# Disease-resistance related sequences in common bean

**M.I. Rivkin, C.E. Vallejos, and P.E. McClean**

**Abstract**: Primers based on a conserved nucleotide binding site (NBS) found in several cloned plant disease resistance genes were used to amplify DNA fragments from the genome of common bean (*Phaseolus vulgaris*). Cloning and sequence analysis of these fragments uncovered eight unique classes of disease-resistance related sequences. All eight classes contained the conserved kinase 2 motif, and five classes contained the kinase 3a motif. Gene expression was noted for five of the eight classes of sequences. A clone from the SB3 class mapped 17.8 cM from the *Ur-6* gene that confers resistance to several races of the bean rust pathogen *Uromyces appendiculatus*. Linkage mapping identified microclusters of disease-resistance related sequence in common bean, and sequences mapped to four linkage groups in one population. Comparison with similar sequences from soybean (*Glycine max*) revealed that any one class of common bean disease-resistance related sequences was more identical to a soybean NBS-containing sequence than to the sequence of another common bean class.

*Key words*: nucleotide binding sites, disease-resistance related sequences, *Phaseolus vulgaris*, *Glycine max*.

**Résumé** : Des amorces inspirées de la séquence d'un site conservé de liaison de nucléotides (NBS) qui est présent chez plusieurs gènes de résistance aux maladies clonés chez des plantes ont été utilisées pour amplifier des fragments d'ADN chez le haricot (*Phaseolus vulgaris*). Le clonage et le séquençage de ces fragments ont permis d'identifier huit classes uniques de séquences apparentées à des gènes de résistance. Les huit classes contiennent le motif kinase 2 conservé et cinq des classes contiennent également le motif kinase 3a. L'expression génique a été documentée chez cinq des huit classes de séquences. Un clone de la classe SB3 était localisé à 17,8 cM du gène *Ur-6*, lequel confère la résistance à plusieurs races du pathogène causant la rouille du haricot, *Uromyces appendiculatus*. La cartographie génétique de ces séquences a permis d'identifier des petits amas de ces séquences apparentées à des gènes de résistance chez le haricot et des séquences ont été localisées sur quatre groupes de liaison chez un croisement. Des analyses comparées réalisées avec des séquences semblables du soya (*Glycine max*) ont révélé que toute séquence du haricot était plus semblable à une séquence comparable du soya qu'à toute autre séquence appartenant à une autre classe chez le haricot.

*Mots clés* : site de liaison de nucléotides, séquences apparentées à des gènes de résistance, *Phaseolus vulgaris*, *Glycine max*.

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## Introduction

Plant resistance (*R*) genes are thought to be one component of the gene-for-gene resistance mechanism in plants (Flor 1956). According to this mechanism, avirulence genes encoded in the genome of the invading pathogen produce products which are recognized by receptor proteins encoded by the *R* genes of the host (Tang et al. 1996; Scofield et al. 1996). Analsyis of the sequences of several *R* genes cloned from different plant species revealed remarkable similarities in general structure, and conservation of specific domains that participate in protein–protein interactions and signal transduction (Staskawicz et al. 1995).

Several classes of sequenced *R* genes encode proteins containing leucine-rich repeat (LRR) domains. These domains have been shown to be involved in protein–protein interactions (Kobe and Deisenhofer 1994). A number of *R* genes with LRR motifs also contain amino acid sequences with a strong similarity to nucleotide binding sites (NBS) (Bent 1996). These domains are characteristic of various proteins with ATP/GTP binding activity and comprise the P-loop, and kinase 2a and 3 motifs (Traut 1994). The role of the NBS in activating the cascade of molecular events leading to disease resistance is considered to be important. Data from site-specific mutagenesis studies suggest that specific mutations that alter key residues involved in nucleotide binding eliminate the function of *Arabidopsis RPS2* and tobacco *N* genes (cited in Bent 1996 and Baker et al. 1997).

The high sequence similarity among NBS-containing *R* genes from different genera that confer resistance to bacte-

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rial, fungal, and viral pathogens (Staskawicz et al. 1995) suggests that amplifying and cloning PCR fragments using primers that target the NBS might identify sequences contained within resistance genes. We have used this approach to isolate eight classes of common bean genomic clones containing NBS sequences. We have analyzed the relationships between these clones and those recently reported for soybean (Kanazin et al. 1996; Yu et al. 1996).

## Materials and methods

### DNA amplification and cloning

DNA was isolated from leaves of the common bean cultivars 'Sierra' and 'Olathe' using the procedure of Doyle and Doyle (1990). The aligned amino acid sequences of known NBS-containing plant disease resistance genes (Staskawicz et al. 1995) were analyzed for the purpose of identifying suitable primers for the PCR amplification of NBS-containing sequences from the common bean. The selected primer sequences B1 and B2 (Table 1) were located at the 5′ and at the 3′ of the conserved region, respectively. DNA amplification was performed in 10 mL reaction mixtures containing 4 hg DNA, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 mM of each primer, and one unit *Taq* DNA polymerase. A Perkin Elmer Model 480 thermocycler was used for the PCR amplifications. After an initial denaturing step at 94°C for 2 min, products were amplified using 40 cycles of the following conditions: 94°C, 1 min; 40°C, 1 min; 72°C, 2 min. Amplification concluded with a final elongation step at 72°C for 5 min. The PCR amplification products were cloned into the plasmid pPCR (Borovkov and Rivkin 1997). Clones obtained from the amplification of 'Sierra' and 'Olathe' DNA were given the prefixes S and O, respectively. The DNA amplification conditions using primers designed to specific classes of bean disease-resistance related sequences (Table 1) were identical to those described above except that the primer annealing temperature was 50°C instead of 40°C.

### DNA sequencing and sequence analysis

DNA sequencing was performed using the LI-COR automated DNA sequencer (LI-COR, Inc.). The sequencing reaction conditions were those suggested by the manufacturer. The DNA sequences and derived amino acid sequences were analyzed using the Molecular Biology Workbench software package (http://bioweb.ncsa.uiuc.edu/). An unrooted phylogenetic tree was built using the CLUSTAL W program (Thompson et al. 1994) that calculates distances based on progressive mutliple alignment and uses the neighbor-joining method (Saitou and Nei 1987) for tree construction.

### cDNA preparation and amplification

mRNA was isolated from growth-chamber grown, two-week old leaves using the procedure of Chomczynski and Sacchi (1987). PolyA+ mRNA was isolated by two cycles of oligo dT (Gibco BRL) chromatography using the procedures recommended by the manufacturer. Double-stranded cDNA was prepared using the procedures described in the Clontech Marathon cDNA Amplification Kit (Clontech Laboratories, Palo Alto, Cal.). The conditions for PCR amplification of double-stranded cDNA were identical to those used for class-specific DNA amplification, except cDNA was substituted for DNA.

### DNA hybridization and linkage analysis

Linkage analysis was performed on two recombinant inbred (RI) populations (Burr et al. 1988). One population consisted of 103 lines derived via single-seed descent ($F_6$) from the cross 'Sierra' × 'Olathe'. Standard conditions for DNA restriction enzyme digestion, blotting, hybridization, and washing were used. The final wash was 0.1 × SSC, 0.005% SDS, 65°C. The second population was developed from the cross 'Jamapa' × 'Calima' (76 lines; single-seed descent through $F_8$). Restriction digestion, blotting, hybridization, and washing were as described by Vallejos et al. (1992). The final wash was with 0.5 × SSPE, 0.1% SDS, 65°C. Linkage analysis of cloned sequences and previously mapped molecular marker loci was carried out with MAPMAKER/Exp 3.0 (Lander et al. 1987; Lincoln et al. 1992). Briefly, marker loci were sorted into linkage groups according to the following linkage criteria: LOD > 4.0 and a maximum distance of 35 cM (Kosambi function); a framework for each group was identified with loci that could be ordered with LOD > 3.0. Loci order was considered significant only if the second most likely order differed by a LOD value of > 2.0.

**Table 1.** Nucleotide sequences of primers used in these experiments. For each primer the suffix 5′ or 3′ designated the region of the common bean NBS to which the primer is complementary. All primers are listed in the 5′ → 3′ direction from left to right.

| Primer | Sequence* |
|---|---|
| B1 | GGIGGIRTIGGIAARACIAC |
| B2 | WTIARIGYIARIGGIARICC |
| OB1-5′ | CAAGCATAAGCCTACCACAC |
| OB1-3′ | CTTGCTCTAGCCATTTATAA |
| OB2-5′ | GGCCAAAATTTGAGCCAGAG |
| OB2-3′ | TTCAACAATGAGTTCCTTCC |
| OB3-5′ | CTTGCATATTCAACCACTCC |
| OB3-3′ | GCTAGATTAGTCTATGAAGC |
| SB1-5′ | CCACAGTATGTGACTACTTC |
| SB1-3′ | AGCCAAATCCATTTGCAATG |
| SB3-5′ | ATACTTCAATACATCAAATG |
| SB3-3′ | GTTTAAAGAAATCTCTCCTC |
| SB4-5′ | ACAATTTCTTTCCCAATCTC |
| SB4-3′ | CTTCAAAGACACCAACACTG |
| SB5-5′ | TTTGCAGCAGCTTATTGCAC |
| SB5-3′ | GTATGACTCCATTTACAAGG |
| SB8-5′ | GAAGCATAAGTTGCTACATG |
| SB8-3′ | CTCTAGCAACGTATAATTTG |
| MAL-5′ | CAAATGCATTATGATTGGCG |
| MAL-3′ | TGAAAAACTGGCCAGAAAAG |

*I = Inosine; R = A or G; W = A or T; Y = C or T.

## Results

### Cloning and sequence analysis of disease-resistance related sequences in common bean

PCR amplification of 'Sierra' and 'Olathe' DNA with primers B1 and B2 generated two major fragments of 0.6 and 0.7 kb. These fragments were cloned and sequenced. In order to determine which of these common bean clones may be related to plant disease resistance genes, we compared their sequences to the NBS sequences of known plant disease resistance genes. These comparisons revealed that the 0.7 kb clones did not resemble the target NBS sequences; these clones were not analyzed further. In contrast, sequence analysis of 14 clones containing the 0.6-kb fragment revealed varying degrees of amino acid identity to the NBS

sequences of some disease resistance genes and were analyzed further.

Comparisons of the 14 sequences led us to group them into eight classes (Table 2). Members of an individual class were at least 90% identical (e.g., clones SB3 and OB9 were 96.5% identical and were considered members of the same class). Each class was designated by the name of a single clone representative of the class. The derived amino acid sequences of individual clones representing each of the eight classes were quite unique. The levels of identity among these classes varied from 51.6% between clones OB1 and SB8, to 18.8% between OB2 and OB3. The derived amino acid sequences of the eight independent classes contained the P-loop encoded in the B1 primer, and a consensus sequence similar to the kinase 2 motif (LVLDD). However, only five of the eight classes contained the kinase 3a motif (FGNGSR) which is common to the NBS domains of some of the known disease resistance genes (Fig. 1).

**Table 2.** Amino acid sequence identities between common bean disease-resistance related sequences and soybean NBS sequences.

| Common bean class | Soybean sequence with highest identity | Common bean class with highest identity |
|---|---|---|
| OB1 (1[a]) | RGA1[b] (67.1[c]) | SB8 (51.6[b]) |
| OB2 (1) | Class *d* (31.0) | SB4 (27.3) |
| OB3 (4) | RGA5 (63.4) | OB1 (37.8) |
| SB1 (1) | RGA6 (52.1) | SB5 (40.8) |
| SB3 (2) | RGA4b (72.0) | SB5 (35.1) |
| SB4 (2) | Class *j* (36.4) | OB3 (30.3) |
| SB5 (1) | Class *i* (53.8) | OB1 (42.5) |
| SB8 (2) | Class *d* (68.7) | OB1 (51.6) |

[a]Number of clones obtained for each class.
[b]The RGA sequences are from Kanazin et al (1996) and the alphabetic class sequences are reported in Yu et al. (1996).
[c]Percent identity.

### Comparison of common bean NBS amino acid sequences and known disease-resistance genes

None of the common bean NBS classes were highly identical to the amino acid sequences of comparable regions of published disease-resistance genes (Fig. 1). The highest level of identity (45.1%) was found between clone SB5 and the tobacco *N* gene (Whitham et al. 1994). Clone SB1 was 30.0% identical to the *Arabidopsis* gene *RPS2* (Bent et al. 1994), and SB5 was 39.6% identical to the *L6* gene of flax (Lawrence et al. 1995). By comparison, *N* and *L6* were 36.0% identical, the highest identity level among the NBS of these three genes.

In contrast, high levels of identity were detected between some of the common bean sequences and those recently published NBS sequences from soybean (Kanazin et al. 1996; Yu et al.1996). The highest level of identity (72.0%) was detected between common bean clone SB3 and soybean clone RGA4b (Table 2). Common bean clone SB8 was 68.7% identical to soybean class *d*. The identity between common bean clone OB1 and soybean clone RGA1 was 67.1%.

Several trends were observed when the common bean, soybean, and resistance-gene sequences were compared. First, when the 31 sequences were characterized, identity among any two sequences ranged from 11.8% (OB3 and soybean class *j*) to 96.3% (soybean classes RGA7 and *j*). Although, several high levels of identity were observed, these were rare. In fact, of all possible comparisons, only 16% (74/465) were greater than 50%. This clearly demonstrates that these NBS sequences represent a divergent class of sequences in legumes.

Progressive multiple alignment (Thompson et al. 1994) of the amino acid sequences of the eight common bean disease-resistance related sequences, 20 soybean NBS sequences (Kanazin et al. 1996; Yu et al. 1996), and the NBS region of three known resistance genes (*N*, *L6*, and *RPS2*) classes were used to generate distance data for the construction of a neighbor-joining (Saitou and Nei 1987) phylogenetic tree (Fig. 2). This tree depicts more clearly the distances between specific common bean and soybean sequences. The tree also demonstrates that, with the exception of SB4 and SB8, each common bean disease-resistance related sequence clustered with a sequence from another species before clustering with another common bean sequence. (The initial placement of SB4 and OB8 in the tree was with a cluster containing a common bean sequence and a sequence from another species.)

A partial explanation for the clustering pattern of these sequences can be found in the sharing of certain amino acid domains. For instance, the sequences FXXXCFL and LQXXLLSELL, recognized by Yu et al. (1996), and III/VTTRD were present in both *N* and SB5, but not in OB2 and *RPS2* sequences. OB2 and *RPS2* instead contained the sequence ENXXKVI/MFTTRS that is not found in other common bean NBS sequences, or in the sequence of the NBS of other cloned resistance genes. SB1 and *L6* both contain a unique SLELFSXHA sequence not found in the other sequences that we compared.

### Expression of common bean disease-resistance related sequences

To determine whether the cloned disease-resistance related sequences from common bean were expressed, double-stranded cDNA was prepared from two-week old leaves of the cultivar Sierra that were grown in a growth chamber. The cDNA was then amplified with primers specific to each of the eight classes of sequences. Each amplification was paired with a control DNA amplification. cDNA amplification products were not detected for SB1, SB3, and SB8 (Fig. 3; lanes 4, 5, and 8, respectively). In addition to the expected product, a major amplification product about 150 bp larger than expected was noted for SB5 (Fig. 3; lane 7). The most abundant amplification products were noted for OB1 and OB3 (Fig 3; lanes 1 and 3, respectively). All amplification products were identical in size to products generated by DNA amplification.

To ensure that the amplification products indeed represented expressed mRNAs found in bean leaves and did not arise from contaminating DNA, control amplifications were performed using primers designed for the gene encoding the *P. vulgaris* malic enzyme (Walter et al. 1994). The 5′ primer was complementary to a sequence in exon 6, and the 3′ primer was complementary to a sequence in exon 10. We obtained the expected ≈350 bp fragment from cDNA amplification and the expected ≈700 bp fragment from DNA am-

**Fig. 1.** Amino acid alignment between common bean disease-resistance related sequences and NBS regions of three known plant disease-resistance genes.

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 OB1 GGMGKTTLALAIYNLVAHH......FDGLCF...LENVRENSNKH.GLQHLQSILLAELVKEKR.........MNITSVQQGISMLQHRLQQK....KVLLVVDDAHKH.........
 SB8 GGMGKTTLALATYNLIAHD......FDGSCF...LQNVREELKKH.GLKHLRDLILSEILGEKN.........INLAE.....SNAEFRDTTKARRKKVLLILDDVDNR.........
 OB3 GGVGKTTIARLVYEAVKEK......FKVSCF...LENIRELSKTN.GLVHIQKEILSHLNVR.R.........MIFVIFMMGKKIIANSLSNK....KVLLVLDDVSDI.........
 SB1 GGMGKTTIAKSICNEIRHE......FKYKSY...LANIREAWGGGRGPVDLQEQLLSDILKT..........KNKVHSTDWGKGKIKEMLCTK....KVLVLLDDVSSV.........
 SB5 GGVGKTTLVKALYDSIYKE......FEGSCF...LSNVRETSNQIKGIEFLQQRLLSEIL.........EDRKIQLGSKEEGTNTIRRSLASK....RVFIVLDDVDNI.........
   N GGVGKTTIARAIFDTLLGRMDSSYQFDGACF...LKDIKENKR...GMHSLQNALLSELLREKANYNNEEDGKHQMASR..........LRSK....KVLIVLDDIDNK.........
  L6 GGIGKTTTAKAVYNKI......SSCFDCCCF...IDNIRETQEKD.GVVVLQKKLVSEILRIDS.......GSVGFNNDSGGRKTIKERVS....RFKILVVLDDVDEK.........
 SB3 GGMGKTTLANTLFKEI......SPQYDAWCHIDDLSKIYLDL....GVTSAQKQLLCQVLNQ.........GNMEIHHVSHGTMLMKNRLRHL....KTLIVLDNVDQV.........
 OB2 GGVGKTTLLKKFNNE..........FLPQKFY..DAVIWVVVSKEADVGSVQQSIGDKLNVPVGKWGGKTIN.DRAIV...........LYNFLKRKKFVLMLDD...........LW
RPS2 GGVGKTTLMQSINNE...........LITKGHQYDVLIWVQMSREFGECTIQQAVGARLGLSWDEKE..TGE.NRALK...........IYRALRQKRFLLLLDD...........VW
 SB4 GGMGKTTLAK................LIFKDTNTDACFPLKMWVCV.......SNDFELRNLLIKILNSTPNSNRENFNNLETEQLQNRLRNTLQVQKFLLVLDDVWNEVPEKWHELK
Cons GG GKTTLA    N           F   CF   L NIRE      G   LQ  LLSELL                        G       L  K    KVLLVLDDV
     P Loop Motif                                                                                    Kinase 2 Motif
 OB1 .EQ.LQAIVGRSDWFGSGSRIIITTRDEQLLASHKVKRTYVVKELNK....NEALQLLTWKAFRTDEVDPSYEEVL...........NSVVAYACGLPFAL
 SB8 .KL.LQAFAGRSDWFGPGSRVIITTRDEQLLKTHEVERTEEVEELNMMILFNCLLGMLSKGKIMIQVTRTSSKHVA...........TY....ASGLPFAF
 OB3 .SQ.LENLGGKREWFGPGSRLIITTRDKHLLKTYGVDMTYKARGLAQ....NEALQLFCLKAFKQDQPKEGYLNLC...........KGVVEYARGLP
 SB1 .EQ.LNDLCGNRDAIGEGSVIIITTRNVRLLREIGVDCVHRVEKM..NEL..ESLELFSWHALRRADPCRDLFELS...........QEVVTYCGGLPLTL
 SB5 .EQ.LENLAGRRFWFGDGSRIIVTTRDQHLL.EVG.QINNRYEVKVLNNQ..ESLELFCQSAFRKSCPEKNYEDLS...........NRAISCCKGLPLAL
   N .DHYLEYLAGDLDWFGNGSRIIITTRDKHLI.EKN.DIIY..EVTALPDH..ESIQLFKQHAFGKEVPNENFEKLS...........LEVVNYAKGLPLAL
  L6 .FKFEDML.GSPKDFISQSRFIITSRSMRVLGTLNENQCKLYEVGSMSKPR..SLELFSKHAFKKNTPPSYYETLA...........NDVVDTTAGLPLT
 SB3 .EQ.LEKLGLHPEYLGAGSRIIIISTDCRILQNYGVNEVYNVKVLDETQ....ALKLFCKRAFKSKDIPKEYEELT...........FDVLKYVKGLPLA
 OB2 ERMDLLKLGIPIPDMENGSKVIFTTRSMEVCRNMEANR.......CIKVEC.....LAQEEAFELFREKVGEETLNSHPEIFPLAQI...LAKECQGLPLTF
RPS2 EEIDLEKTGVPRPDRENKCKVMFTTRSIALCNNMGAEY.......KLRVEF.....LEKKHAWELFCSKVWRKDLLESSSIRRLAEI...IVSKCGGLPLAL
 SB4 EIIDVGVEGSEILV.........TTRSHAVATVMHTKSSNSYLLQCLSEED..SLSLFVKYASEDGMEKHPE...........LLEIGKEIVKKCGGLPLAF
Cons     L  L G    FG GSR IITTRD  LL                      L LF   AF         E L              VV    GLPLA
                   Kinase 3a Motif
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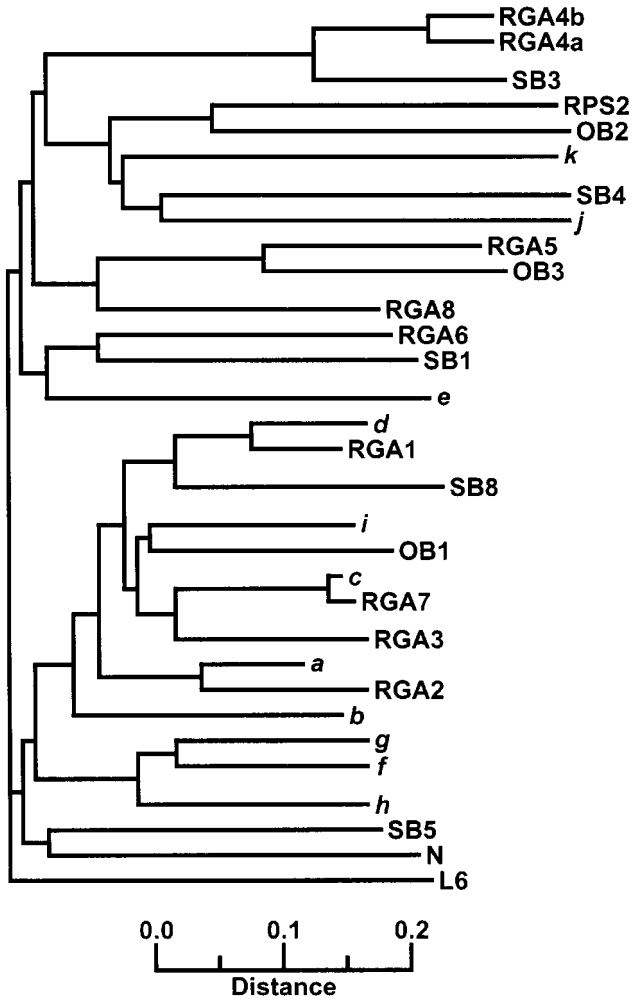
**Fig. 2.** Neighbor-joining analysis phylogenetic tree based on the alignment of common bean and soybean NBS sequences, and the NBS domains of the tobacco *N* gene, the *L6* gene from flax, and the *RPS2* gene from *Arabidopsis*.




plification (Fig. 3; lane 9). The fact that the ≈700 bp fragment was not detected during cDNA amplification strongly indicates genomic DNA was not contaminating the cDNA preparation. Therefore, these experiments provide qualitative evidence of gene expression for five of the eight classes of common bean disease-resistance related sequences.

### Linkage relationship among disease-resistance related sequences

The genomic organization and linkage relationships of the NBS containing clones were investigated in a recombinant inbred population obtained from the cross 'Jamapa' × 'Calima'. These linkage relationships are shown in Fig. 4. Four clones, OB1, SB1, SB3, and SB5, cosegregated and mapped 6 cM from OB2 on linkage group *F*. OB3 mapped to linkage group *I*. Clustered with OB3 are OB5, OB6, and OB7, three members of the OB3 NBS class. Clone SB8 recognized multiple loci. Two of these, SB8a and SB8c, mapped to linkage groups *D* and *B*, respectively. It was not

**Fig. 3.** Amplification of common bean DNA and cDNA with primers to specific classes of disease-resistance related sequences. Each pair of lanes consists of DNA (left) and cDNA (right) amplification with the following primer sets (Table 1). 1 = OB1; 2 = OB2; 3 = OB3; 4 = SB1; 5 = SB3; 6 = SB4; 7 = SB5; 8 = SB8; 9 = malic enzyme.

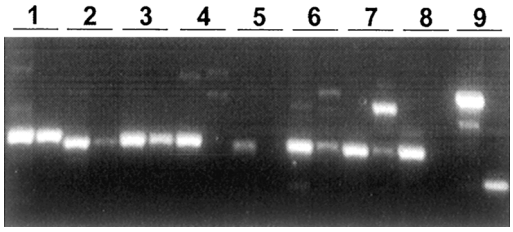
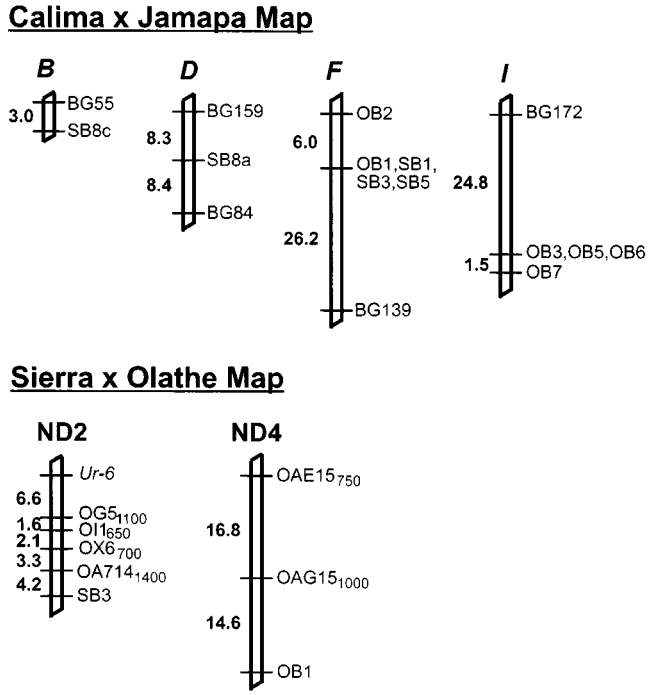



**Fig. 4.** Genetic linkage groups containing common bean disease-resistance related sequences. Linkage groups *B, D, F,* and *I* are from a RI population of the cross 'Calima' × 'Jamapa' and linkage group ND2 and ND4 are from a RI population of the cross 'Sierra' × 'Olathe'.




possible to map SB4 because it yielded a complex hybridization pattern indicative of a large multigene family.

### Linkage between disease-resistance related sequences and bean rust-resistance complex loci

*Ur-3* and *Ur-6*, segregated in a RI population of the cross 'Sierra' × 'Olathe' and map to linkage group ND2 of the RAPD molecular map developed in our laboratory (Ewing and McClean, unpublished results). To determine whether any of the disease-resistance related sequences were linked to these disease-resistance loci, we searched for polymorphisms between 'Sierra' and 'Olathe'. Hybridization of SB3 to *Eco*RI digests of the parents identified one polymorphism. Linkage analysis detected SB3 at 17.8 cM from the *Ur-6* complex locus on linkage group ND2. Class specific PCR products from 'Sierra' and 'Olathe' were screened by

digesting with one of nine resitriction enzymes. Digestion of fragments generated using class-specific PCR primers with the restriction enzyme *Msp*I identified a second polymorphism. Using this polymorphism, OB1 was mapped to linkage group ND4.

## Discussion

We have exploited the sequence homologies among the NBS regions of *R* genes of different species to design primers for the PCR amplification and cloning of eight distinct classes of NBS-like sequences from the common bean. Several features of these sequences suggest they are related to the NBS domains of known disease-resistance genes. First, all of the bean sequences contain a P-loop and a kinase 2-like consensus sequence. Five of the eight classes contain the kinase 3a motif found in the *N* gene, but not in *RPS2* or *L6*. Furthermore, the amino acid sequence identities of bean clones and *R* genes ranged from 22.5% to 45.1%.

Alignment of NBS sequences of common bean and soybean with the *N*, *RPS2*, and *L6* genes reveals that we have indeed cloned a diverse array of sequences. This analysis also suggests that common bean clones OB3, SB3, and OB1 are closely related to soybean sequences RGA5, RGA4b, and RGA1, respectively. Kanazin et al. (1996) and Yu et al. (1996) suggest these classes of NBS sequences may represent subfamilies of *R* genes, and our data suggest that these subclasses share a common ancestor.

Our results (Fig. 2) support the observation of Yu et al. (1996) that the NBS sequences can be classified into two groups. One group consists of sequences related to the *Arabidopsis RPS2* gene and includes classes *j* and *k* as reported by Yu et al. (1996). But unlike that paper, we found their class *e* was also related to the *RPS2* sequences. A second group of sequences is related to the NBS sequence of the tobacco *N* gene. In comparison to the *RPS2* group, Fig. 2 also demonstrates that except for the RGA4a, RGA4b, and SB3 relationships, the *N*-related sequences are more similar than *RPS2*-related sequences. Finally, the fact that each RGA sequence of Kanazin et al. (1996) clustered with one of the two groups consisting of other soybean and common bean sequences suggests an ancestral relationship of the legume NBS sequences.

Linkage and sequence analyses detected two common bean clusters of NBS domain containing sequences. One cluster found in linkage group *F* contains OB1, SB1, SB3, and SB5. The highest identity among any pair within this cluster was only 43% (OB1 vs. SB5). This cluster consists of members of both the *RPS2* and *N* groups. An examination of the data of Kanazin et al. (1996) reveals that a cosegregating cluster on soybean linkage group J also consists of *RPS2* (RGA2) and *N* (RGA5) sequences.

Located on common bean linkage group *I* is a cluster of three sequences (OB3, OB5, and OB6) that cosegregate as a group and map 1.5 cM from OB7. All four of these sequeces are greater than 90% identical and are members of the OB3 group.

The observation that some regions of legume genomes contain tightly-linked clusters of NBS sequences that are highly identical, whereas other clusters consist of highly-diverged NBS sequences suggests that multiple modes of sequence dispersion are occurring in legumes. Further in-depth analysis of multiple NBS-containing genomic regions will be necessary to understand the evolution of these related but quite unique sequences.

This pattern of clustering is often reported for many plant *R* genes (Bent 1996). The clusters of NBS-containing soybean sequences identified by Kanazin et al. (1996) and Yu et al. (1996) also mapped in the vicinity of the known resistance genes. Common bean sequence SB3 is tightly linked to other classes of NBS sequences in the 'Calima' × 'Jamapa' mapping population and maps 17.8 cM from *Ur-6* locus on the ND2 linkage group based on the 'Sierra' × 'Olathe' population.

In addition to *Ur-6*, the *Ur-3* bean rust-resistance locus also maps on ND2. Furthermore, correlative data suggests that *Ur-4*, another major bean rust resistance locus, also maps to ND2 (Ballantyne 1978). Finally, recent mapping data has also placed the common bean mosaic virus-resistance genes *bc-1* and *bc-u* on ND2 (C. Strausbaugh et al., unpublished). This evidence appears to suggest that common bean, like soybean, contains NBS sequences interspersed with known disease-resistance genes. Therefore, further mapping of bean-resistance genes and the disease-resistance related sequences, and merging the RAPD and RFLP maps, should provide a more definitive picture of the proximity of disease-resistance related sequences to known resistance genes in common bean.

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