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G.M. Timmerman-Vaughan · T.J. Frew · N.F. Weeden

Characterization and linkage mapping of R-gene analogous DNA sequences in pea (*Pisum sativum* L.)

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Abstract Pea (*Pisum sativum* L.) sequences that are analogous to the conserved nucleotide binding site (NBS) domain found in a number of plant disease resistance genes (R-genes) were cloned. Using redundant oligonucleotide primers and the polymerase chain reaction (PCR), we amplified nine pea sequences and characterised their sequences. The pea R-gene analog (RGA)-deduced amino acid sequences demonstrated significant sequence similarity with known R-gene sequences lodged in public databases. The genomic locations of eight of the pea RGAs were determined by linkage mapping. The eight RGAs identified ten loci that mapped to six linkage groups. In addition, the genomic organization of the RGAs was inferred. Both single-copy and multicopy sequence families were present among the RGAs, and the multicopy families occurred most often as tightly linked clusters of related sequences. Intraspecific copy number variability was observed in three of the RGA sequence families, suggesting that these sequence families are evolving rapidly. The genomic locations of the pea RGAs were compared with the locations of known pea R-genes and *sym* genes involved in the pea-rhizobia symbiosis. Two pea RGAs mapped in the genomic region containing a pea R-gene, *Fw*, and four pea RGAs mapped in regions of the genome containing *sym* genes.

Key words NBS-LRR · Disease resistance · *sym* genes · Legume-rhizobia symbiosis

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G.M. Timmerman-Vaughan (✉) · T.J. Frew
New Zealand Institute for Crop & Food Research Limited,
Private Bag 4704, Christchurch, New Zealand

N.F. Weeden
Department of Horticultural Science, Cornell University,
Geneva NY 14456, USA

Present address:
N.F. Weeden, Department of Plant Sciences,
Montana State University, Bozeman MT 59717-7622, USA

Introduction

Plant disease resistance genes (R-genes) have been cloned and characterized from both mono- and dicotyledonous plants (reviewed in Hammond-Kosack and Jones 1997). Based on amino acid sequence similarities, these resistance genes have been grouped into classes that represent their predicted functions. The NBS-LRR class, containing most of the R-genes cloned to date, is characterized by the presence of two conserved sequence elements, a nucleotide binding site (NBS) near the N-terminus and a leucine-rich repeat (LRR) near the C-terminus. The NBS-LRR class R-genes are proposed to act as receptors in signal transduction pathways that operate in response to pathogen attack. The NBS and LRR sequence elements, as well as other elements found in these R-genes (leucine zipper and Toll-like sequences), are similar to domains found in known receptor proteins from mammals, yeast, and *Drosophila* (reviewed in Staskawicz et al. 1995; Hammond-Kosack and Jones 1997). Genes from the NBS-LRR class condition resistance to bacterial, fungal, and viral pathogens, and to aphid and nematode pests, and have been cloned from a number of plants including *Arabidopsis thaliana* (Bent et al. 1994; Mindrinos et al. 1994; Grant et al. 1995), flax (Lawrence et al. 1995), tobacco (Whitham et al. 1994), tomato (Milligan et al. 1998), and rice (Yoshimura et al. 1998). These R-genes condition dominantly inherited, pathogen strain-specific resistance that is characterized by the development of the hypersensitive response and systemic acquired resistance.

A polymerase chain reaction (PCR)-based strategy for isolating putative R-gene sequences has been devised based on conserved sequence motifs within the NBS (Leister et al. 1996; Kanazin et al. 1996). Through the use of redundant oligonucleotide primers designed to bind to P-loop and Conserved Domain 2 (CD2) motifs (defined in Grant et al. 1995), DNA sequences analogous to R-gene NBS regions have been amplified from plant species including potato (Leister et al. 1996), soybean (Kanazin et al. 1996), barley and rice (Leister et al. 1998), wheat and barley (Seah et al. 1998), and *A. thali-*

ana (Aarts et al. 1998). For the above examples, the genomic locations of the R-gene analogous (RGA) sequences were mapped using molecular linkage maps, thereby permitting the locations of RGAs and authentic R-genes to be compared. In all the above cases, at least some RGAs showed close genetic linkage with known R-genes, and most of the *A. thaliana* RGAs were tightly linked to known R-genes (Aarts et al. 1998). Therefore, genomic regions likely to contain R-genes may be identified by cloning and mapping RGAs. Furthermore, the RGA sequences themselves may represent candidate R-genes. For example, an *A. thaliana* RGA proved to be part of the cloned RPP5 gene (Aarts et al. 1998).

Peas (*Pisum sativum* L.) are economically important crops that are grown throughout the world. Pests and diseases constrain pea production, affecting yield and seed quality. Disease resistance genes are known in peas, but relatively few have been placed on molecular linkage maps (Weeden et al.1996; Dirlewanger et al. 1994). Most of the pea disease resistance genes placed on molecular linkage maps show recessive inheritance; for example, *sbm-1* (Timmerman et al. 1993), *er-1* (Timmerman et al. 1994), and *mo* (Weeden et al. 1996; Gilpin et al. 1997), therefore NBS-LRR sequences might be unlikely candidates for these genes. Dominantly inherited pea disease resistance genes include *Fw*, *Fnw*, and *En*.

As legumes, peas interact with symbiotic rhizobia to form nitrogen-fixing root nodules. The rhizobia-plant interaction shares many similarities with plant defence responses (reviewed in Baron and Zambryski 1995), including the exchange of signals; the induction of defence-like enzymes, ion fluxes, and reactions; the presence of a cellular hypersensitive response; and the involvement of gene-for-gene interactions affecting strain specificity. Rhizobia produce species-specific Nod-factors that signal the nodulation developmental pathway at low concentrations and with high specificity (reviewed by Cohn et al. 1998). Plant receptors for the Nod-signals trigger pathways and responses similar to defence responses and therefore may involve proteins with structural and functional similarities to receptor-like R-genes, including those of the NBS-LRR class. Plant Nod-signal receptors have not been identified.

To identify pea genomic regions likely to contain R-genes or NBS-class genes involved in other plant-microbe interactions, and to obtain candidate R-gene sequences, we cloned and characterized nine pea RGAs. Eight of these RGAs have been localized on molecular linkage maps related to the "standard" pea genetic linkage map using anchor loci (Weeden et al. 1996, 1998), thereby enabling us to determine whether NBS-class sequences occur in the same regions as known pea disease resistance or symbiosis genes. In addition, we characterized the genomic organization of the pea RGAs.

Materials and methods

PCR amplification of RGA sequences

Three pea varieties were used as sources of total DNA for PCR amplification of RGA sequences: OSU442–15 (Baggett and Hampton 1977), B880–221 (originally from Dr. G.A. Marx, as maintained by NFW), and 3176-A26 (A. Russell, Crop & Food Research, Christchurch, New Zealand). Total DNA was extracted from young leaf tissue as described previously (Timmerman et al. 1993). Two primers designed from consensus P-loop sequences were used, which we designated P-soy (5'-GGIGGIGGTGG-IAAIACIAC-3'; Kanazin et al. 1996) and P-spud (5'-GGTG-GGGTTGGGAAGACAACG-3'; Leister et al. 1996). Three primers designed from CD2 sequences were used: CD2-soy (ARI-GCTARIGGIARICC; Kanazin et al. 1996), CD2-spud1 (5'-CA-ACGCTAGTGGCAATCC-3'), and CD2-spud2 (5'-IAGIGCIAG-IGGIAGICC-3') (Leister et al. 1996). The primer combinations P-soy and CD2-soy, P-soy and CD2-spud1, and P-spud and CD2-spud2 were used. PCR amplifications were carried out in a total volume of 50 µl in 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 200 µ*M* of each of the four dNTPs, 1.0 µ*M* of each primer, 40 ng of total pea DNA, and 1.25 U of *Taq* polymerase (Boehringer-Mannheim). Reactions were capped with two drops of mineral oil. Amplification conditions were 1 cycle at 94°C for 2 min, followed by 40 cycles of 94°C for 45 s, 45°C for 45 s, and 72°C for 1 min, then a final extension of 72°C for 8 min. After electrophoresis through 1% agarose, 1% NuSieve agarose (FMC) in 1 × TBE, amplification products were visualized by staining with ethidium bromide.

PCR fragment cloning and sequence analysis

The major band of approximately 550 bp found in each amplification made using the primer combinations described above was excised from the agarose gels. DNA was extracted using the Qiaex II Gel Extraction Kit (Qiagen) following the manufacturer's instructions. PCR products were cloned using the pGEM-T vector system (Promega). To differentiate individual clones, we PCR-amplified the inserts using Forward and Reverse primers, digested PCR products with either *Rsa*I or *Hin*fI restriction endonucleases, and analyzed the banding patterns on 1% agarose, 1% NuSieve gels. Automated nucleotide sequencing was performed at the University of Otago (Dunedin, New Zealand) using an ABI373 sequenator (Applied Biosystems) and at the University of Waikato (Hamilton, New Zealand) using an ABI377 sequenator (Applied Biosystems). The predicted protein sequences were compared against the non-redundant combined databases (Release date May 5, 1998) using Gapped BLASTX 2.0.5 (Altshcul et al. 1997) and the BLOSUM62 matrix.

Linkage mapping of RGA sequences

The methods for restriction fragment length polymorphism (RFLP) analysis were described previously (Timmerman et al. 1993). On parental blots, five restriction endonucleases were used to find polymorphisms: *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, and *Xba*I. After hybridization with RGAs, Southern blots were washed at 65°C twice with 2× SSC, 0.1% SDS for 15–30 min each wash; twice with 1× SSC, 0.1% SDS for 15 min each wash; and once with 0.5× SSC, 0.1% SDS for 5 min. Three populations were used to map RGAs. The Primo × OSU442–15 F_2 population of 102 individuals was described previously (Timmerman-Vaughan et al. 1996; McCallum et al. 1997; Gilpin et al. 1997), as was the JI1794 × Slow population of 51 recombinant inbred lines (RILs) developed by N. Weeden (Weeden et al. 1993; Timmerman-Vaughan et al. 1996). A third F_2 population of 148 individuals was developed by crossing Rovar (Cebeco, Lelystad, The Netherlands) with breeding line 3176-A26 (A Russell, Crop & Food Research, Christchurch, New Zealand). Linkage maps in this cross were

computed using 84 polymorphic RFLP and RAPD (randomly amplified polymorphic DNA) loci (unpublished data) as described previously (Gilpin et al. 1997) using MAPMAKER/EXP version 3.0 (Lincoln et al. 1992). LOD threshold values for marker assignment to linkage groups were set at LOD ≥ 3.0.

Results and discussion

Amplification and cloning of pea RGAs

Two primer combinations resulted in amplification of the nine RGAs described in this study: P-soy and CD2-spud1, and P-soy and CD2-soy. The amplification products from all three primer combinations included a 550-bp band, but the sequence composition of that band was different for each combination.

Using the P-soy and CD2-spud1 primer pair, we found eight RGAs after screening the inserts in 291 recombinant pGEM-T clones for different restriction fragment patterns and sequencing the unique inserts. Gapped BLASTX database searching found that four of the sequence-characterized inserts contained retrotransposon-like sequences (data not presented), while the remaining 10 clones contained eight RGA sequences. Based on the restriction patterns of the 291 clones, about 70% had restriction patterns identical to the cloned RGAs, while approximately 30% had restriction patterns like the retrotransposon-like sequences. The RGA1.1 insert was sequenced from OSU442–15 once and from B880–221 twice, and was identical each time. RGA1.5 was amplified from OSU442–15, while RGA2.23, RGA2.26, RGA2.65, RGA2.75, RGA2.97, and RGA2.159 were amplified from B880–221. The relative success in cloning RGAs from B880–221 is probably due to the quantity of purified insert and the success of the ligation reaction.

One RGA was amplified using the P-soy and CD2-Soy primer combination. The insert fragments in 10 clones were characterized by restriction digestion with *Rsa*I, and all revealed the same pattern. Two clones were sequenced, RGA-G3A amplified from OSU442–15 and RGA-G45A from 3176-A26, and were identical except for one base. Since *Taq* polymerase was used, this base change could be a PCR artifact.

No RGAs were found using the P-spud and CD2-spud2 primer pair. Eleven insert fragments with different restriction digestion patterns were sequence-characterized. Nine contained retrotransposon-like sequences, and two inserts failed significantly to match sequences in the non-redundant databases (data not presented).

Characterization of pea RGA sequences

All nine pea RGA sequences contained single open reading frames (ORFs). The fragment lengths, GenBank accession numbers, and BLASTX scores indicating the significance of amino acid sequence similarities to known R-genes are presented in Table 1. Comparisons of the deduced amino acid sequence similarities between the pea RGAs are presented in Table 2. This analysis revealed that the pea RGAs fall into two groups. Gapped BLASTX scores reveal that RGA1.1, RGA1.5, RGA2.26, and RGA2.159 share a high degree of sequence similarity. RGA2.97 was also similar to this group of RGAs, but with lower Gapped BLASTX scores (Table 2). A second group of four pea RGAs (RGA2.23, RGA2.65, RGA2.75 and RGA-G3 A) share a high degree of sequence similarity (Table 2). Very high Gapped BLASTX scores were obtained when the following pea RGAs were compared with RGAs from *Glycine max* or *Phaseolus vulgaris* (BLASTX scores in parentheses): RGA1.1 (128), RGA2.23 (203), RGA2.65 (152), RGA2.75 (155), RGA1.59 (145), and RGA-G3A (175). These strong similarities suggest conservation within the legumes of NBS-class sequences that might be involved in plant-pathogen or plant-microbe interactions.

Organization of pea RGA sequence families

To determine the genomic organization of the RGA families, we analyzed the RFLP segregation patterns for the

Table 1 Similarity of pea RGA sequences to known R-genes

Clone	Insert size (bp)[a]	Accession number[b]	Known R-gene with similarity to the clone[c]
RGA1.1	540	AF123695	I2C-2 (138), I2C-1 (126), XA1 (118), RPM1 (71)
RGA1.5	507	AF123696	I2C-2 (109), I2C-1 (97), XA1 (87)
RGA2.23	513	AF123697	M (93), L6 (85), N (82), RPP5 (77)
RGA2.26	534	AF123698	I2C-1 (96), I2C-2 (95), XA1 (95), RPM1 (67)
RGA2.65	513	AF123699	M (105), N (104), RPP5 (101), L6 (89)
RGA2.75	516	AF123700	M (58)
RGA2.97	513	AF123701	RPS2 (127)
RGA2.159	519	AF123702	I2C-2 (145), I2C-1 (138), XA1 (116), PRF (73), RPM1 (72)
RGA-G3A	510	AF123703	M (81), RPP5 (78), L6 (76), N (67)

[a] Size of the cloned insert including the primer sequences
[b] Genbank accession number for the sequence excluding primer sequences
[c] BLASTX scores for the insert sequences between the primers are shown in parentheses. Scores greater than 60 are presented, except for RGA2.75 where the best similarity to a known R-gene had a score of 58

Table 2 Similarity of the deduced amino acid sequences for pea RGAs based on comparison of Gapped BLASTX scores. Similarity scores greater than 60 are italicized

RGA1.1	RGA1.5	RGA2.26	RGA2.159	RGA2.97	RGA2.23	RGA2.65	RGA2.75	RGA-G3A	
*	*133*	*116*	*225*	*64*	37	40	38		RGA1.1
	*	*98*	*177*	48	36	37			RGA1.5
		*	*123*	57	36	45			RGA2.26
			*	57		42			RGA2.159
				*					RGA2.97
					*	*152*	*215*	*64*	RGA2.23
						*	*169*	*92*	RGA2.65
							*	*76*	RGA2.75
								*	RGA-G3A

Table 3 RGA family organization

Clone	RGA family organization[a]	Are faintly hybridizing bands present?
RGA1.1	Single copy[b]	No
RGA1.5	Single copy	No
RGA2.23	Single copy	Yes
RGA2.26	Multiple band linked cluster	Yes
RGA2.65	Multiple band linked cluster (RGA2.65B) Independently segregating single band (RGA2.65A)	Yes
RGA2.75	Single copy	Yes
RGA2.97	Multiple band linked cluster	Yes
RGA2.159	Single copy	No
RGA-G3A	Multiple band linked cluster	Yes

[a] Determined by examining segregation of polymorphic bands on Southern blots containing mapping progeny DNA
[b] Accession JI1794 contained two copies
[c] JI1794 DNA contains one less hybridizing band than the other five parental lines

strongly hybridizing bands in the polymorphic mapping families (Table 3). Four of the sequences (RGA1.5, RGA2.23, RGA2.75, and RGA2.159) hybridized to single bands, therefore identify single-copy families. RGA1.1 hybridized to a single band in all the parental lines except JI1794, for which two bands hybridized. The remaining four RGAs (RGA2.26, RGA2.97, RGA-G3A, and RGA2.65) labelled multiple bands and therefore may identify multicopy families. Segregation patterns indicated that the hybridizing sequences in the RGA2.26, RGA2.97, and RGA-G3A sequence families exist as tightly linked clusters and that the RGA2.65 family contains both a multiple sequence cluster and a single unlinked member. Two loci were resolved by crossovers in the RGA2.26 family microcluster on linkage group I in the Primo × OSU442–15 mapping population (Fig. 1).

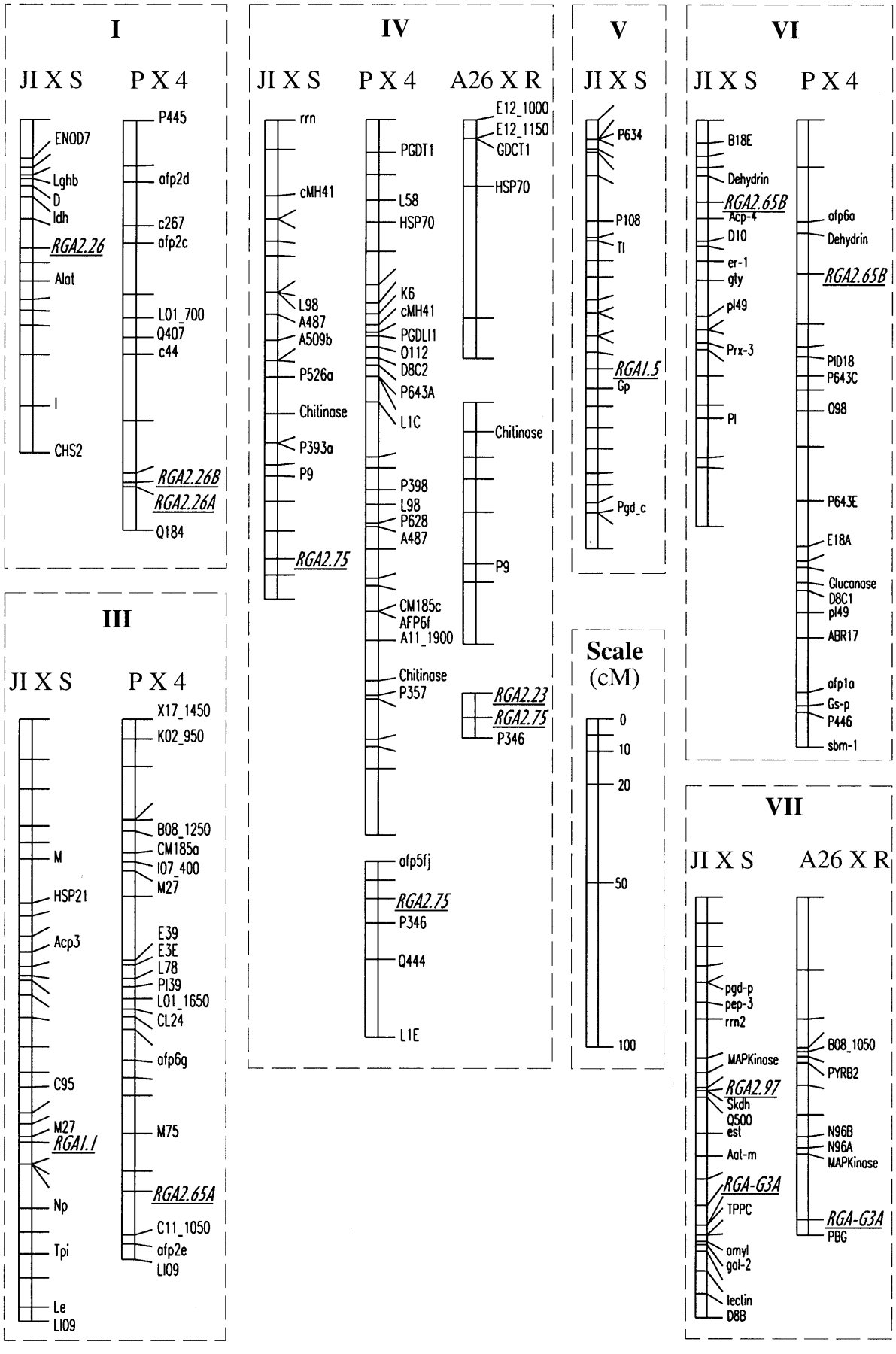
Variability in the number of hybridizing bands was observed on parental and progeny blots probed with RGA1.1 and RGA2.97 (Table 3 footnotes). RGA1.1 labelled two bands in accession JI1794 but was single-copy in the other parental lines. The hybridization banding pattern produced by RGA2.97 was identical for five of the parental lines examined for polymorphism but differed for JI1794 which contained one less hybridizing band than the other lines. The variation in the number of hybridizing bands for the RGA2.97 and RGA-G3A families could result either from restriction site polymorphism or from a locus-dependent insertion or deletion event involving each of these two sequences within *P. sativum* germplasm. Variation in the sequence copy number within pea germplasm has been observed previously but was relatively rare (Ellis et al. 1992; Gilpin et al. 1997).

In other plant species, RGAs show similar patterns of genomic organization, including the clustering of related sequences and intraspecific copy number variation (in rice and barley, Leister et al. 1998). In both soybean (Kazanin et al. 1996) and potato (Leister et al. 1996), RGAs occur most often as multicopy families which typically are so tightly linked that they are not resolved in typical mapping populations. Multicopy families with unlinked family members similar to the RGA2.65 sequence family organization also have been observed. Single-copy families also exist in soybean and potato. Soybean RGA "microclusters" contain only a single R-gene homolog (Kazanin et al. 1996). Based on the limited number of RGAs examined in our study, the pea RGA "microclusters" also contain the members of a single RGA family. In rice and barley, however, Leister et al. (1998) found mixed R-gene homolog clusters containing quite dissimilar sequences.

Linkage mapping of pea RGAs

The genomic locations of eight RGAs were mapped (Fig. 1). RGA2.159 was not polymorphic. The eight RGAs detected ten genetic loci on six linkage groups. RGAs mapped to linkage groups I (RGA2.26), III (RGA1.1 and RGA2.65 A), IV (RGA2.75 and RGA2.23), V (RGA1.5), VI (RGA2.65B), and VII (RGA2.97 and RGA-G3 A), as described on the "standard" linkage map of the pea genome (Weeden et al. 1996, 1998). In Fig. 1, named loci re-

Fig. 1 Linkage maps showing the position of RGA loci on pea linkage groups. Maps from the 'JI1794' × 'Slow', Primo × OSU442–15, and 3176-A26 × Rovar crosses are indicated as *JI × S*, *P × 4*, and *A26 × R*, respectively. The scale presented represents centiMorgans, calculated in Kosambi units ▶


I
JI X S
P X 4
ENOD7
Lghb
D
Idh
RGA2.26
Alat
I
CHS2
P445
afp2d
c267
afp2c
L01_700
Q407
c44
RGA2.26B
RGA2.26A
Q184
III
JI X S
P X 4
M
HSP21
Acp3
C95
M27
RGA1.1
Np
Tpi
Le
LI09
X17_1450
K02_950
B08_1250
CM185a
I07_400
M27
E39
E3E
L78
PI39
L01_1650
CL24
afp6g
M75
RGA2.65A
C11_1050
afp2e
LI09
IV
JI X S
P X 4
A26 X R
rrn
cMH41
L98
A487
A509b
P526a
Chitinase
P393a
P9
RGA2.75
PGDT1
L58
HSP70
K6
cMH41
PGDLI1
O112
D8C2
P643A
L1C
P398
L98
P628
A487
CM185c
AFP6f
A11_1900
Chitinase
P357
afp5fj
RGA2.75
P346
Q444
L1E
E12_1000
E12_1150
GDCT1
HSP70
Chitinase
P9
RGA2.23
RGA2.75
P346
V
JI X S
P634
P108
TI
RGA1.5
Gp
Pgd_c
Scale
(cM)
0
10
20
50
100
VI
JI X S
P X 4
B18E
Dehydrin
RGA2.65B
Acp-4
D10
er-1
gly
pI49
Prx-3
PI
afp6a
Dehydrin
RGA2.65B
PID18
P643C
O98
P643E
E18A
Glucanase
D8C1
pI49
ABR17
afp1a
Gs-p
P446
sbm-1
VII
JI X S
A26 X R
pgd-p
pep-3
rrn2
MAPKinase
RGA2.97
Skdh
Q500
est
Aat-m
RGA-G3A
TPPC
amyl
gal-2
lectin
D8B
B08_1050
PYRB2
N96B
N96A
MAPKinase
RGA-G3A
PBG

late these maps to previously published maps (Weeden et al. 1996; Gilpin et al. 1997), while the positions of pedigree-specific RAPD and AFLP markers are indicated but not named for the sake of clarity. The RGAs were mapped in more than one cross when possible to demonstrate the stability of the map location, and to provide more loci that relate the locations of the RGAs to the "standard" pea linkage map.

Localization of pea RGAs to an R-gene containing genomic region

By mapping pea RGAs, we may identify the locations of genes for disease or pest resistance. In many species, R-genes are found in tightly linked clusters (reviewed in Pryor and Ellis 1993). RGAs in potato (Leister et al. 1996), soybean (Kanazin et al. 1996), barley and rice (Leister et al. 1998), and *A. thaliana* (Aarts et al. 1998) show linkage to R-genes. In our study only two sequences, RGA1.1 and RGA2.65A, map to a region of linkage group III (Fig. 1) known to contain the disease resistance gene *Fw*, a dominant gene for resistance to *Fusarium oxysporum* Race 1. *Fw* is linked to *Np* on linkage group III (Weeden et al. 1996, 1998), but there is no more information on the location of *Fw* based on molecular markers in the published literature. To date, the R-genes in the NBS-LRR class that have been characterized encode dominantly inherited pest or disease resistance (Hammond-Kosack and Jones 1997). Most of the disease resistance genes located on the pea "standard" map (i.e., *er-1*, *sbm-1*, and *mo*) show recessive inheritance, therefore NBS-LRR sequences might be unlikely candidates for these genes. The JI1794 × Slow and Primo × OSU442–15 crosses segregate for the disease resistance genes *er-1* (Timmerman et al. 1994), and *mo* and *sbm-1*(Gilpin et al. 1997), respectively. Genes *mo* and *sbm-1* are not linked to the RGAs mapped in this study, and *er-1* is only distantly linked to RGA2.65B.

Localization of RGAs and host symbiosis genes to similar genomic regions

Five pea RGA loci map to regions of linkage groups I, IV, and VII that contain symbiosis (*sym*) genes. Pea *sym* genes have been characterized by producing mutants defective in nodulation (Kneen et al. 1984, 1987, 1990). The mutant phenotypes show recessive inheritance. The linkage relationships of a number of *sym* genes have been determined in crosses other than those used in our study by analyzing the joint segregation of *sym* genes with anchor loci, usually isozymes or morphological markers (Kneen et al. 1994; Weeden et al. 1996). On linkage group I, the RGA2.26A and RGA2.26B loci map below c267, c44 and *Idh* (Fig. 1) in the same region as *sym*5 and *sym*19. The *sym*5 mutation is involved in the ethylene response that occurs during nodulation (Fearn and LaRue 1991). The RGA2.23 and RGA2.75 loci are linked and map to linkage group IV, distal to P9 (Fig. 1), in the genomic region containing *sym*9 (Kneen et al. 1994; Weeden et al. 1998). On linkage group VII, RGA2.97 is tightly linked to *Skdh* in the JI1794 × Slow population (Fig. 1). The *sym*11 locus was mapped to that region in another cross but was approximately 15 ± 7 cM from *Skdh* (Kneen et al. 1994). On the JI1794 × Slow map, marker distances near *Skdh* are compressed in comparison with other linkage maps (data not presented), suggesting that a microinversion or some other mechanism that represses recombination may act in this cross. RGA-G3A maps between *Aat-m* and *Amy* on linkage group VII, near the TPP-C locus (Fig. 1), in the same region as *sym*15, which also mapped between *Aat-m* and *Amy*, 10 ± 4 cM from *Amy* (Kneen et al. 1994).

Summary

Seven of the NBS-class sequences described in this study map to pea genomic regions on linkage groups I, III, IV, and VII that contain genes involved in plant-microbe interactions. Two RGAs map to the region carrying a dominant gene for resistance to fusarium wilt. The remaining five NBS-class sequences map to regions where the closest host-microbe interaction gene of interest is a *sym* gene involved in rhizobia-plant symbiosis. Pea NBS-class sequences also map to regions of linkage groups V and VI, implying that these genomic regions might also contain genes involved in plant-microbe interactions.

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