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Isolation and characterization of a full-length resistance gene homolog from soybean

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Abstract Using mixed resistance gene analogs as probes, a putative resistance gene (*KR1*) was isolated from soybean and characterized further. The KR1 protein consists of a Toll/interleukin receptor (TIR) domain, a nucleotide binding site (NBS) domain, an imperfect leucine-rich repeat (LRR) domain and two C-terminal transmembrane segments. Due to these features, *KR1* represents a distinct member in the TIR-NBS-LRR class of resistance genes. Southern-blot analysis indicated that there were several *KR1*-related sequences within the soybean genome, and two polymorphic loci were mapped onto linkage group L. *KR1* was induced by SA treatment and soybean mosaic virus (SMV) infection in the resistant line (Kefeng 1). An orthologue (*NR1*) and a homologue (*NR2*) of the *KR1* gene were also identified in the SMV susceptible-line Nannong1138-2. Sequencing analysis revealed that *NR2* was highly homologous to *KR1* and *NR1*, but had a 21-bp deletion. Moreover, the *NR1*, *NR2* transcription and the ratio of *NR1*/*NR2* was up-regulated by viral infection in Nannong1138-2. These results indicated the complexity of the regulatory mechanism in the plant responses to SMV infection.

Keywords Soybean · Resistance gene (R gene) · Soybean mosaic virus (SMV) · *KR1*

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Introduction

Plants have developed complicated defense mechanism during evolution to resist the harmful pathogens they encountered. The mechanism involves the interaction of the plant resistance (R) gene product with the component from the pathogen. This interaction further activates the signal transduction pathway, thus leading to defense responses. These defense responses include a hypersensitive response that results in localized cell death, and other general responses such as strengthening of the cell wall, formation of phytoalexin etc. (Hutcheson 1998).

Isolation of the R genes is beneficial to the engineering of disease resistance in economically important crops. Until now, by using map-based cloning and transposon tagging, more than 30 R genes have been isolated and characterized from different plant species (Hammod-Kosack and Jones 1997; Hulbert et al. 2001). These R gene products can be classified into five classes based on their structural features and the majority fell into the TIR-NBS-LRR class (Hammod-Kosack and Jones 1997; Ellis et al. 2000). In this class, the protein is composed of a Toll/interleukin receptor (TIR), a nucleotide binding site (NBS) and several leucine-rich repeats (LRRs). This structural specificity makes it possible to isolate potential R genes by the homology-based cloning technique, especially from those plants with a relatively large genome (Leister et al. 1996). A large number of plant resistance gene analogs (RGAs) have been identified from different plant species (Ohmori et al. 1998; Seah et al. 1998; Creusot et al. 1999; Leister et al. 1999; Li and Chen 1999; Mago et al. 1999; Rivkin et al. 1999). Linkage analysis showed that these RGAs were distributed throughout the genome and existed in microclusters. Some RGAs have been demonstrated to be linked with the known R genes, and at least 21 resistance loci were linked with RGAs in *Arabidopsis thaliana* (Aarts et al. 1998; Speulman et al. 1998). A R gene *Lr10* was also isolated from wheat by RGA screening (Fenillet et al. 1997).

Soybean is one of the important crops in China and is seriously harmed by soybean mosaic virus (SMV). Pre-

viously we have found a molecular marker that is linked to the SMV R gene (Zhang et al. 1999). A new defense gene *SbPRP* and four RGAs were isolated from the SMV-resistant variety Kefeng1 (He et al. 2001a, 2002). Other genomic research was also performed (He et al. 2001b; Wu et al. 2001). In the present study, the cloned analogs (He et al. 2001a) were used to obtain homologous sequences from the soybean library. A full-length cDNA *KR1* of the R gene homologue was obtained. Sequencing analysis indicated that it is a new gene of the TIR-NBS-LRR class. Its genomic organization and mapping was investigated by RFLP analysis, and its expression in response to SMV infection and other treatments was examined by the RT-PCR method. The possible functions of the *KR1* gene and its homologues in the SMV susceptible line were also discussed.

Materials and methods

Plant materials and nucleic acid extraction

Seeds of the soybean [*Glycine max* (L.) *Merr.*] SMV-resistant cultivar Kefeng1 were grown in the greenhouse or in pots filled with vermiculite. Leaves were harvested for DNA extraction with the method described previously (Chen et al. 1991).

Two-week-old seedlings were used in the following treatments. Samples from all the treatments were harvested at the indicated times and stored at –70 °C for RNA isolation. Total RNA isolation was performed following the description by Zhang et al. (1995). For salicylic acid (SA) treatments, seedlings were sprayed with 2.0 mM of SA until running off. For virus inoculation, each leaf of the soybean seedling was gently rubbed with carborundum and inoculated with a suspension of the soybean mosaic virus (SMV) strain Sa by using a soft brush. For wounding treatment, attached leaves were cut and leaves from the uncut seedlings were used as controls.

Construction and screening of soybean cDNA library

Total RNA from Kefeng1 leaves, which have been treated with 2.0 mM of salicylic acid for 24, 36, 48 and 72 h respectively, was combined and used for mRNA isolation by the PolyA Tract mRNA Purification System III (Promega). A cDNA library was constructed with the ZAP-cDNA Gigapack III Gold Cloning Kit according to the instruction manual (Stratagene), except that cDNA size fractionation was performed on a 1.0% agarose gel of low gelling temperature. The second-strand cDNA was ligated with the Uni-ZAP XR vector and then packaged with the Gigapack III Gold Packaging Extract (Stratagene). After screening, the inserts in the corresponding positive plaques were excised into the pBluescript SK phagemid according to the manufacturer's instruction (Stratagene).

The membranes for hybridization were prepared according to the instruction manual (Stratagene). DNA was crosslinked to the membranes under UV light for 3 min. Hybridization was performed for 16 h at 65 °C with α-^{32}P-dCTP-labeled soybean resistance analogs as a mixed probe (He et al. 2001a). The filters were washed with 2 × SSC, 0.1% SDS, and 1 × SSC, 0.1% SDS, for 15 min and 10 min at 68 °C, respectively.

Southern hybridization and RFLP mapping

Genomic Southern-blot analysis was carried out as described previously (Chen et al. 1991). Hybridization was performed for 16 h at 65 °C with α-^{32}P-dCTP-labeled full-length *KR1* cDNA as a probe. The filters were washed with 2 × SSC, 0.1% SDS; 1 × SSC, 0.1% SDS and 0.5 × SSC, 0.1% SDS for 15 min at 68 °C, respectively. The mapping population is a RIL population NJRIKY from a cross between Kefeng1 and Nannong1138-2 (He et al. 2001b; Wu et al. 2001). The linkage location of *KR1* was analyzed by the software Mapmaker 3.0 version.

RT-PCR analysis

Total RNA was treated with DNAase I (GIBCOL) to remove the genomic DNA contamination. The first strand of cDNA was synthesized by using 4 µg of total RNA with cDNA synthesis Kit (Promega) in a 20 µl reaction volume. The specific primers were designed according to the 3′ sequence of *KR1*. The sense primer is 5′-ACAAATTCCCAGCCATTGC-3′ and the antisense primer is 5′-TGTTCTCTCTTATCTGCATC-3′. The expected length of the amplified fragment is 609 bp. The total volume of PCR reaction is 25 µl, containing 1 µl of the first-strand cDNA, 0.8 µM of each primer, 1 × PCR Buffer, 0.4 µM of dNTP and 1 U of *Taq* DNA polymerase. The reaction was denatured at 94 °C for 5 min, and then subjected to 30 cycles of 94 °C denaturation for 1 min, 56 °C annealling for 1 min and 72 °C extension for 1 min, plus a final extension at 72 °C for 10 min. The PCR products were separated on an agarose gel and quantified using the Imaging DensitoMeter (Model GS-670, Bio-Rad). A soybean *Tubulin* gene, amplified with primers 5′-AACCTCCTCCTCATCGTACT-3′ and 5′-GACAGCATCAGCCATGTTCA-3′, was used as a control in the experiment. The experiments were repeated three times with the same results and one of them was presented. The ratio of the target band intensity to the *Tubulin* band intensity represented the relative expression level of the target gene.

Sequencing analysis

The sequences of the cDNA clones were determined using the *Taq* Dye Primer Cycle Sequencing Kit (Amersham) and the ABI 373A automatic sequencer. The nucleotide and amino-acid sequences were compared with those released in GenBank databases by using the BLAST analysis program. The structure of the KR1 domain was analyzed with the EMBL Database (http://smart.embl-heidelberg.de). The nucleotide sequence of the *KR1* has been deposited in the GenBank database under the accession number AF327903. The RT-PCR fragments were cloned into the pGEM-T vector according to the manufacturer's protocols (Promega). Sequencing analysis was performed as described above. The accession number of *NR2* in Genbank is AF410848.

Results

Isolation and structure analysis of soybean *KR1* gene

A soybean cDNA library was constructed using mRNA from a cultivar of Kefeng1 (SMV resistant). The library was screened by the mixed probes of soybean RGAs, which were cloned in a previous study (He et al. 2001a). Two positive clones were obtained after screening 8 × 10^6 plaques, and the one with the longest insert was sequenced and analyzed further. The nucleotide sequence of this cDNA is 3,672 bp in length containing a 3,372 bp open-reading frame that was flanked by 5′- and 3′-untranslated regions of 99 bp and 201 bp, respectively. The deduced polypeptide showed similarity to other R genes, thus it was designated as *KR1* (*K*efeng1 *R*esistance gene) (Fig. 1). The KR1 protein shared 23.3% identity with the N protein encoded by the tobacco mosaic virus (TMV) resistance gene from tobacco (Whitham et al. 1994), 20.6% identity with the L6 protein encoded by the flax

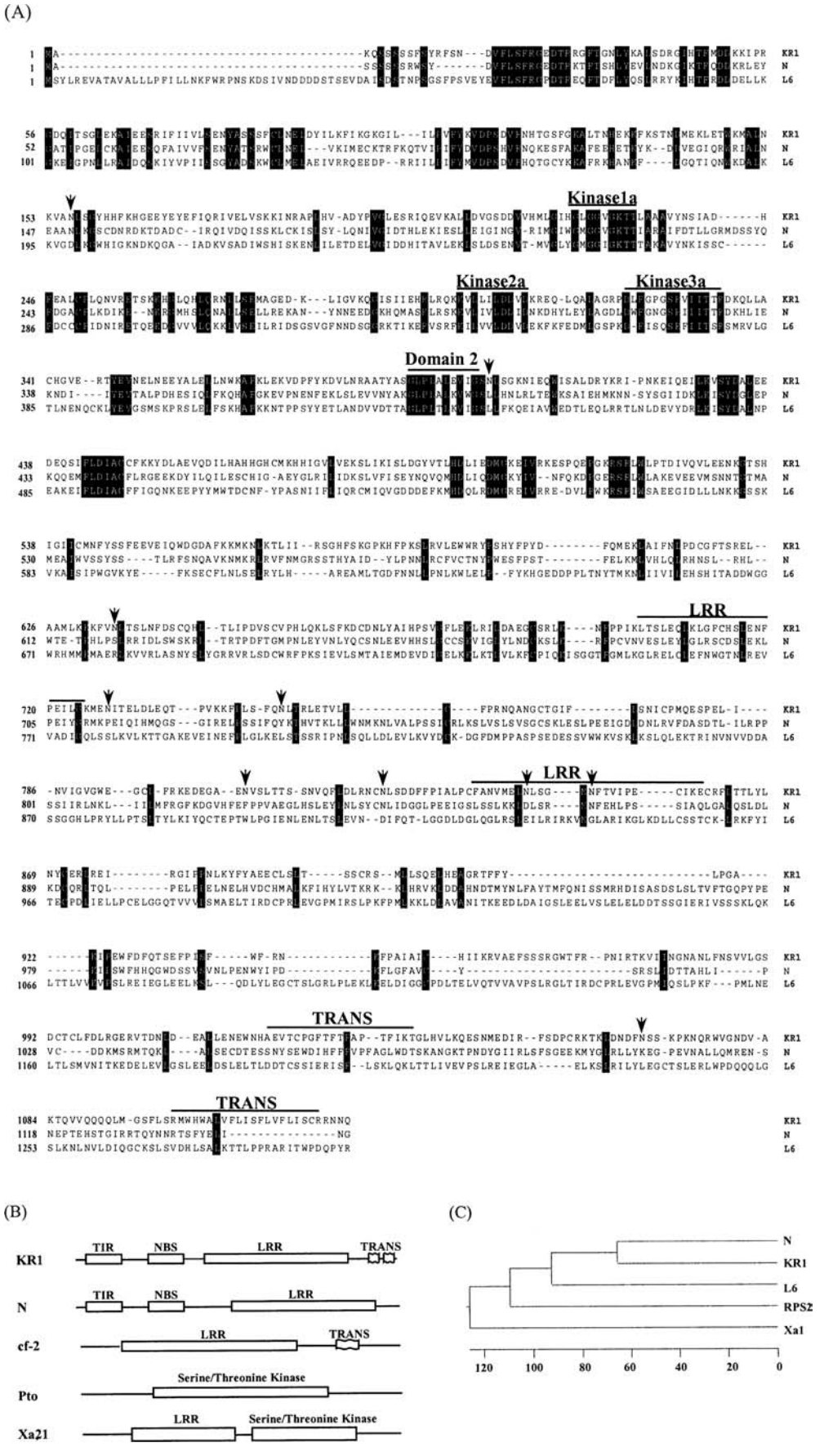

Fig. 1A–C Comparison of the KR1 protein with other proteins encoded by different resistance genes. **A** Comparison of the deduced amino-acids sequences from *KR1*, tobacco *N* and lini *L6*. *Dashes* were introduced for maximum alignment. *Arrows* indicate the potential N-linked glycosylation sites. Kinase 1a, 2a, 3a, NBS domain 2, two perfect LRRs and two transmembrane regions (TRANS) are *overlined*. Identical residues are shaded in *black*. **B** Schematic representation of the structure of the KR1 protein and other plant resistance gene products. The names of each domain are indicated. The domain structure was analyzed in the EMBL Database (http://smart.embl-heidelberg.de). **C** Cluster analysis of KR1 with other resistance gene products. The sequences cited here are tobacco N (U15605), Lini L6 (U27081), rice Xa1 (AB002266) and Arabidopsis RPS2 (U14158). The length of each pair of branches represents the distance between sequence pairs. The units at the bottom of the tree indicate the number of subtitution events

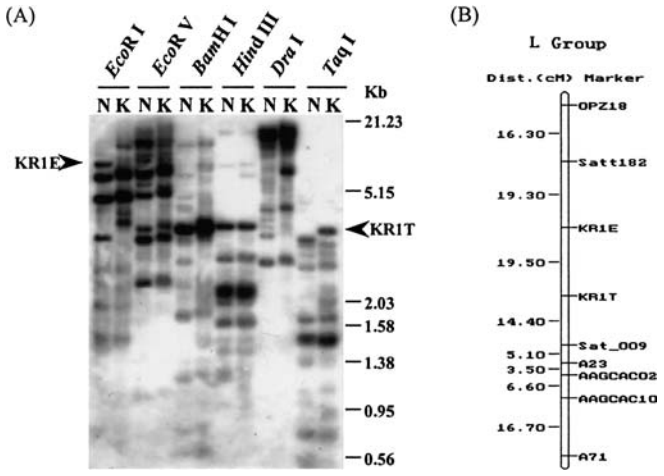

Fig. 2A, B Southern hybridization and mapping analysis of the *KR1* gene. **A** Southern hybridization analysis of *KR1*. Ten micrograms of genomic DNA from Kefeng1 (*K*) and Nannong1138-2 (*N*) were digested with restriction enzymes. After electrophoresis on a 0.8% agarose gel, fragments were transferred onto Hybond N^+ nylon membrane and hybridized with the labeled *KR1* probe. *Numbers* on the right indicate the size of DNA markers. Two polymorphic DNA fragments (KR1E and KR1T) in this study were indicated by *arrows*. **B** Mapping of the *KR1* gene. The polymorphic loci in both *Eco*RI- and *Taq*I-digestion (KR1E and KR1T) were mapped on linkage group L of the soybean genome

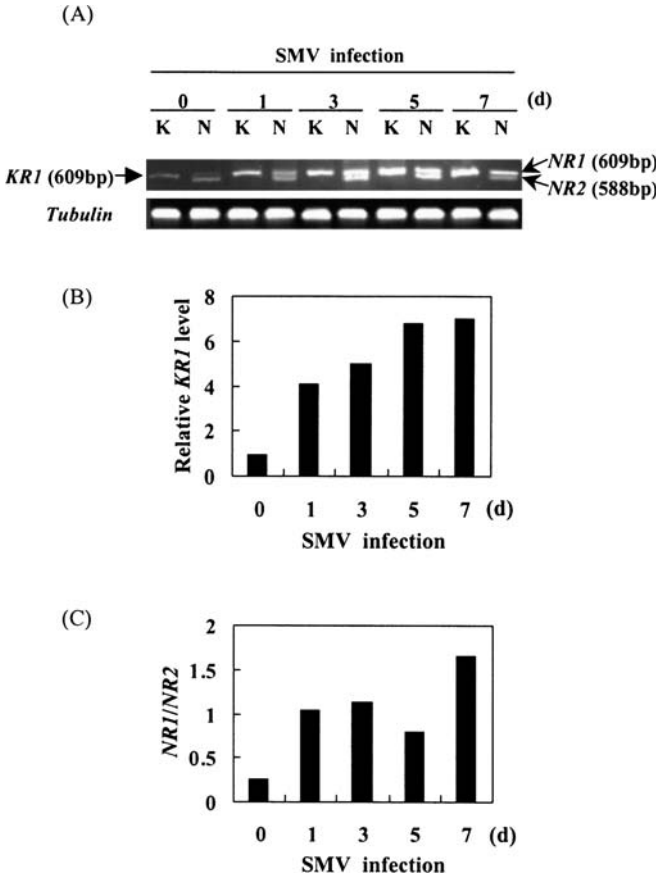

Fig. 3A–C Expression of the *KR1* gene in response to viral infection in SMV-resistant and susceptible lines of soybean. **A** RT-PCR analysis of *KR1* expression in response to SMV infection. The products were separated on a 3.5% agarose gel. *K* stands for Kefeng1 and *N* for Nannong1138-2. The *KR1* fragment (609 bp) from Kefeng 1 was indicated by an *arrow on the left side*. The two fragments from Nannong 1138-2 were designated *NR1* (609 bp) and *NR2* (588 bp) respectively, and indicated by *arrows on the right side*. *Tubulin* was amplified as a control. **B** Quantification of the *KR1* expression in resistance line Kefeng 1. The DNA bands intensities were quantified and the *KR1* expression level was represented by the ratio of *KR1* band intensity to *Tubulin* band intensity. The value at 0 (d) was arbitrarily set to 1 and other values were compared with it. **C** The change of the ratio (*NR1*/*NR2*) in susceptible line Nannong 1138-2. The quantitation was as in **B** and the ratio of *NR1*/*NR2* was plotted against the infection time

rust resistance gene (Lawrence et al. 1995), 13.9% identity with the RPS2 protein encoded by a resistance gene from *Arabidopsis thaliana* (Bent et al. 1994), and 10.9% identity with the Xa1 protein encoded by a rice resistance gene (Yoshimura et al. 1998). KR1 clustered with the N protein but not the others (Fig. 1C). By comparison with the R gene products, several domains were identified in KR1, including a TIR domain (amino acids of 15–157), a NBS domain (amino acids of 197–450) and an imperfect LRR domain (amino acids of 566–885) (Fig. 1A and B). These domains distinguished KR1 from products of other classes of R genes, e.g. the tomato *cf-2* (Dixon et al. 1996), the tomato *Pto* (Martin et al. 1993) and the rice *Xa21* (Song et al. 1995), which have an extra-cytoplasmic LRR domain or a serine/throenine kinase domain, or both (Fig. 1B). The TIR domain is conserved among the compared proteins. After the TIR domain, the conserved sequences of GLGGVGKTT (amino-acids 225–233), KVLLILDDVD (amino-acids 299–308), DLIGPGSRVIITRDK (amino-acids 320–335) and GLPLAL (amino-acids 388–393) were identified, which corresponded to the kinase 1a (P-loop), kinase 2a, kinase 3a and NBS domain-2 consensus motifs, respectively (Wang et al. 1999; Graham et al. 2000). LRR domains were also identified. However, most LRR motifs are imperfect except for two of them (amino-acids 702–725 and amino-acids 837–859) (Fig. 1A). There are ten potential N-linked glycosylation sites NXS/T in the KR1 protein, which may interact with other proteins in a defense reaction signal-transduction pathway as suggested by Cai et al. (1997). In C-terminal regions, two potential transmembrane segments (TRANS, amino-acids 1,020–1,040 and amino-acids 1,100–1,120) were also identified. This special feature made KR1 a distinctive type among members of the TIR-NBS-LRR class of proteins. It is interesting to note that the sequence LVFLIS was repeated in the second TRANS segment (Fig. 1A and B). The reason for this is unknown.

Southern hybridization and linkage location of soybean *KR1*

Southern-hybridization analysis was performed to investigate the genomic organization of *KR1*. The genomic

Fig. 4A, B A Comparison of the nucleotide sequences from the DNA fragment *KR1* (609 bp), *NR1* (609 bp) and *NR2* (588 bp) in Fig. 3A. The *sequences underlined* are the specific primers for RT-PCR. **B** Comparison of the deduced partial amino-acid sequences from KR1, NR1 and NR2. *Dashes* indicate the gap in sequences. The *underlined sites* indicate the divergences among *KR1, NR1* and *NR2* or KR1, NR1 and NR2, in both **A** and **B**

(A)

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1    ACAAATTCCCAGCCATTGCTATTTGTCATATTATCAAACGGGTGGCTGAGTTTTCCTCATC   KR1
1    ACAAATTCCCAGCCATTGCTATTTGTCATATTATCAAACGGGTGGCTGAGTTTTCCTCATC   NR1
1    ACAAATTCCCAGCCATTGCTATTTGTCATAGTATTAAACGGGTGCCTGAGTTTTCCTCATC   NR2

62   GAGAGGTTGGACATTTAGACCCAACATTAGAACCAAGGTGATCATCAATGGCAATGCAAAT   KR1
62   GAGAGGTTGGACATTTAGACCCAACATTAGAACCAAGGTGATCATCAATGGCAATGCAAAT   NR1
62   AAGAGGTTGGACATATAGACCCGACATTAGAACCAAGGTGATCATCAATGGCAATGCAAAT   NR2

123  TTGTTTAACTCTGTGGTGTTGGGAAGTGATTGTACATGTCTTTTTGATCTGCGGGGTGAAA   KR1
123  TTGTTTAACTCTGTGGTGTTGGGAAGTGATTGTTCATGTCTTTTTGATCTGCGGGGTGAAA   NR1
123  TTGTTTTACTCTATGGACTTGGGAAGTGATTGTTCAGTTCTTTTTGATCCGCGGGATGATA   NR2

184  GAGTAACAGATAATTTAGATGAAGCACTTTTAGAAAATGAATGGAACCATGCAGAAGTTAC   KR1
184  GAGTAACAGATAATTTAGATGAAGCACTTTTAGAAAATGAATGGAACCATGCAGAAGTTAC   NR1
184  AAGTAACAGATAATTTAGATGAAGCACTTTTAGAAAATGAATGGAACCATGCAGAAGTTAC   NR2

245  ATGTCCAGGTTTTACTTTTACTTTTGCCCCAACCTTTATAAAAACTGGACTACATGTATTG   KR1
245  ATGTCCAGGTTTTACTTTTACTTTTGCCCCAACCTTTATAAAAACTGGACTACATGTATTG   NR1
245  ATGTCCAGGTTTTACTTTTACTTTTGCCCCAACCTTTATAAAAACTGGACTACATGTATTG   NR2

306  AAACAGGAAAGTAACATGGAGGATATTCGATTCTCTGATCCTTGTAGAAAAACAAAATTGG   KR1
306  AAACAGGAAAGTAACATGGAGGATATTCGATTCTCTGATCCTTGTAGAAAAACAAAATTGG   NR1
306  AAACAGGAAAGTAACATGGAGGATATTCGATTCTCTGATCCTTGTAGAAAAACAAAATTGG   NR2

367  ATAATGATTTCAATAGTTCAAAACCAAAAAATCAAAGATGGGTGGGCAATGATGTTGCCAA   KR1
367  ATAATGATTTCAATAGTTCAAAACCAGAAAATCAAAGATGGGTGGGCAATGATGTTGCCAA   NR1
367  AGAATCATTTCAATAGTTCAAAACCAGAAAATCAAAGATGGGTGGGCAATGATGTTGCCAA   NR2

428  GACACAAGTTGTGCAGCAGCAACAACTTATGGGCTCTTTTTTGTCACGCATGTGGCACTGG   KR1
428  GACACAAGTTGTGCAGCAGCAACAACTTATGGGCTCTTTTTTGTCACGCATGTGGCACTGG   NR1
428  GACACAAGTTGTGCAGCAGCAACAACTTATGGGCTCTTTTTTGTCACGCATGTGGCACTGG   NR2

489  GCACTAGTGTTTCTCATTTCTTTCCTAGTGTTTCTCATTTCTTGCCGCCGCAACAATCAAt   KR1
489  GCACTAGTGTTTCTCATTTCTTTCCTAGTGTTTCTCATTTCTTGCCGCCGCAACAATCAAt   NR1
489  GCACTAGTGTTTCTCATTTCT----------TGCCGCCGCAACAATCAAt              NR2

550  gattggataatgtgctagaggattcaagttcaatggatttgatgcagataagagagaaca   KR1
550  gattggataatgtgctagaggattcaagttcaatggatttgatgcagataagagagaaca   NR1
529  gattggataatgtgctagaggattcaagttcaatggatttgatgcagataagagagaaca   NR2
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(B)

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1    KFPAIAICHIIKRVAEFSSSRGWTFRPNIRTKVIINGNANLFNSVVLGSDCTCLFDLRGER  KR1
1    KFPAIAICHIIKRVAEFSSSRGWTFRPNIRTKVIINGNANLFNSVVLGSDCSCLFDLRGER  NR1
1    KFPAIAICHSIKRVPEFSSSRGWTYRPDIRTKVIINGNANLFYSMDLGSDCSVLFDPRDDK  NR2

62   VTDNLDEALLENEWNHAEVTCPGFTFTFAPTFIKTGLHVLKQESNMEDIRFSDPCRKTKLD  KR1
62   VTDNLDEALLENEWNHAEVTCPGFTFTFAPTFIKTGLHVLKQESNMEDIRFSDPCRKTKLD  NR1
62   VTDNLDEALLENEWNHAEVTCPGFTFTFAPTFIKTGLHVLKQESNMEDIRFSDPCRKTKLE  NR2

123  NDFNSSKPKNQRWVGNDVAKTQVVQQQQLMGSFLSRMWHWALVFLISFLVFLISCRRNNQ   KR1
123  NDFNSSKPENQRWVGNDVAKTQVVQQQQLMGSFLSRMWHWALVFLISFLVFLISCRRNNQ   NR1
123  NHFNSSKPENQRWVGNDVAKTQVVQQQQLMGSFLSRMWHWALVFLIS-------CRRNNQ   NR2
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DNAs from SMV-resistant line Kefeng1 and SMV-susceptible line Nannong1138-2 were digested with *Eco*RI, *Eco*RV, *Dra*I, *Bam*HI, *Hin*dIII and *Taq*I, respectively. As shown in Fig. 2A, there were several hybridizing bands in each digestion and these bands represented the *KR1* gene or its homologous sequences in soybean. The polymorphisms were detected between the two cultivars in all the six digestions. *Eco*RI and *Taq*I-digestions were selected to analyze a population of the soybean recombinant inbred lines NJRIKY, which was derived from a cross between Kefeng 1 and Nannong1138-2 (He et al. 2001b; Wu et al. 2001). As a result, two DNA fragments corresponding to two polymorphic loci (KR1E and KR1T) were mapped to the linkage group L (Fig. 2B).

Expression of the *KR1* gene in susceptible and resistant cultivars in response to SMV infection

The RT-PCR method was used to examine *KR1* gene expression in response to SMV infection because its transcripts were undetectable by Northern analysis. The products of RT-PCR were separated on a 3.5% agarose gel and it can be seen from Fig. 3A that there was one expected fragment (609 bp) in Kefeng 1 (SMV-resistant). However, there were two DNA bands in Nannong 1138-2 (SMV-susceptible) and the corresponding genes were designated *NR1* and *NR2* respectively. The *KR1* expression was steadily increased in Kefeng 1 (Fig. 3B). In Nannong1138-2, the *NR2* fragment was prevalent in un-

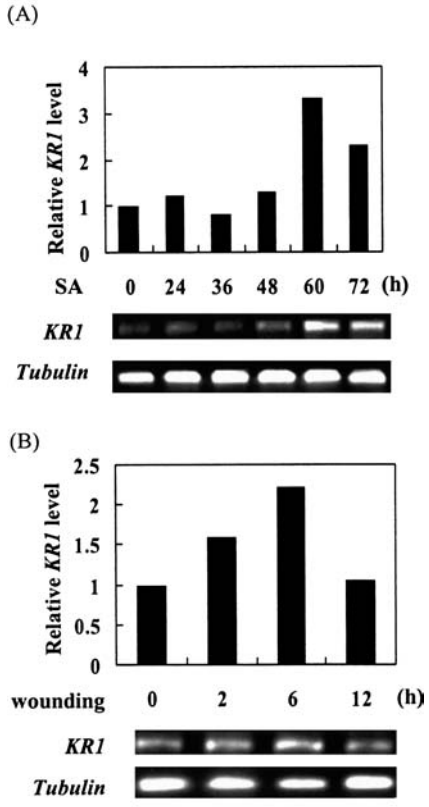

Fig. 5A, B Expression of the *KR1* gene in response to salicylic acid and wounding treatment. **A** The time course of *KR1* expression upon SA treatment. SA treatment was performed by spraying with SA solution. The RNA from the treated materials was subjected to RT-PCR analysis. The band intensity was quantified and the relative mRNA levels were represented, determined by the ratio of the *KR1* intensity to the *Tubulin* intensity. The value at 0 (h) was arbitrarily set to 1 and all other values were compared with it. **B** Expression of the *KR1* gene in response to wounding. Attached leaves were cut and collected at the indicated times after initation of the wounding treatments. The leaves from the uncut seedlings were used as a control. RT-PCR analysis and the quantification were performed as in **A**

inoculated Nannong1138-2 seedlings, whereas the *NR1* fragment gradually became prevalent in inoculated Nannong1138-2 seedlings and finally predominated over the *NR2*. The ratio of *NR1*/*NR2* was substantially up-regulated by SMV infection (Fig. 3C).

The three DNA fragments from both Kefeng 1 and Nannong1138-2 in Fig. 3A were cloned, sequenced and compared (Fig. 4A, B). It showed that the fragment from Kefeng 1 is the expected sequence of *KR1* (609 bp). The large fragment (*NR1*) in Nannong1138-2 is almost identical to *KR1* (609 bp) except for two base differences. This difference led to two amino-acids changes, with one from Thr and Ser, and one from Lys to Glu. The variation possibly reflected the molecular divergence among different cultivars. The small fragment (*NR2*) in Nannong 1138-2 was obviously different from *KR1* and *NR1*. There were 16 sites of single-base divergence between *NR1* and *NR2*, leading to 14 corresponding differences in amino acids between NR1 and NR2. A deletion of 21 bp in the 3′ end was also detected in *NR2* when compared with *NR1* (Fig. 4A), resulting in a deletion of seven amino acids (FLVFLIS) in the second transmembrane segment of NR2 (Fig. 4B). This result indicated that NR2 is different from KR1 and NR2 but highly homologous. The occurrence of the *NR2* gene in the susceptible line may suggest its involvement in the SMV susceptibility.

KR1 expression in response to salicylic acid and wounding

The relationship of salicylic acid (SA) with plant disease resistance has been extensively studied (Delaney et al. 1994; Durner et al. 1997). RT-PCR analysis was thus performed to investigate the *KR1* expression in response to SA treatment. The results presented in Fig. 5A indicated that the *KR1* mRNA accumulation increased upon SA treatment, and reached a maximum at 60 h after the initiation of the experiment and then declined. Wounding, as one mechanical factor, influenced a series of gene expressions during the pathogen attack. *KR1* expression, as indicated in Fig. 5B, was also induced by wounding in the first 6 h and then decreased. This probably indicates that *KR1* functioned an the early stage of the wounding response.

Discussion

In the present study, we have isolated a R gene homologue (*KR1*) from SMV resistant variety Kefeng 1, and *KR1* encodes a protein of the TIR-NBS-LRR type. A number of RGAs and one full-length R gene homolog, LM6, have been isolated from soybean (Kanazin et al. 1996; Yu et al. 1996; Graham et al. 2000).

Although *KR1* is different from other R gene homologues from soybean, all of these sequences encode putative proteins with domain features of the (TIR)-NBS-LRR class of genes. The NBS domain is necessary for the function of ATP or GTP binding. This domain may function as a kinase and activate other proteins (Whitham et al. 1994; Lawrence et al. 1995). LRR domains play roles in protein-protein interaction. The TIR domain is highly homologous to the receptor of signal transduction in mammals and *Drosophila* (Hashimoto et al. 1988; Sims et al. 1989) but may not be necessary in these genes. In addition to the domains described above, the KR1 protein has two putative transmembrane segments in its C-terminal end (Fig. 1B). The presence of these two transmembrane segments may suggest that KR1 is localized on the cell-membrane system. However, precisely where KR1 is localized remains to be identified.

Some R genes were expressed constitutively and at a very low level (Hammond-Kosack and Jones 1997). However, several R genes were induced by SA or pathogens, as in the case of *Xa1* and *Pib* from rice (Yoshimura et al. 1998; Wang et al. 1999), DD6 from soybean

(Seehaus and Tehhaken 1998) and *RPW8* from *A. thaliana* (Xiao et al. 2001). In the present study, *KR1* was up-regulated by the signal molecule of SA, implying that *KR1* may play roles in a SA-dependent defense response pathway. *KR1* expression was also induced by wounding, and wound-induced *KR1* expression peaked at a very early stage of the treatment. This expression pattern is different from that observed for SA treatment, which exhibited a higher expression at a later stage of the treatment. This difference in the time course of expression may reflect the different roles played by KR1 in the different responses.

KR1 expression was steadily induced upon SMV infection in the resistant line. Therefore *KR1* was possibly involved in the soybean resistance to SMV. One orthologue (*NR1*) and one homologue (*NR2*) were also identified in the SMV susceptible line Nannong1138-2. *NR1*, *NR2* and the ratio of *NR1*/*NR2* were up-regulated by SMV infection. It is possible that *KR1* and *NR1* functioned in the resistance responses in resistant and susceptible lines respectively. However, due to the presence of NR2 in the susceptible line, the NR2 may form a heterodimer with NR1 and interfere with the normal function of the NR1 homodimer, hence leading to the reduced resistance of SMV. The dimerization of the resistance protein during the resistance response has been suggested previously (Song et al. 1995; Dixon et al. 1996). Alternatively, *NR2* was possibly not localized at the right place due to the deletion of seven amino acids in the transmembrane segments and leads to the loss of resistance to SMV in the susceptible line. Another possibility exists that NR2 functioned in a negative regulatory mechanism in the plant response to SMV infection. Further research on transgenic plants, biochemical properties and the linkage with SMV resistance will reveal the functions of KR1, NR1 and NR2 in defense responses.

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