Physical and Genetic Mapping of Wheat Kinase Analogs and NBS-LRR Resistance Gene Analogs

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ABSTRACT

Conserved motifs within resistance genes have been utilized in polymerase chain reaction (PCR)-based strategies to isolate resistance gene analogs (RGAs) and resistance gene-like (RGLs) sequences from many plant species. RGAs have the potential to serve as closely linked markers for marker-assisted breeding or even as resistance gene candidates. The objectives of this study were to clone, sequence, and map RGAs and kinase analogs (KA). Three motifs in nucleotide binding site-leucine-rich repeat (NBS-LRR) resistance genes and two conserved motifs within R-gene kinases were used to design degenerate primers and amplify RGAs and KAs from wheat (*Triticum aestivum* L.). Eight NBS-LRR and 26 KAs were isolated. The clones were physically mapped to chromosomes by means of wheat nulli-tetrasomic lines. The probes detected 137 fragments that could be assigned to 20 of the 21 chromosomes; nearly half of the fragments mapped in the B genome. None of the fragments mapped to chromosome 4D. Genetic mapping of clones showed simple and complex loci indicating both single and multigene families. The RGAs and KAs will be useful as markers for mapping resistance gene loci.

Disease resistance genes (R-genes) have been cloned from a number of plant species and can be categorized into four classes on the basis of the conserved amino acid sequences of their protein products. The classes include the NBS-LRR genes, which contain a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) motif, extracellular LRR genes, protein kinase genes, and receptor kinase genes (Bent, 1996; Dangl, 1995; Hammond-Kosack and Jones, 1997). In recent studies, conserved amino acid sequences have been exploited for the design of oligonucleotide primers that are used in a PCR-based technique to isolate resistance gene analogs (RGAs) and resistance gene-like (RGL) sequences. This technique has been used in soybean [*Glycine max* (L.) Merr.], rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (Collins et al., 1998; Kanazin et al., 1996; Mago et al., 1999). RGAs contain sequences that are similar to known R-gene sequences and often map within specific regions of the genome that are known to contain R-genes. To demonstrate further the potential of RGAs, Dodds et al. (2001) proved that an RGA cosegregating with the flax *N* locus was the *N* gene. Therefore, RGAs and RGLs have the potential to serve as closely linked markers for marker-assisted breeding strategies, or they could be candidate R-genes themselves.

Common or bread wheat is an allohexaploid ($2n = 6x = 42$, AABBDD), with a haploid genome size of 1.6×10^{10} base pairs. *Triticum aestivum* arose from a hybridization event between the tetraploid wheat *T. turgidum* L. ($2n = 4x = 28$, AABB) and the diploid wheat *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD) about 8000 yr ago (McFadden and Sears, 1946). Genetic mapping in wheat can be difficult because of polyploidy and low levels of polymorphism because of its recent origin from a few hybridization events. However, because of polyploid buffering, bread wheat can tolerate deficiencies of whole chromosomes, chromosome arms, and subarm regions, allowing the development of a vast array of cytogenetic stocks (Sears 1954, 1966; Endo and Gill, 1996). Use of these aneuploid stocks for inter- and intrachromosomal mapping in wheat is a powerful approach and has been used to develop cytogenetic physical maps of the 21 chromosomes of wheat (Delaney et al., 1995a, b; Gill et al., 1993, 1996a,b; Hohmann et al., 1994; Mickelson-Young et al., 1995).

Four RGL sequences have been described in wheat that map in regions known to contain R-genes. The *Cre3*, *Yr10*, and *Lr21* loci contain sequences with motifs similar to NBS-LRR class genes (Lagudah et al., 1997; Spielmeyer et al., 2000; Huang and Gill, 2001). The *Lr10* locus contains both an NBS-LRR and a receptor kinase gene that is membrane spanning (Feuillet et al., 1997; Wicker et al., 2001). Even though these genes cosegregate with the R-gene locus, none has been demonstrated to be the R-gene. In comparison with dicotyledonous species, only a few RGAs have been isolated and mapped in wheat. Spielmeyer et al. (1998) used degenerate and nested primers to isolate RGAs that mapped to all homeologous groups of wheat. Their study only evaluated NBS-LRR type genes and the nested PCR approach limited the type of genes isolated. Seah et al. (1998) used a similar approach to isolate five distinct NBS RGAs from wheat and barley (*Hordeum vulgare* L.), of which two were mapped to wheat chromosomes 2A and 2B. Chen et al. (1998) did not clone any RGAs or KAs, but used the conserved motifs to design primers and identified polymorphisms associated with resistant wheat, rice and barley lines.

Several R-genes have been cloned that contain kinase domains. *Pto* from tomato (*Lycopersicon esculentum*

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Abbreviations: KA, kinase analogs; ITMI, International Triticeae Mapping Initiative; NT, nullisomic-tetrasomic line; PCR, polymerase chain reaction; R-gene, resistance gene; RGA, resistance gene analog; RGL, resistance gene-like.

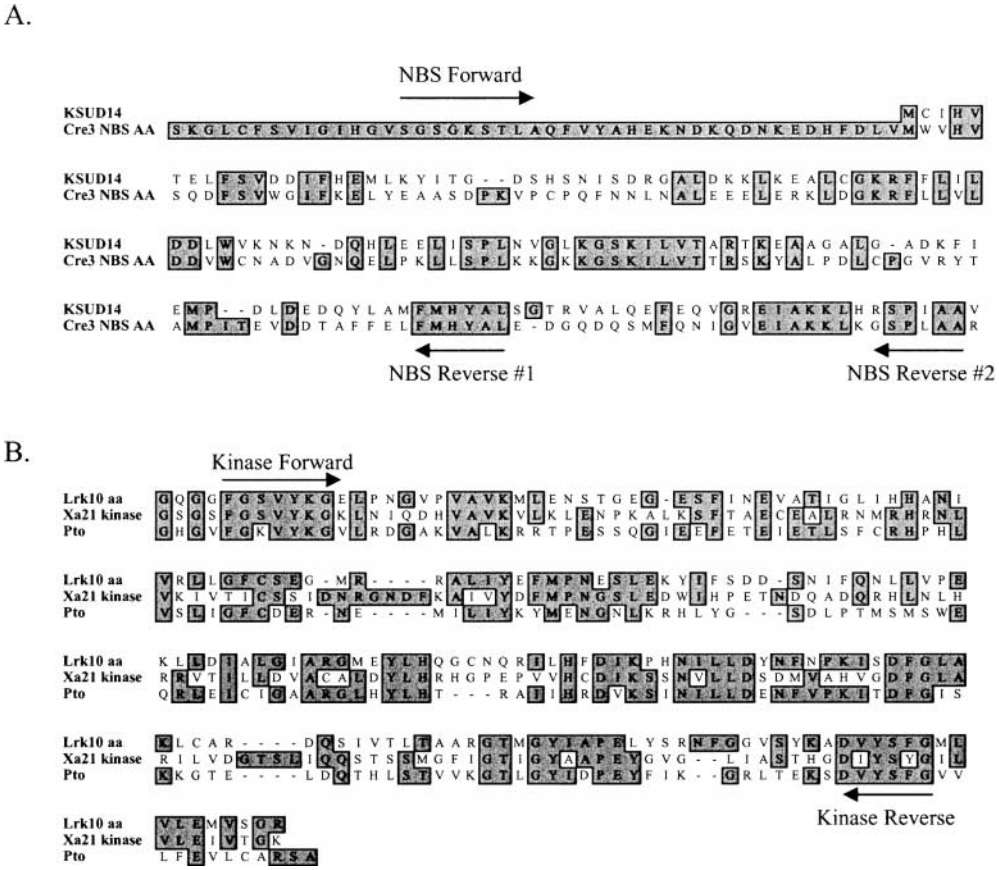

Fig. 1. ClustalW alignments of nucleotide binding site-leucine-rich repeat (A) and kinase (B) containing resistance genes. Arrows identify the conserved motifs used to design primers. Positional identities of amino acids are highlighted in gray and dashes indicate computer generated gaps needed for alignments.

Mill.)(Loh and Martin, 1995) and *PBS1* from *Arabidopsis* (Swiderski and Innes, 2001) are serine–threonine protein kinases that provide resistance to different strains of *Pseudomonas syringae. Xa21* from rice (Song et al., 1995) is a receptor kinase that provides resistance to *Xanthomonas oryzae* pv. *oryzae. Xa21* has a serine–threonine kinase as well as an LRR region. *Rpg1*, recently cloned from barley, has two tandem protein kinase domains and represents a new type of R-gene (Brueggeman et al., 2002). Use of conserved kinase domains to isolate RGLs is difficult because of the numerous kinase families found in plant genomes. However, kinase genes have been shown to cluster next to and around R-gene regions. *Pto* is positioned next to *Prf* in tomato (Salmeron et al., 1996). In wheat, *Lrk10* maps in the region of *Lr10* on chromosome group 1 (Feuillet et al., 1998). Brooks et al. (2002) found a kinase sequence between two RGAs in a genomic clone from *Ae. tauschii*. Isolating kinase-like genes could be a useful source of molecular markers.

The objectives of this study were to clone, sequence, and map physically and genetically RGAs and RGLs from wheat. In this study, eight new wheat NBS RGAs and 26 KAs were isolated by means of degenerate PCR primers on the basis of conserved amino acid sequences of several cloned R-genes. The RGAs and KAs were assigned to specific chromosomes within the wheat genome using nullisomic-tetrasomic (NT) lines. Subsets of these probes were also mapped in the recombinant inbred population developed by members of the International Triticeae Mapping Initiative (ITMI).

MATERIALS AND METHODS

Genetic Materials

The hard red winter wheat cultivar Jagger and the *Ae. tauschii* accession TA2460 were used as sources of DNA for PCR amplification. NT lines developed from wheat cv. Chinese

Table 1. Conserved regions, identified by arrows in Fig. 1, were used to design degenerate primers. Forward (for) and reverse (rev) primers were used to amplify genomic DNA fragments 300–500 base pairs in length. Primers are listed in 5′–3′ orientation from left to right.

Primer	Sequence 5′–3′†
NBSfor1‡	GGIGGIGTIGGIAAIACIAC
NBSfor2	GGIGTIWSIGGIWSIGGIAA
NBSfor3	GGNGGNGTHGGIAAGACNAC
NBSfor4	GGNTYNGGIAARACWACIC
NBSrev1‡	ARIGCTARIGGIARICC
NBSrev2	ARIGCRTARTGCATRAA
Kinase for1	TTYGGNAARGTITAYAARGG
Kinase for2	TTYGGNWSNGTITAYAARGG
Kinase rev1	ACICCRAAISWRTANACRTC
Kinase rev2	ATICCRAAISWRTANACRT

† Codes of mixed bases are based on IUPAC standards, except for I, which is inosine.

‡ Primer sequences for NBSfor1 and NBSrev1 were taken from Kanazin et al., 1996

Table 2. Nucleotide binding site-leucine rich repeat (NBS-LRR) resistance gene analogs (RGAs) and kinase analogs isolated from Jagger and TA2460. Predicted proteins were compared to GenBank and the top alignment results are listed.

Probe†	Source	Alignment	Probability scores
		NBS-LRR	
KSU940	Jagger	(AF032684) NBS-LRR type resistance protein (*Hordeum vulgare*)	1e-71
KSU941	″	(AY048863) resistance protein (*Glycine max*)	1e-90
KSU942	″	(AF087521) resistance protein (*Triticum aestivum*)	3e-35
KSU943	″	probable cyst nematode resistance gene-wheat	9e-32
KSU944	″	(T06219) probable cyst nematode resistance gene-wheat	3e-43
KSU945	″	(AF446141) NBS-LRR class RGA (*Aegilops tauschii*)	2e-48
KSU946	″	(AF446141) NBS-LRR class RGA (*Aegilops tauschii*)	9e-59
KSU947	″	(AF158635) V6 (*Aegilops ventricosa*)	5e-38
		Kinase	
KSUK948	Jagger	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	3e-67
KSUK949	″	(AC079037) Putative wall-associated protein kinase (*Oryza sativa*)	3e-49
KSUK950	″	(AP003758) putative receptor-type protein kinase LRK1 (*Oryza sativa*)	1e-53
KSUK951	″	(AC098565) Putative wall-associated protein kinase (*Oryza sativa*)	4e-53
KSUK952	″	(AF230499) serine/threonine protein kinase (*Oryza sativa*)	1e-73
KSUK953	″	(AP003934) protein kinase-like [*Oryza sativa*]	9e-62
KSUK954	″	(X95909) receptor like protein kinase (*Arabidopsis thaliana*)	1e-63
KSUK955	″	(NM_115241) serine/threonine-specific kinase like protein (*Arabidopsis thaliana*)	2e-68
KSUK956	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	3e-67
KSUK957	″	(AC092173) Putative wall-associated kinase 1 (*Oryza sativa*)	1e-77
KSUK958	″	(AC098566) Putative wall-associated protein kinase (*Oryza sativa*)	3e-67
KSUK959	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	9e-60
KSUK960	″	(AC092173) Putative wall-associated kinase 1 (*Oryza sativa*)	1e-74
KSUK961	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	3e-69
KSUK962	″	(AP003758) putative receptor-type protein kinase LRK1 (*Oryza sativa*)	7e-69
KSUK963	″	(U51330) rust resistance kinase *Lr10* [*Triticum aestivum*]	7e-93
KSUK964	″	(AL133298) receptor-like protein kinase homolog (*Arabidopsis thaliana*)	4e-82
KSUK965	″	(AP003021) putative wall-associated kinase 1 (*Oryza sativa*)	5e-65
KSUK966	″	(AP003021) putative wall-associated kinase 1 [*Oryza sativa*]	3e-68
KSUK967	″	(AC083944) Putative protein kinase (*Oryza sativa*)	2e-58
KSUK968	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	4e-66
KSUK969	TA 2460	(AF230514) serine/threonine protein kinase (*Oryza sativa*)	2e-79
KSUK970	″	(U51330) rust resistance kinase *Lr10* (*Triticum aestivum*)	1e-84
KSUK971	″	(AF172282) receptor-like protein kinase (*Oryza sativa*)	2e-82
KSUK972	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	4e-48
KSUK973	″	(AC113336) Putative wall-associated protein kinase (*Oryza sativa*)	4e-52

† Sequence for probes have been deposited into Genbank. Accession numbers are AF445764–AF445797, respectively.

Spring (Sears 1954, 1966) were used for initial chromosome assignments of probes. A few of the NT Southern blots contained lines ditelosomic for chromosome arms 2AS (line dt2AS) and 2AL (line dt2AL). A subset of 59 recombinant inbred lines from the ITMI mapping population derived from a cross between the hard red spring wheat cv. Opata and the synthetic hexaploid W-7984 (Nelson et al., 1995c) was obtained from the Wheat Genetics Resource Center at Kansas State University (provided by M. E. Sorrells, Cornell University, Ithaca, NY) and used for genetic mapping of RGAs and RGLs.

Cloning and Analysis of Sequences

Degenerate primer sets designed from conserved motifs (Fig. 1; Table 1) were used in PCR amplifications of genomic DNA from Jagger and the *Ae. tauschii* accession TA2460. Amplifications included 100 ng of genomic DNA, 10 pmol of each primer, 1× *Taq* polymerase buffer without Mg (Sigma, St. Louis, MO), 2.5 m*M* $MgCl_2$ (Sigma), 1 m*M* of dNTPs, and 1.25 units of *Taq* polymerase (Sigma). Products were amplified with an PTC-200 thermocycler (MJ Research, Inc.,Waltham, MA) and cycling program of an initial denaturing of 92°C for 3 min, and 35 cycles of denaturing at 92°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min. PCR products were ligated into the TA vector pCR2.1 (Invitrogen, Carlsbad, CA) and 25 random clones per ligation reaction were PCR amplified. The amplification products were then digested with restriction enzymes *Rsa*I and *Hae*III (American Allied Biochemical, Aurora, CO) and separated on 2.0% (w/v) agarose gels. Clones with unique banding patterns were sequenced at the Kansas State University Sequencing Facility with an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed by BLASTX (Altschul et al., 1997) and the MacVector DNA analysis package version 7.1.1 (Accelrys, San Diego, CA).

RFLP Analysis

DNA extraction and preparation, Southern blotting, and RFLP analysis were performed according to Faris et al. (2000). All RGA and RGL clones were PCR amplified from plasmids and ^{32}P-labeled according to Faris et al. (2000). DNA from the wheat NT lines was digested with one of the three restriction enzymes *Eco*RI, *Eco*RV, or *Hin*dIII. DNA of the two parents of the ITMI mapping population was digested with either *Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, or *Xba*I. Probes revealing polymorphism between the parents of the population were mapped onto a subset of the ITMI population. Linkage relationships were calculated using the MAPMAKER program with the Kosambi mapping function (Kosambi, 1944) and a minimum LOD score of 2.0. Markers were placed on an existing framework map constructed by the ITMI (Marino et al., 1996; Nelson et al., 1995a,b,c; Van Deynze et al., 1995) by the 'TRY' command.

RESULTS AND DISCUSSION

Cloning and Sequencing of Kinase and NBS Analogs

The wheat *Cre3* (Lagudah et al., 1997) and maize *Rp1-D* (Collins et al., 1999) sequences were aligned and degenerate primer sets were designed for conserved

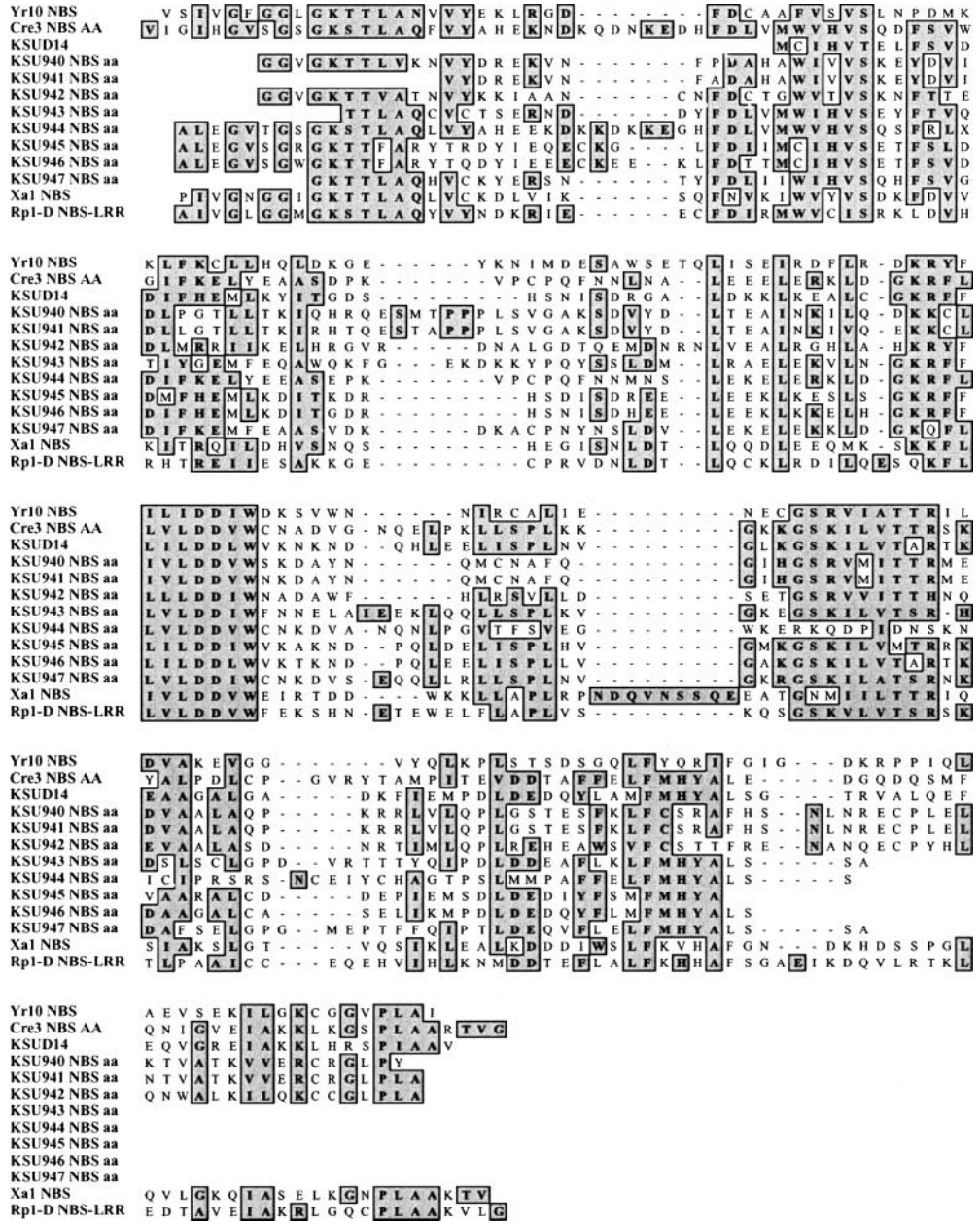

Fig. 2. ClustalW alignments of amino acid (aa) sequences from the nucleotide binding site (NBS) RGAs cloned from Jagger and TA2460. These clones were compared with three NBS sequences from wheat *Yr10*, *Cre3*, and KSUD14, *Xa1* from rice and *Rp1-D* from maize. Positional identities are highlighted in gray and computer generated gaps used for alignments are indicated by dashes.

motifs in the NBS regions of these genes (Fig. 1A). The *Ae. tauschii*-derived RFLP clone KSUD14 was previously sequenced (Huang and Gill, 2001) and found to contain an RGL sequence but not a P-loop domain. Therefore, primers were designed from the P-loop domains of *Cre3* and other R-genes. Only two NBS-LRR clones, KSU941 and KSU942, were isolated from wheat by means of the primers of Kanazin et al. (1996), possibly because the domain 2 motif (GLPLA) is not common in cereals. This has also been observed in maize (S. Hulbert, personal communication). Therefore, primers were designed to the FMYHAL motif, 22 amino acids upstream of the putative GLPLA motif. With these primers, six RGA clones were isolated. The sequences for the eight NBS-LRR clones were aligned with other plant RGAs in GenBank (Table 2) and found to have no perfect alignments with sequences in the database signifying that the clones are unique.

Sequence comparisons were made with other cereal NBS-LRR genes that have been cloned (Fig. 2). These included *Xa1* from rice (Accession no. AB200226; Yoshimura et al., 1998), *Rp1-D* from maize (AF107293; Collins et al., 1999), and *Yr10* (AF149114; Laroche, A., unpublished), *Cre3* (AF052641; Lagudah et al., 1997) and KSUD14 (AF257240; Huang and Gill, 2001) from wheat. Conservation of motifs was evident in the P loop, kinase 2a, kinase 3, and domain 2. Within the KSU clones, KSU940 and KSU941 were most similar to each other,

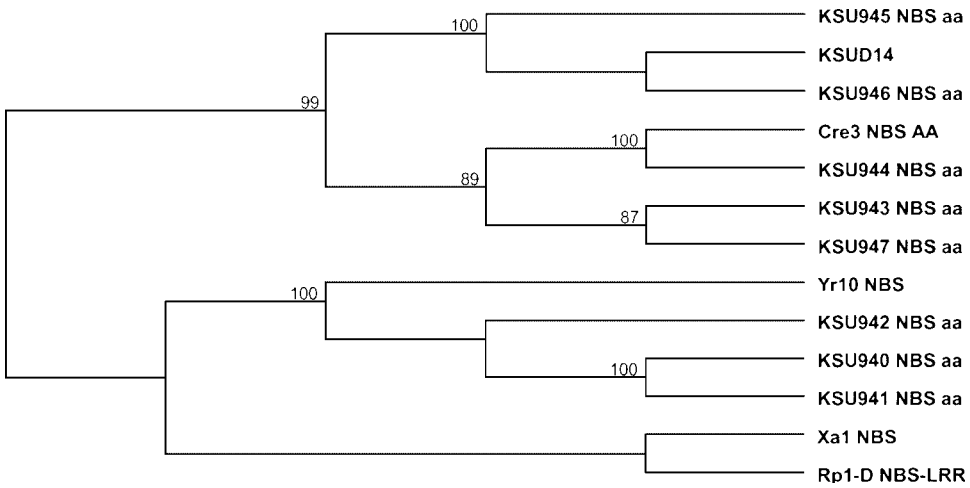

Fig. 3. An NBS protein tree derived from comparing predicted amino acid sequences of 8 NBS RGAs cloned from Jagger with three NBS genes from wheat, one from rice and one from maize. The tree was constructed using the nearest neighbor method. Percent occurrences during bootstrap analysis (from 1000 cycles) are indicated at each branch point.

with only nine amino acid differences. However, all of the RGAs were significantly different from the R-genes used in comparison. Phylogenetic analysis, using 1000 cycles of bootstrapping and midpoint rooting, determined that KSU945 and KSU946 were most similar to KSUD14. KSU943, 944, and 947 were most similar to *Cre3* and KSU940, 941, 942 were most similar to *Yr10* (Fig. 3).

Kinase genes are often found physically linked to NBS-LRR genes during sequencing of R-gene loci (Loh and Martin, 1995; Feuillet et al., 2001). Kinase sequences also resemble, or are part of R-gene proteins. These linkages led to the aligning of *Lrk10* (Feuillet et al., 1997), *Xa21* (Song et al., 1995), and *Pto* (Loh and Martin, 1995) by means of ClustalW in MacVector. Conserved residues were found in subdomains II and IX of these kinases (Fig. 1B) to which degenerate primers were designed. Using the four kinase primer combinations described in Table 1, 26 unique, nonredundant clones were isolated from both Jagger and TA 2460 (Table 2). Putative proteins were derived by computer for all of the clones and then submitted to GenBank for BLAST analysis. The top BLAST hits for each clone are listed in Table 2. The resulting probability scores ranged from e^{-48} to e^{-93} indicating that the clones were similar to other proteins in other species.

The majority of the clones aligned to 14 wall kinases, three serine–threonine protein kinases, and five receptor kinases. Two clones, however, KSUK963 and KSUK970, aligned with *Lrk10* from wheat with probability scores of e^{-93} and e^{-84}, respectively. This verifies that the target sequences could be obtained from genomic DNA by means of these primers. Clones KSUK948, 956, 959, 961, 968, 972, and 973 aligned to a cluster of putative wall kinases found on a single bacterial artificial chromosome (BAC) isolated from chromosome 10 from rice (AC113336). KSUK971 aligned with a putative receptor-like kinase on the same rice BAC from chromosome 4 containing a putative *Xa21*-like sequence (AF172282). Except for KSUK951 and KSUK960, which aligned to gene-poor regions of rice, the other clones aligned to sequence with several predicted open reading frames.

Bootstrap analysis was used to determine relationships of the wheat KA clones with other kinases. Four wall kinases, in addition to *Rpg1*, *Xa21*, *Pto*, *Lrk10*, *Lrk19*, *Lrk33* (Feuillet et al., 2001), and *PBS1* from *Arabidopsis* were used in the comparisons. Vallad et al., (2001) had previously used bootstrap analysis to determine that five *Pto*-like kinase families from pea were distinctly different from other kinases. They also found that kinase subdomains VIa, VIb, VIII, and IX of the *Pto*-like class are unique to plant species. Our analysis determined that the wheat KAs can be separated into two distinct groups (Fig. 4). The first contain a majority of KAs aligning with wall kinases, but none of the R-genes were placed in this group. The second group has four KAs clustered with *Lrks* from wheat and four clustered separately from an R-gene. KSUK948 and KSUK964 fell within the branch with *Pto* and *PBS1*. Clustering of *PBS1* with *Pto* is contrary to Swiderski and Innes (2001) who found that *Pto* and *PBS1* did not fall into the same subfamiliy. It is possible that our analysis did not include enough different kinase families to be able to separate *Pto* and *PBS1*. These results suggest that the second group of KAs are more like R-genes than the first group.

Nullisomic-Tetrasomic Aneuploid Mapping

Initial mapping of RGAs and KAs by Southern analysis revealed hybridization patterns ranging from simple, involving the detection of a few fragments, to complex, and consisting of a large number of fragments. The fragments were assigned to specific chromosomes by mapping onto NT lines. Using 8 RGA and 26 KA clones as probes, we detected 246 fragments for an average of 7.2 fragments per probe. A total of 137 fragments were assigned to individual chromosomes, providing a mapping efficiency of 56%. The failure to assign 44% of the fragments to chromosomes presumably was due to a

lack of intergenomic polymorphism or the comigration of fragments.

The greatest number of fragments were assigned to the B genome (48%), followed by the D genome (28%) and the A genome (24%). Group 1 chromosomes contained the largest number of RGL loci with chromosomes 1B and 1D containing eight and 11 loci, respectively (Table 3). The fewest number of RGLs mapped to the group 4 chromosomes (Table 3). The 137 fragments detected a minimum of 92 loci, which were assigned to 20 of the 21 chromosomes of wheat (Table 3). KA clones, KSUK951 and KSUK955, each mapped to six different chromosomes, exhibiting the greatest genome-wide mapping profiles compared to all other clones (Table 3).

The number of fragments detected by each of the 34 clones varied from one to 14. The theoretical expectation is three loci corresponding to each chromosome of a homeologous group. This expectation was met by only six probes, and for four of these (KSUK948, KSUK950, KSUK954, KSUK964) all the orthologous loci were mapped to individual chromosomes, indicating these loci can be considered highly conserved. RGA probe KSU943 detected only one fragment on chromosome 4B, and RGA probe KSU944 detected two fragments on chromosome 2A. These loci could represent examples of genome diploidization, which involves loss of orthologous loci following polyploidization (Feldman et al., 1997).

The vast majority of the probes detected both orthologous and paralogous loci. Several types of distribution patterns were observed (Table 3). RGA KSU940 detected both orthologous and paralogous loci on the same homeologous chromosome group. Of the six fragments this probe detected, one was mapped onto 1A, two onto 1B, and three onto 1D. It appears that of the three fragments mapped on 1D, one is an ortholog and the other two fragments are paralogs that probably originated by tandem duplication events. However, it is also possible that the restriction enzymes used, cleaved within a particular locus resulting in multiple bands mapping to the same chromosome. Hybridizing these probes onto DNA digested with different enzymes would allow testing of this hypothesis.

Loci detected by KA probe KSUK952 mapped onto homeologous groups 5 and 6. These may represent examples of ancient duplication events before the divergence of the A, B, and D genome progenitor species. In this case, it is difficult to distinguish between orthologs and paralogs. Some probes, such as KSUK951, detected loci conserved on a particular homeologous group, in addition to loci located at random throughout the genome. This pattern probably indicates more recent duplication events in the progenitor species, or duplication following polyploidization. Finally, several probes, such as KSUK968, KSUK969, and KSUK970, detected a large number of fragments of which only a few could be assigned map positions. These sequences presumably have many repeated copies dispersed throughout the genome.

KA probes KSUK953, KSUK963, and KSUK970 each had a common fragment lacking in both lines dt2AS and N3BT3D. This pattern was explained by a previous study (Devos et al., 1999) that reported on the presence of small terminal deletions in chromosome 3B of line dt2AS. Therefore, we presume that these probes detect loci on chromosome 3B. A similar observation

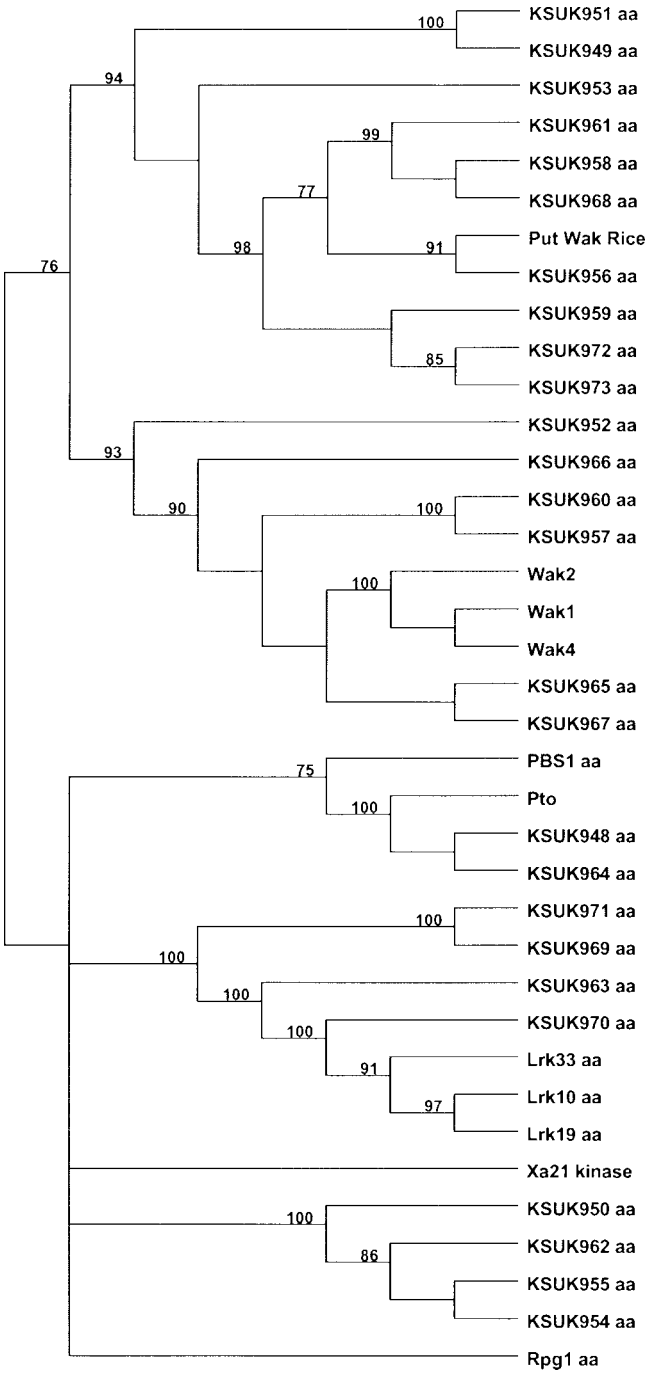

Fig. 4. A protein kinase tree constructed from comparing 26 kinase analogs cloned from Jagger and TA2460 with kinase resistance genes and other kinase sequences from rice, *Arabidopsis*, and wheat. Other kinases included, three wall associated kinases (wak) from *Arabidopsis* (Wak1, AJ009696; Wak2, AY062531; Wak4, AJ009695), *Lrk10*, *Lrk19*, *Lrk33*, and a putative (put) rice wak (AC113336). The tree was constructed using the nearest neighbor method and rooting was based on the midpoint. Percent occurrences during bootstrap analysis (from 1000 cycles) are indicated at each branch point.

Table 3. RGA and KA clones were mapped onto chromosomes using nullisomic-tetrasomic (NT) analysis. Included are restriction enzymes used for Southerns, number of fragments found, their positions, and the number of unmappable fragments.

Probe	Restriction enzyme for NT analysis	Total fragments	Chromosome assignment	Unmappable fragments
			NBS-LRR	
KSU940	*Eco*RI	6	1A,1B,1D	0
KSU941	*Eco*RI	4	1B,1D	2
KSU942	*Eco*RI	5	1B,1D	1
KSU943	*Eco*RV	1	4B	0
KSU944	*Eco*RI	5	2A	3
KSU945	*Hin*dIII	2	1B,2D	0
KSU946	*Eco*RI	11	1B,1D	8
KSU947	*Eco*RI	3	5B†,7A†	2
			Kinase	
KSUK948	*Eco*RI	3	2A†,2B,2D,5B†	0
KSUK949	*Eco*RV	4	6B,6D,7A	1
KSUK950	*Hin*dIII	3	1A,1B,1D	0
KSUK951	*Eco*RI	10	1A,1B,1D,3D,6D,7B	1
KSUK952	*Eco*RI	12	5A,5B,5D,6A,6B	3
KSUK953	*Eco*RI	10	3B,6A,6B,6D	3
KSUK954	*Hin*dIII	3	3A,3B,3D	0
KSUK955	*Eco*RI	13	1D,2B,5A,5D,6A,6B	5
KSUK956	*Eco*RI	6	5A,5B	3
KSUK957	*Eco*RI	6	5A,5B,5D	0
KSUK958	*Eco*RI	8	4A,5B,6B	3
KSUK959	*Eco*RI	7	1A,1B,4B	2
KSUK960	*Eco*RI	6	7A,7B,7D	3
KSUK961	*Eco*RI	9	6A,6B	5
KSUK961	*Eco*RI	6	7A	4
KSUK963	*Eco*RI	13	1D,2B,3B,3D,5D	2
KSUK964	*Eco*RI	3	7A,7B,7D	0
KSUK965	*Eco*RI	11	2A†,2B,5B†	5
KSUK966	*Eco*RI	7	6A,6B,6D	2
KSUK967	*Eco*RI	8	3D,5B	6
KSUK968	*Eco*RI	12	5B,6B	9
KSUK969	*Eco*RI	13	1D	12
KSUK970	*Eco*RI	14	1A,3B,5D	11
KSUK971	*Eco*RI	13	1D,2D,3A,7B	8
KSUK972	*Eco*RI	6	5B,5D	3
KSUK973	*Eco*RI	3	5B	2

† Ambiguous results nullisomic-tetrasomic lines in which a fragment could be assigned to either of two chromosomes

was made for KA probes KSUK948 and KSUK965, each of which detected identical polymorphisms for lines dt2AS and N5BT5D, and RGA probe KSU947 which detected identical polymorphisms for lines N5BT5D and N7AT7D. Again, Devos et al. (1999) reported that nulli-5B lines are known to contain a number of aberrations resulting in regions missing from chromosomes other than 5B. This phenomenon can explain the surprising hybridization patterns observed with KSUK948, KSUK965 and KSU947, but still makes it difficult to assign these probes onto a particular chromosome.

Genetic Mapping

The ITMI reference population was used to map several probes in relation to framework markers. In total, 10 clones, resulting in 26 loci, were successfully mapped onto 13 chromosomes in the population (Table 3, Fig. 5). All 10 clones showed polymorphisms with at least three of the five restriction enzymes on the parental survey filters. These clones subsequently were mapped onto population filters digested with the restriction enzyme that presented either the most clear polymorphic fragment, or the greatest number of polymorphic fragments on parent filters. The clones detected an average of 12 fragments, indicating the presence of either a multigene family or multiple copies of the sequence in the genome. However, not all loci detected by the 10 clones were mapped in the population because of lack of polymorphism or weak polymorphisms that were difficult to score in the population. In some cases, markers could not be placed on the framework map at a LOD > 2.0 and were therefore placed in the most likely intervals. (Fig. 5).

RGA mapping in the ITMI population resulted in a single locus being mapped for each of the four KA clones KSUK953, KSUK958, KSUK952, and KSUK961, while another three clones, KA KSUK951 and RGAs KSU944 and KSU947, each had two loci mapped in the population (Fig. 5). Although KA probe KSUK960 detected four loci, two of which resided on chromosome 7D, none of these loci were linked to each other or conserved within a homeologous group. The two remaining clones KSUK965 and KSU946, each detected a multigene family along with additional sequences in the genome (Table 3, Fig. 5).

Two types of complex loci were seen in this study. The first complex locus involved an RGA probe that detected multiple RFLPs, some of which were linked. RGA KSU946 detected loci located on all three group one chromosomes, in addition to sequences on chromosomes 2B and 3A. Nine loci were detected by KSU946, resulting in the greatest number of loci mapped compared to all other clones (Fig. 5). KA probe KSUK965 also detected two loci linked at approximately 3 centimorgans (cM) on chromosome 2B (Fig. 5).

The second type of complex locus was characterized by a clones that mapped in close proximity to each other

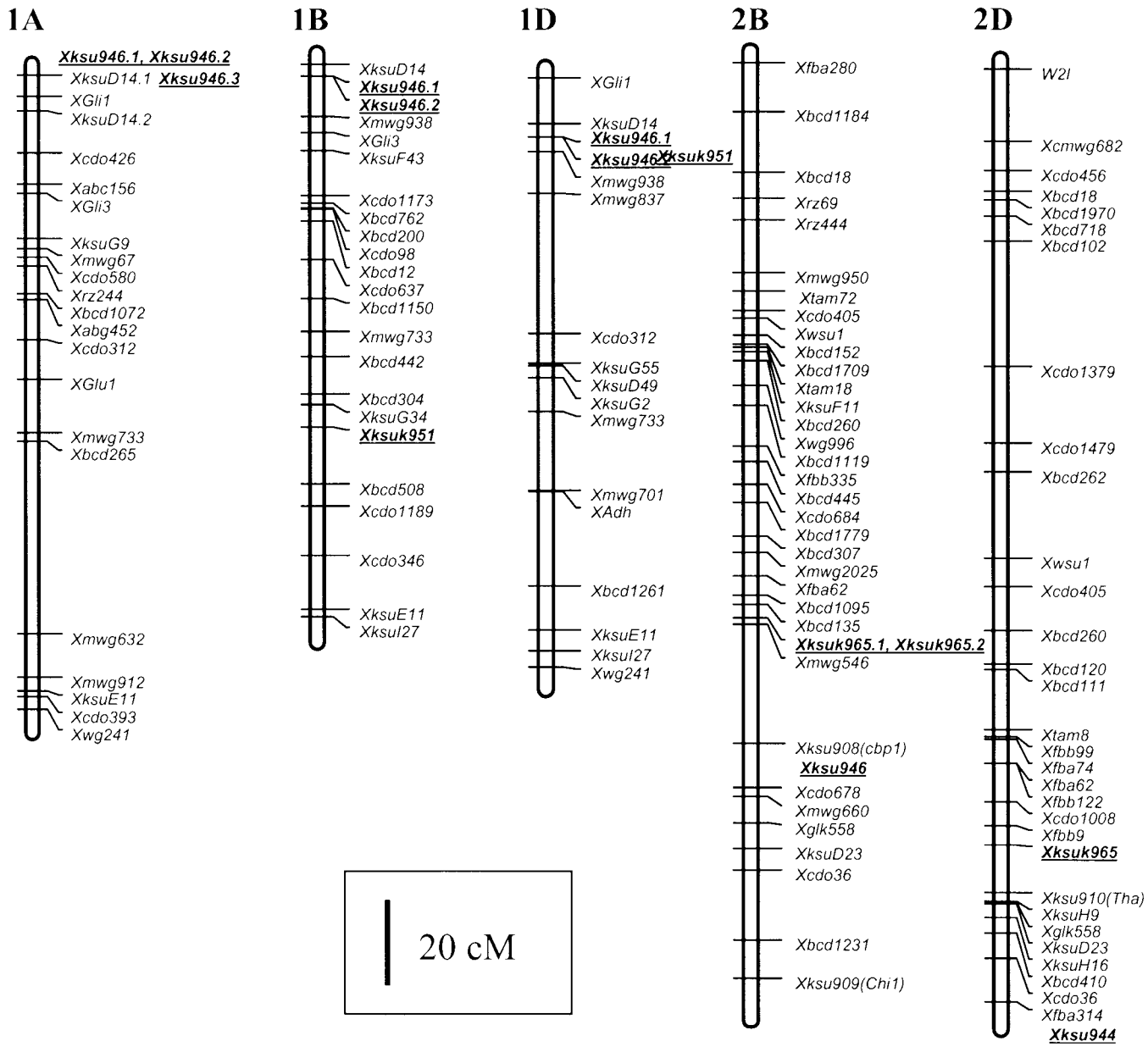

Fig. 5. Linkage maps of NBS RGAs and kinase RGLs. This map is based on the framework map of the ITMI population using LOD > 2.0. Markers are highlighted in bold. Markers that could not be placed with confidence were placed in the most likely interval.

and appeared to represent an R-gene complex. This was seen with KA markers *Xksuk958-6B*, *Xksuk953-6B* and *Xksuk960-6B*, all of which mapped distal to the marker *Xbcd342* on the end of the short arm of chromosome 6B (Fig. 5). Cosegregating loci *Xksu946-1D.1* and *Xksu946-1D.2*, mapped 7cM from the locus *Xksuk951-1D* on chromosome 1D. Finally, loci *Xksu947-7A* and *Xksuk961-7A* were 9 cM apart on the end of the short arm of chromosome 7A. The different RGA an RGL members that comprise these resistance complexes suggest functional associations among the different members and therefore have remained clustered.

Map positions of loci from this study were compared to positions of known resistance genes, RGA loci, and defense response genes. Several such associations were observed, including the RGA probe KSU946, which detected homoeoloci on the distal regions of the group 1 chromosomes and mapped in close proximity to the markers *XksuD14-1D.1* and the prolamin storage proteins. This region is known to contain several important loci including the leaf rust resistance genes *Lr10, Lr21*, and *Lr26*, and the quantitative trait loci *Qphs.cnl* (Anderson et al., 1993) and *QTsc.ndsu* (Faris et al., 1997). Also RGA probe KSU944 detected a locus, *Xksu-944-2D* distal to the marker *Xfba314* on chromosome 2DL in the region known to contain the *Cre3* cereal cyst nematode (CCN) locus. A number of the RGA loci from this study mapped to regions where other wheat, maize, and barley RGA loci were placed by Spielmeyer et al. (1998). These probes were mapped to the distal regions of the short arms of the group 1 chromosomes and the distal regions of chromosomes 4AL and 7AL. The lack of sequence similarity indicates different genes. The possibility therefore exists that some of the RGA

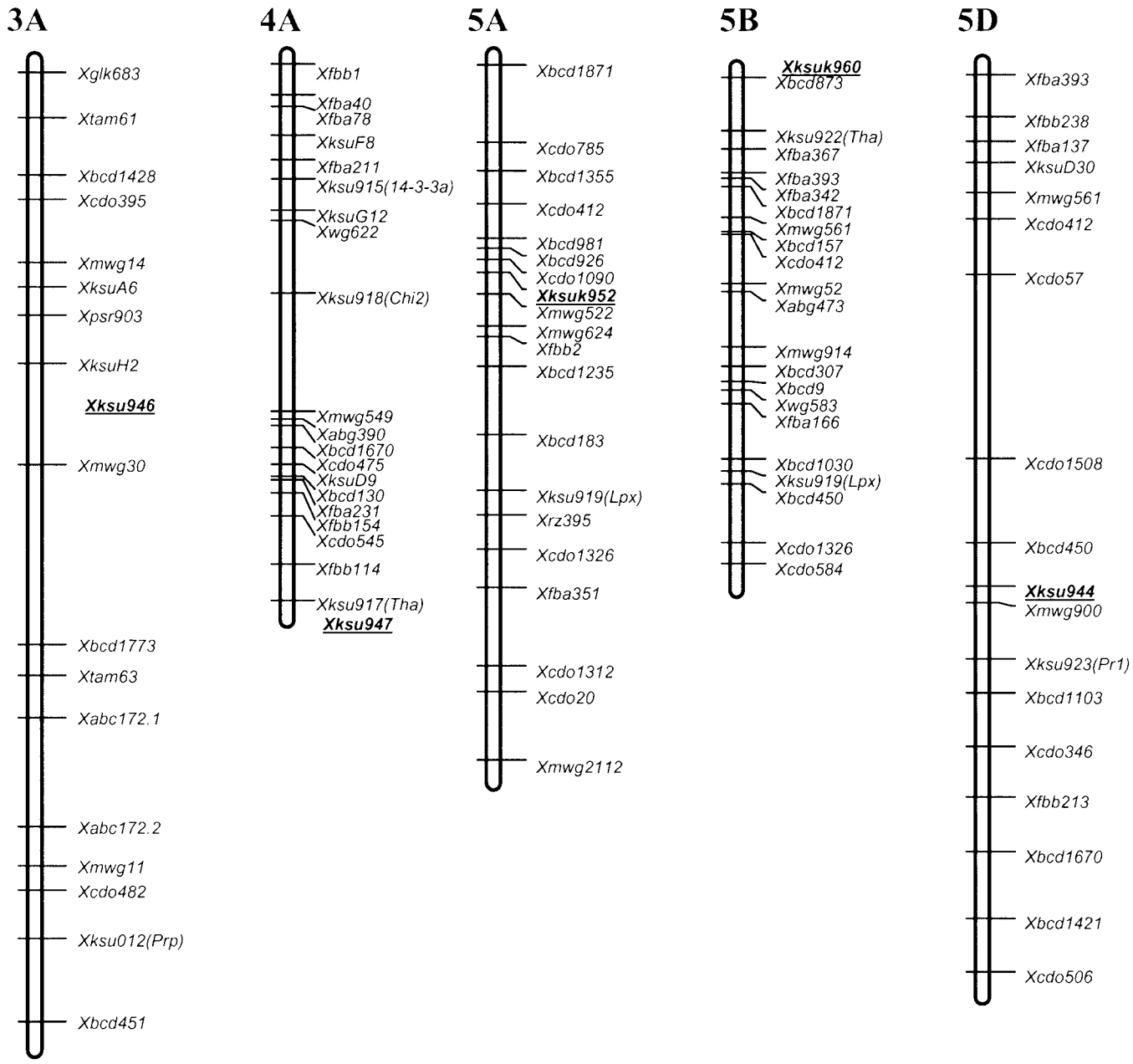

Fig. 5. Continued.

probes identified different members of the same gene family or R-gene complex.

Finally, a significant number of the loci mapped in regions where several defense response genes have been placed previously onto chromosomes 1B, 1D, 2B, 3A, 4A, and 5B, in a study done by Li et al. (1999). RGA and KA loci on chromosomes 1D and 2B mapped within 10 cM of defense response loci, whereas loci on chromosomes 1B, 3A, 4A, and 5B mapped 10 to 16 cM from defense response loci. These findings suggest the possibility for the existence of large defense response/resistance loci, although this hypothesis can only be confirmed by more extensive mapping.

CONCLUSIONS

In this study, we have used a PCR-based technique to isolate a collection of 34 novel clones containing NBS and kinase-like sequences from wheat. Though the isolation of NBS-LRR RGAs has been repeated previously, the clones that are reported were isolated by means of different conserved motifs than used previously in wheat (Seah et al., 1998; Chen et al., 1998; Spielmeyer et al., 1998). The clones also represent new, previously unknown RGL sequences that can be used for mapping. Also in this study are 26 KA containing similar domains as other cloned kinase class resistance genes. With the recent cloning of *Rpg1* from barley (Brueggeman et al., 2002) and *PDS1* from *Arabidopsis* (Swiderski and Innes, 2001) there is now more support for using KAs as markers for mapping R-genes. Chen et al. (1998) have already shown that using primers designed from kinase regions provide a higher level of polymorphism in wheat than do RGA primers. These clones provide new information about kinases in the wheat genome as well as provide a new source of markers for mapping.

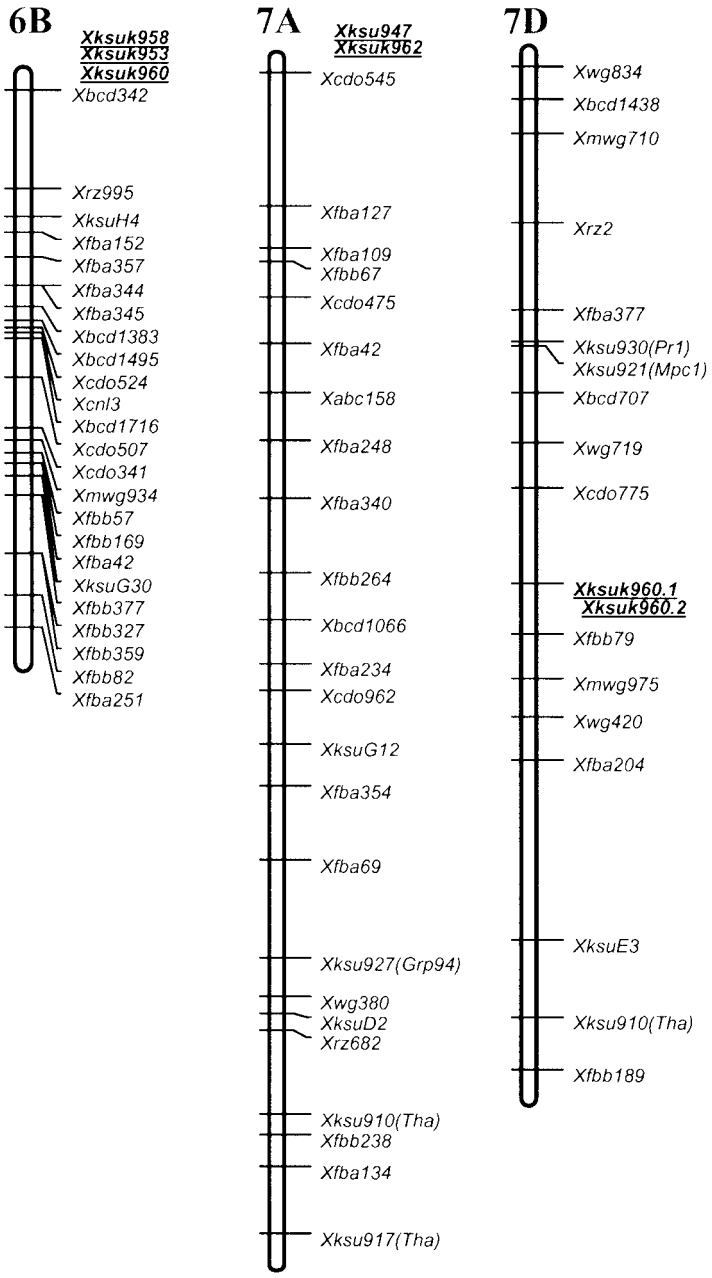

Fig. 5. Continued.

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