.
42. Hulbert, S., Pryor, T., Hu, G., Richter, T. & Drake, J. (1997) in *The Gene-for-Gene Relationship in Plant-Parasite Interactions*, eds. Crute, I. R., Holub, E. B. & Burdon, J. J. (British Society for Plant Pathology, CAB International, Oxon, U.K.), pp. 27–43.
43. Grant, M. R., Godiard, I., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W. & Dangl, J. L. (1995) *Science* **269,** 843–846.
44. Engels, W. R. & Preston, C. R. (1984) *Genetics* **107,** 657–678.
45. SanMiguel, P., Tikhonov, A., Jin, Y. K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P. S., Edwards, K. J., Lee, M., Avramova, Z. & Bennetzen, J. L. (1996) *Science* **274,** 765–768.
46. Xiao, J., Fulton, T., McCouch, S., Tanksley, S., Kishimoto, N., Ohsawa, R., Ukai, Y. & Saito, A. (1992) *Rice Genet. Newslett.* **9,** 124–128.
47. Yoshimura, S., Nelson, R., Yoshimura, A., Mew, T. W. & Iwata, N. (1992) *Rice Genet. Newslett.* **9,** 136–138.
48. Khush, G. S. & Kinoshita, T. (1991) in *Rice Biotechnology*, eds. Khush, G. S. & Toenissen, G. H. (CAB International and IRRI, Wallingford, Oxon, U.K.), pp. 83–108.
49. Abenes, M. L. P., Tabien, R. E., McCouch, S. R., Ikeda, R., Ronald, P., Khush, G. S. & Huang, N. (1994) *Euphytica* **76,** 81–87.
50. Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C. & Nelson, R. J. (1994) *Genetics* **136,** 1421–1434.
51. Xinghua, L., Duanpin, Z., Yuefeng, X., Qifa, Z. & Heping, G. (1996) *Int. Rice Res. Notes* **21,** 30.
52. Saghai Maroof, M. A., Zhang, Q. & Biyashev, R. M. (1994) *Theor. Appl. Genet.* **88,** 733–740.
53. Feuerstein, U., Brown, A. H. D. & Burdon, J. J. (1990) *Plant Breeding* **104,** 318–324.