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Specific resistances against *Pseudomonas syringae* effectors AvrB and AvrRpm1 have evolved differently in common bean, soybean, and *Arabidopsis*

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Summary

- In plants, the evolution of specific resistance is poorly understood. *Pseudomonas syringae* effectors AvrB and AvrRpm1 are recognized by phylogenetically distinct resistance (*R*) proteins in *Arabidopsis* (Brassicaceae) and soybean (*Glycine max*, Fabaceae). In soybean, these resistances are encoded by two tightly linked *R* genes *Rpg1-b* and *Rpg1-r*. To study the evolution of these specific resistances, we investigated AvrB- and AvrRpm1-induced responses in common bean (*Phaseolus vulgaris*, Fabaceae).
- Common bean genotypes of various geographical origins were inoculated with *P. syringae* strains expressing AvrB or AvrRpm1. A common bean recombinant-inbred-line (RIL) population was used to map *R* genes to AvrRpm1.
- No common bean genotypes recognized AvrB. By contrast, multiple genotypes responded to AvrRpm1, and two independent *R* genes conferring AvrRpm1-specific resistance were mapped to the ends of linkage group B11 (*Rpsar-1*) and B8 (*Rpsar-2*). *Rpsar-1* is located in a region syntenic with the soybean *Rpg1* cluster. However, mapping of specific *Rpg1* homologous genes suggests that AvrRpm1 recognition evolved independently in common bean and soybean.
- The conservation of genomic position of AvrRpm1-specific genes between soybean and common bean suggests a model whereby specific clusters of *R* genes are predisposed to evolve recognition of the same effector molecules.

Keywords

AvrB; AvrRpm1; *Phaseolus vulgaris* (common bean); *Pseudomonas syringae* pv. *phaseolicola*; resistance gene evolution

Introduction

In their natural environment, plants encounter a vast array of pathogenic microorganisms such as fungi, oomycetes, bacteria, viruses and nematodes. To combat these microbial pathogens, plants use a two level innate immune system (Chisholm *et al.*, 2006; Jones & Dangl, 2006). The first layer is the recognition of Microbial- or Pathogen-Associated Molecular Patterns (MAMP or PAMP), and is referred to as PAMP-Triggered Immunity (PTI) (Boller & Felix, 2009). To overcome PTI, pathogens have evolved effector proteins that are translocated into host cells, where they interfere with signaling pathways required for induction of PTI. In turn, plants evolved specific resistance (*R*) proteins that detect the presence of individual pathogen effectors, resulting in Effector Triggered Immunity (ETI) (Innes, 2004; Dangl & McDowell, 2006). Most *R* proteins contain a nucleotide-binding site and leucine-rich repeats (NB-LRR) and are encoded by large gene families in plants (Meyers *et al.*, 2005; Collier & Moffett, 2009). NB-LRR proteins can be subdivided into two major subfamilies, the Toll-Interleukin 1 Receptor (TIR) NB-LRR (TNL) and Coiled-Coil (CC) NB-LRR (CNL), depending on their N-terminal domain (Rairdan *et al.*, 2008). TNL and CNL are encoded by two phylogenetically distinct classes of genes (Meyers *et al.*, 2003; Ameline-Torregrosa *et al.*, 2008; Kohler *et al.*, 2008). In plant genomes, NB-LRR sequences can be distributed as single loci, such as *RPM1* in *Arabidopsis* (Grant *et al.*, 1995), but are more often grouped into complex loci as exemplified by *Arabidopsis* where two-thirds of them are organized in tightly linked clusters (Meyers *et al.*, 2003; Leister, 2004; McDowell & Simon, 2006 and 2008). The available data suggest that different *R* genes can follow strikingly different evolutionary trajectories, such as the fast evolving (Type I) and the slow evolving (Type II) *R* genes identified at the lettuce *Dm3* CNL cluster (Kuang *et al.*, 2004). It is not yet clear what mechanisms underlie these apparently disparate rates of *R* gene evolution.

Bacterial strains belonging to the species *Pseudomonas syringae* attack a broad range of plant species, which provides an opportunity to study the evolution of resistance against this pathogen in different plant lineages. Three strains of this bacterium have been sequenced, and the *P. syringae* effector repertoire has been extensively studied during the last decade (Mansfield, 2009). In *Arabidopsis*, RPM1-interacting protein 4 (RIN4) is a point of convergence between PTI and ETI and is an example of the guard hypothesis (Chisholm *et al.*, 2006). RIN4 functions as a regulator of PAMP signaling, and is manipulated by at least three *P. syringae* effectors (AvrRpm1, AvrB and AvrRpt2) to promote virulence (Belkadir *et al.*, 2004; Kim, HS *et al.*, 2005; Kim, MG *et al.*, 2005). AvrB-RIN4 or AvrRpm1-RIN4 interactions are correlated with the phosphorylation of RIN4 and activation of resistance mediated by RPM1, a CNL protein (Mackey *et al.*, 2002). By contrast, AvrRpt2 induces RIN4 cleavage and activation of RPS2, a distantly related CNL (Axtell & Staskawicz, 2003; Mackey *et al.*, 2003; Day *et al.*, 2005). Interestingly, AvrRpt2 suppresses RPM1 function by inducing RIN4 disappearance in *RPM1 rps2* plants (Mackey *et al.*, 2003). In the *Arabidopsis* genome, *RPM1* is isolated and confers dual resistance specificity to AvrB and AvrRpm1 (Mackey *et al.*, 2002 and 2003), while in soybean resistance to these effectors is encoded by *Rpg1-b* and *Rpg1-r*, respectively, two *RPM1*-unrelated CNL genes tightly linked at the complex *Rpg1* resistance cluster. AvrRpt2 also suppresses *Rpg1-b* function in soybean, providing indirect evidence that a RIN4 homolog may be required for *Rpg1-b* function (Ashfield *et al.*, 2003). Despite the functional similarities between *Rpg1-b* and *RPM1*, these genes belong to distinct evolutionary lineages of CNL that diverged before the monocot-dicot split, suggesting that the ability to recognize AvrB and AvrRpm1 arose independently in *Arabidopsis* and soybean through convergent evolution (Ashfield *et al.*, 2004; McDowell, 2004).

We have previously sequenced and analyzed *c.* 950 kb of common bean (*Phaseolus vulgaris*) genomic DNA that is syntenic to the soybean *Rpg1* resistance cluster (Innes *et al.*, 2008; Wawrzynski *et al.*, 2008). This common bean sequence assembled into five contigs, but the locations of these contigs in the common bean genome were not determined. In both species, the sequenced regions contain a large *Rpg1*-related CNL cluster that appeared before the radiation of legume species ~50 Mya (Innes *et al.*, 2008). Phylogenetic analysis of this cluster revealed that there has been a high frequency of duplication and loss of CNL sequences in both lineages subsequent to the *Glycine–Phaseolus* split that occurred ~20 Mya, and this process has led to different physical distributions of *Rpg1*-related CNL subfamilies in common bean and soybean (Innes *et al.*, 2008). Given this high rate of CNL gene turnover, we wished to determine whether an *Rpg1-b*- and/or *Rpg1-r*-like gene is present in common bean, and if so, whether such genes have been maintained since the divergence of soybean and common bean. Here we report that multiple common bean genotypes possess *Rpg1-r* like genes (*i.e.* that confer recognition of AvrRpm1), while no tested genotypes contained *Rpg1-b*-like genes (*i.e.* conferring recognition of AvrB). Furthermore, genetic mapping in common bean uncovered two unlinked genes with AvrRpm1 specificity, one of which mapped to the *Rpg1*-syntenic region. We discuss these findings in the context of CNL evolution.

Materials and Methods

Plant materials

The geographical origins of wild and cultivated common bean (*Phaseolus vulgaris* L.) genotypes are presented in Table 1. Seventy-seven F11 recombinant-inbred-lines (RILs) derived from a cross between BAT93 and JaloEEP558 were used for mapping experiments (Freyre *et al.*, 1998; Geffroy *et al.*, 2000). We have generated 102 additional F9 BAT93xJaloEEP558 RILs that were also used to map resistance specificities and molecular markers (Supporting Information Table S1). The near-isogenic-lines (NILs) differing at the *Co-2* anthracnose resistance locus (P12R and P12S) are described in Geffroy *et al.* (1998). P12R results from the introgression of the *Co-2* anthracnose resistance gene from Cornell49242 in the Andean ‘Processor’ background, and is a BC12-F15 line.

Bacterial strains

The *Pseudomonas syringae* pv. *phaseolicola* strain 1448AN race 6 (*Psp*) has been described previously (Fillingham *et al.*, 1992). *Psp(avrB)* carries the plasmid pVB01 described in Innes *et al.* (1993) and *Psp(avrRpm1)* carries the plasmid pVSP61/*avrRpm1* described in Ashfield *et al.* (1995). *Psp(avrRpt2)* and *Psp(avrRpt2::Ω)* carry the plasmids pLH12 and pLH12::Ω (with an omega insertion in *avrRpt2*) respectively (Innes *et al.*, 1993).

Pathogenicity tests and *in planta* bacterial population counts

Bean pods were inoculated with a suspension of 5×10^8 cells ml⁻¹, as described in Harper *et al.* (1987). Bean plants were inoculated using a 2 ml syringe (without needle) to infiltrate a bacterial suspension of 1×10^7 cells ml⁻¹ (for phenotypic tests) or 1×10^4 cells ml⁻¹ (for bacterial population counts) into the underside of fully expanded cotyledonary leaves. Bacterial multiplication was examined following the procedure described in Katagiri *et al.* (2002). Leaf disks from two independent replicate plants were pooled for each tissue sample, and three samples were examined for each time point. Resistance phenotypes induced by *Psp(avrRpm1)* in cotyledonary leaves of the 179 BAT93xJaloEEP558 RILs were scored in two independent experiments as fully resistant (R) and susceptible (S). An intermediate phenotype (I) described in (Fillingham *et al.*, 1992), corresponding to maceration associated with hypersensitive response (HR) limited to areas adjacent to main veins, was also identified.

Genetic mapping

Markers used in this study are described in Table S1. A PCR-based approach was used to map the previously sequenced contigs from the common bean G19833 genotype using specific oligonucleotide primers (Innes *et al.*, 2008). CNL1 and CNL3/4 probes were mapped by Southern Blot hybridization experiments as described in Geffroy *et al.* (1998). Chi-squared (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios. MAPMAKER software version 3.0 (Lander *et al.*, 1987) was used to map segregating markers as described in Geffroy *et al.* (2000). The maximum likelihood procedure described in Geffroy *et al.* (2008) was used to map the two independent *R* genes (*Rpsar-1* and *Rpsar-2*) encoding AvrRpm1 resistance specificity in the common bean genome. *Rpsar-2* was also mapped using MAPMAKER software by coding the intermediate (I) phenotype as susceptible (S).

Annotation and microsynteny analysis

We have previously sequenced and annotated an *c.* 1 Mbp region encompassing the *RpgI* locus on soybean chromosome 13 (GmChr13) and ~950 kb of syntenic DNA from common bean (localized in this study to linkage group (LG) 11, corresponding to chromosome 11 (PvChr11) (Pedrosa-Harand *et al.*, 2009; Fonsêca *et al.* 2010)), and ~250 kb from a homoeologous region (H3) on soybean chromosome 6 (GmChr6) (Innes *et al.*, 2008). Here, we have sequenced a 239,262 bp contig of two overlapping bacterial artificial chromosome clones, Pva1-105k5 (FQ032806) and Pva1-120g2 (FQ032805), from the region syntenic to soybean H3 on chromosome 5 of common bean (PvChr5). Sequencing and assembly were performed at the Genoscope (Evry, France), and subsequent annotation was performed as described in Innes *et al.* (2008). Putative homologous sequences from *Arabidopsis* were searched by performing a TBLASTN search with the annotated soybean gene structures from the GmChr13 region excluding CNL, retroelement, and kinase sequences, against the available genomic sequence of *Arabidopsis* (<http://www.Arabidopsis.org/>) with a maximum E-value of $1e^{-10}$. Two microsyntenic regions were retrieved on *Arabidopsis* chromosome 3 (AtChr3) and 5 (AtChr5), based on the arbitrary criterion of finding at least three pairs of homologous genes within a maximum physical distance of six unrelated genes between them. The identified region on AtChr3 is located between *RPM1*, and two *RpgI*-related CNL (At3g14460 and At3g14470). To check for more diffuse microsynteny in this region, we aligned large portions of 2.7 Mbp each from the genomic sequences of soybean (<http://soybase.org/>) (Schmutz *et al.*, 2010) and *Arabidopsis* (<http://www.Arabidopsis.org/>), centered on the regions of interest. Synteny images were generated using custom Perl scripts kindly provided by Steven B Cannon and the GD-SVG image library. Gene correspondences were calculated using BLASTALL (Altschul *et al.*, 1997) on peptide sequences from CNL- and repeat-filtered FGENESH gene calls (Salamov & Solovyev, 2000). Genes were considered homologues if their BLAST-based E-values were $< 1e^{-10}$. Only top hits were considered.

Database searches and phylogenetic analyses

RIN4-homologous sequences from common bean and other plant species were retrieved using a TBLASTN search (1×10^{-10} e-value cutoff) against (1) the PUT (Putative unit transcript) of plantGDB for common bean (Dong *et al.*, 2004), with subsequent EST contig assembly using cap3 (Huang & Madan, 1999), or (2) the whole genome of other species listed in Fig. 5 (<http://www.phytozome.net>). Multiple alignment of amino acid sequences from the NBS portion of CNLs, or the complete sequence of RIN4 homologues was performed with MAFFT version 6 using the L-INS-I strategy (Katoh *et al.*, 2005) using default parameters. These alignments were subjected to Bayesian analysis using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). MrBayes analysis was run with a Jones amino acid substitution model, following a gamma distribution. We performed paired runs

with four chains each and ran the analysis for 10 million generations with sampling every 100 generations. The prior for each analysis was of equal probability. All runs started with a random tree. After elimination of the first 50% of runs, which included the burn-in phase, the remaining iterations were summarized in a consensus tree with posterior probabilities as nodal support. The resulting phylogenetic tree was displayed using MEGA (Tamura *et al.*, 2007). Ks was determined using the yn00 algorithm implemented in PAML 4.3 (Yang, 2007).

Results

The genomic region containing the *Co-2* resistance locus in common bean shares synteny with the soybean genomic region containing *Rpg1-b*

To map the *Rpg1* syntenic region(s) in the common bean genome, we developed five PCR-based markers distributed across the five BAC contigs from common bean (Fig. 1b, Table S1). All five markers mapped to the end of common bean Linkage Group (LG) B11 in a 2.6 cM interval that included SCH20, a SCAR marker linked to the *Co-2* anthracnose resistance gene (Fig. 1a,b) (Geffroy *et al.*, 1998). This order is in agreement with the order previously predicted by the microsynteny analysis between soybean and common bean at the *Rpg1* locus (Fig. 2) (Innes *et al.*, 2008), confirming that despite four gaps in the common bean sequence, these regions are collinear within their whole length, and that no major rearrangement has occurred within this locus since the split between *Phaseolus* and *Glycine* 20 Mya (Lavin *et al.*, 2005). In order to orientate these five contigs relative to the telomere, three Leg markers (Leg043, Leg220, and Leg208), previously shown to map at the same end of LG B11 (Hougaard *et al.*, 2008) and the SCH20 marker were scored on 179 BAT93xJaloEEP558 RILs. Marker 47b16-f1 is the most proximal of the five markers, mapping at 15.5 cM from Leg043. It is followed in order by markers 68o6-f1, 34g17-f1, which co-segregates with SCH20, 76g17-f1, and 118d24-r05, the most distal marker, co-segregating with Leg220 (Fig. 1b).

Two *Rpg1*-related CNL subfamilies with distinct physical distribution exist in common bean

Previous phylogenetic analysis showed that the CNL sequences from the *Rpg1* cluster form four distinct clades, (CNL1 through CNL4; Figs. 1 and 2) that diverged prior to the split between *Phaseolus* and *Glycine* (Innes *et al.*, 2008). Sequences belonging to CNL2 were not found in common bean, suggesting that they were lost in common bean after the divergence between *Phaseolus* and *Glycine* (Innes *et al.*, 2008). Hybridization of specific CNL probes to restricted genomic DNA from common bean genotypes BAT93 and JaloEEP558 revealed that CNL3 and CNL4 probes cross-hybridized to common bands, whereas CNL1 hybridized to a unique set. Both CNL1 and CNL3/4 probes produced complex hybridization patterns and high levels of polymorphisms between BAT93 and JaloEEP558 (data not shown). We mapped six *EcoRI* polymorphic bands for CNL1, and three *EcoRI* and six *HaeIII* polymorphic bands for CNL3/4. All CNL1 bands co-segregated with marker 34g17-f1 except the CNL1Ec band, which mapped between markers 34g17-f1 and 76g17-f1 (Fig. 1b). Thus all CNL1 family members are located within 1 cM of each other on common bean LG B11. The CNL3/4 family members mapped to a 2.9 cM interval at a position distal to the CNL1 members, except for CNL3/4He, which mapped to a position 7.9 cM proximal to the CNL1 cluster (Fig. 1b).

AvrB resistance specificity is absent from common bean, whereas AvrRpm1 resistance specificity is present in common bean

To test whether common bean possesses *R* genes with AvrRpm1 and/or AvrB recognition specificities, we inoculated a large set of wild and cultivated common bean genotypes of

various geographical origins with *P. syringae* pv. *phaseolicola* (*Psp*) strains expressing either AvrB or AvrRpm1. After infiltration of cotyledonary leaves, 17 out of the 36 tested genotypes displayed a resistant phenotype in response to *Psp(avrRpm1)*, indicating that at least some common bean varieties can recognize AvrRpm1. Interestingly, six additional genotypes displayed a reduced (intermediate) resistance phenotype, with water soaking at the center, and HR confined near the main veins (Table 1). In contrast to the high frequency of AvrRpm1 recognition among common bean genotypes, all tested genotypes were susceptible to *Psp(avrB)*, suggesting that specific recognition of AvrB is absent in common bean.

AvrRpm1-specific resistance maps to the Co-2 region in common bean

AvrRpm1 resistance specificity maps to the *Rpg1* locus in soybean (Ashfield *et al.*, 1995). We therefore tested whether AvrRpm1 recognition mapped to the *Rpg1* syntenic region in common bean (*i.e.* the *Co-2* resistance locus). To this end, two near isogenic lines (NILs) for the *Co-2* anthracnose resistance gene (P12R and P12S) were used (see Materials and Methods). Three days after inoculation with *Psp(avrRpm1)*, Cornell49242, which was the source of the *Co-2* anthracnose resistance gene for the P12R NIL, showed clear susceptible symptoms (water soaking and maceration at the infection point), while P12S, which carries the susceptible *co-2* allele, displayed a strong hypersensitive response (HR), indicative of resistance (Fig. 3a). Significantly, *Psp(avrRpm1)* induced an intermediate phenotype in P12R, with maceration at the center, and HR limited to areas adjacent to main veins (Fig. 3a). Since NILs are, theoretically, genetically identical except in the introgressed region, the phenotype difference between P12R and P12S suggests that a gene (*Rpsar-1* for Resistance to *Pseudomonas syringae* effector AvrRpm1 number 1) conferring resistance to AvrRpm1 is located in the vicinity of *co-2* in P12S. Furthermore, the intermediate phenotype of P12R coupled with the susceptibility of Cornell49242, suggests an effect of the 'Processor' genetic background (the recurring parent for the P12S line). Analysis of bacterial growth within leaves confirmed the phenotype observations, with bacterial growth in P12R being intermediate between P12S and Cornell49242 (Fig. 3b).

Hybridization experiments using the CNL1 or CNL3/4 as probes showed that the CNL1 hybridization pattern is entirely conserved between P12R and Cornell49242, except for one band that is specific for P12R (red asterisks on Fig. 3c), whereas P12S has a completely different hybridization pattern (Fig. 3c). This strongly suggests that all CNL1 subfamily members were introgressed from Cornell49242 to P12R, together with *Co-2*. By contrast, the CNL3/4 hybridization pattern is entirely conserved between P12S and P12R, whereas Cornell49242 has a completely different hybridization pattern, other than the non-polymorphic bands (Fig. 3c), suggesting that no CNL3/4 members were introgressed from Cornell49242 to P12R. Together, these results further confirm that the CNL1 and CNL3/4 subfamilies are differentially distributed along the *Rpg1* syntenic region, and most importantly, suggest that both *Co-2* and *Rpsar-1* genes are members of the CNL1 subfamily.

AvrRpm1 resistance specificity is encoded by two independent genes in common bean JaloEEP558 genotype

To confirm the mapping of *Rpsar-1* at the *Co-2* locus, we scored a set of RILs derived from a cross between BAT93 (susceptible) and JaloEEP558 (resistant) for resistance to *Psp(avrRpm1)* using the leaf inoculation assay. Phenotypes were scored as fully resistant (R), intermediate (I), or susceptible (S). The phenotypic distribution of the 179 RILs (92 R, 46 I, 41 S), conformed to a ratio of 2R:1I:1S, suggesting that resistance was controlled by two independent genes, one with strong effect and the second with intermediate effect. The *R* gene with intermediate effect (*Rpsar-1*) mapped to the end of LG B11, cosegregating with the SCH20 marker and with CNL1 family members (Fig. 1b). The second *R* gene, referred

to as *Rpsar-2* and responsible for the fully resistant phenotype, mapped to the end of LG B8. To more precisely map *Rpsar-2*, additional available markers Leg741 and Leg451 (Hougaard *et al.*, 2008) and two SCAR markers (SAS13 and Dj1kSCAR) known to be linked to the *Co-4* anthracnose resistance gene (Adam-Blondon, 1994; Young *et al.*, 1998) were scored on the 179 RILs. These analyses revealed that *Rpsar-2* is located at the end of LG B8, 4.9 cM away from SAS13 and distal to all tested markers (Fig. 1c). These results differ from the results obtained from the NILs in that the NIL data indicated a gene of strong effect for resistance to *Psp(avrRpm1)* at the *co-2* locus, and a gene of intermediate effect elsewhere in the genome. These differences likely stem from the use of different parents in these mapping populations (see the Discussion section).

The type III effector AvrRpt2 suppresses recognition of AvrRpm1 in common bean

The *P. syringae* type III effector AvrRpt2 suppresses recognition of AvrB and AvrRpm1 in both *Arabidopsis* and soybean (Mackey *et al.*, 2003; Ashfield *et al.*, 2004). To test whether AvrRpm1 recognition specificity is also altered by AvrRpt2 in common bean, we co-inoculated *Psp(avrRpm1)* and *Psp(avrRpt2)* into pods of 25 RILs that were susceptible to *Psp(avrRpt2)*, and displayed either a full resistance phenotype or an intermediate phenotype after inoculation with *Psp(avrRpm1)* in leaves. When inoculating a 1: 1 mixture of *Psp(avrRpm1)* and *Psp(avrRpt2)*, an HR reaction was clearly visible 120 h post inoculation (Fig. 4), suggesting that AvrRpt2 is not able to suppress recognition of AvrRpm1 in common bean. However, when inoculating *Psp(avrRpt2)*, waiting for 2 h, and then inoculating *Psp(avrRpm1)* at the same inoculation point, the HR phenotype was completely lost. This was not observed after inoculation with *Psp(avrRpt2::Ω)* instead of *Psp(avrRpt2)*, confirming that AvrRpt2 is responsible for loss of the resistance phenotype. These results suggest that the suppression of AvrRpm1 resistance specificity by AvrRpt2 in common bean is time dependent. We observed suppression of HR in all tested RILs, regardless of their phenotype after inoculation with *Psp(avrRpm1)* (i.e. resistant or intermediate), indicating that recognition of AvrRpm1 by both *Rpsar-1* and *Rpsar-2* is suppressed by AvrRpt2.

RIN4 homologues contain two highly conserved domains including AvrB and AvrRpt2 target sites

In *Arabidopsis*, Mackey *et al.*, (2003) showed that AvrRpt2 interferes with RPM1 function by inducing RIN4 disappearance. Whole genome data mining indicates that RIN4 homologues exist in various plant species. Interestingly, analysis of common bean EST sequences showed that two *RIN4* homologues are expressed in common bean. Phylogenetic analysis revealed two rounds of duplication of *RIN4* homologues leading to two *RIN4* paralogs in common bean and four in soybean (Fig. 5a). An analysis of nucleotide substitution rates at silent sites (K_s) shows that these two rounds of duplication are consistent with the two whole genome duplication events that occurred in legumes, respectively before and after the divergence between *Phaseolus* and *Glycine* (Shoemaker *et al.*, 2006). Indeed, the mean K_s value for (Gm_RIN4_b and Gm_RIN4_a) and (Gm_RIN4_c and Gm_RIN4_d) was 0.16 ± 0.06 , in agreement with previous analyses of gene duplicates derived from the most recent whole genome duplication event in soybean where $K_s = 0.149$ (Schlueter *et al.*, 2006). Similarly, a mean K_s value of 0.64 ± 0.06 was estimated for the older *RIN4* duplication event, in agreement with previous results between homoeologous gene pairs from the first polyploidy event in soybean where $K_s \approx 0.59$ (Schmutz *et al.*, 2010). Together, these results suggest that all homoeologous versions of *RIN4* homologues were maintained after polyploidization in soybean and common bean. Significantly, alignment of these RIN4 homologues revealed two highly conserved domains including the previously identified AvrB binding site (BBS), (Kim, HS *et al.*, 2005; Desveaux *et al.*, 2007) and two previously identified AvrRpt2 cleavage sites (RCS1 and RCS2), (Chisholm

al., 2005; Kim, HS *et al.*, 2005) (Fig. 5b), suggesting that these RIN4 homologues may be targets of AvrB and AvrRpt2.

Rpg1-related and *RPM1*-related CNL coexist in a homoeologous region of the *Rpg1* locus. Soybean and common bean share an ancient whole genome duplication estimated to have occurred 50–60 Mya (Shoemaker *et al.*, 2006). The *Rpg1* homoeologous (H3) region derived from this event was partially sequenced in soybean and was shown to possess three CNL sequences (Innes *et al.*, 2008), referred to as gm132_155, gm132_159, and gm132_161. In common bean, we have sequenced two overlapping BAC clones (~240 kb) corresponding to this ancient duplicated region. We mapped this contig to the end of LG B5 in the common bean genome (Fig. 1a). After annotation, we found one CNL (pva120g2_H3) collinear to and sharing *c.* 57% amino acid identity with the three aforementioned soybean CNLs (Fig. 2b). All of these H3 CNL sequences from either soybean or common bean share < 27% amino acid identity with *Rpg1-b*. Because the complete soybean genome sequence is now available (Schmutz *et al.*, 2010), TBLASTN analysis using *Rpg1-b* as a query against the soybean whole genome shotgun sequence assembly revealed one *Rpg1*-related CNL sequence (gm132_307) located only ~650 kb from gm132_161 on GmChr06. To assess the evolutionary relationships among these CNLs, we performed phylogenetic analysis using the NB domain of all CNLs from the *Rpg1* homoeologous regions of soybean and common bean, all CNL sequences identified in the *Arabidopsis* genome, all CNL sequences identified in the common bean genome, and a representative set of CNLs from *Medicago truncatula* (Ameline-Torregrosa *et al.*, 2008). gm132_307 fell into the *Rpg1* clade, suggesting that *Rpg1*-related sequences were present at this locus prior to the ancient legume whole genome duplication (Fig. 2a). Surprisingly, this analysis also demonstrated that *RPM1* is the *Arabidopsis* sequence most closely related to gm132_155, gm132_159, and gm132_161 in soybean, and pva120g2_H3 in common bean. Moreover, TBLASTN analysis using *RPM1* as query against the soybean whole genome shotgun assembly identified gm132_155 as the soybean sequence most closely related to *RPM1*, with 32% amino acid identity. Together, these results indicate that *RPM1*-related and *Rpg1*-related CNL coexist within ~650 kb on GmChr6 (an alignment of these sequences is presented in Fig. S1). The presence of *RPM1*-related CNL in the H3 region of both soybean and common bean suggests that *RPM1*-related CNL were present at this locus prior to the divergence between *Phaseolus* and *Glycine*, 20 Mya (Lavin *et al.*, 2005).

The *RPM1*-related and *Rpg1*-related CNL are located in regions sharing synteny relationships between *Arabidopsis* and legumes

In *Arabidopsis*, the two sequences most closely related to soybean *Rpg1-b* (At3g14460 and At3g14470) are clustered in a region located only 2.6 Mb away from *RPM1* on AtChr3 (Fig. 2b). This physical proximity of *Rpg1*-related sequences and *RPM1* in *Arabidopsis* parallels our observations that *Rpg1*- and *RPM1*-related CNL are also physically close in soybean. Thus, we wondered if those two regions from soybean and *Arabidopsis* could be syntenic regions. Interestingly, microsynteny was observed between the *Rpg1* locus and the AtChr3 region located between the two *Rpg1*-related sequences (At3g14460, At3g14470) and *RPM1* (Fig. 2b). We have found another region sharing microsynteny with the *Rpg1* locus on AtChr5. These two regions from AtChr3 and AtChr5 are homoeologous regions coming from the *Arabidopsis* whole genome duplication dated from 24 to 40 Mya (Fig. 2b) (Blanc *et al.*, 2003).

Discussion

We have mapped the soybean *Rpg1* syntenic region at the *Co-2* resistance locus in common bean. Our results indicate that CNL1 and CNL3/4 subfamilies are linked, but physically separated from each other in common bean (Figs 1, 3c), while they are intermixed in

soybean, suggesting that tandem duplications have prevailed in common bean while soybean has undergone a more complex process. Furthermore, the absence of the CNL2 subfamily in common bean indicates that soybean and common bean have diverged in the qualitative content of *Rpg1*-related CNL (Innes *et al.*, 2008). These differences of both CNL distribution and content between closely related species at a single locus parallels the striking differences observed between more distant plant species at the genome level (McHale *et al.*, 2006; Ameline-Torregrosa *et al.*, 2008). Indeed, dozens of *Rpg1*-related CNL in common bean and soybean correspond to only five CNL from a single clade in *Medicago*, which is itself included in a superclade that expanded in legumes, but not in *Arabidopsis* (Fig. 2a) (Ameline-Torregrosa *et al.*, 2008).

Given that two genes located at the soybean *Rpg1* locus are involved in specific resistance to AvrB and AvrRpm1 (Ashfield *et al.*, 1995), and that there is rapid birth and death of CNL genes at this locus in soybean and common bean (Innes *et al.*, 2008), we wondered if these specific resistances appeared before the divergence between soybean and common bean *c.* 20 Mya (Lavin *et al.*, 2005). Our results show striking similarities between soybean and common bean. First, the resistance specificities to AvrB and AvrRpm1 are genetically separable in common bean as observed in soybean (Ashfield *et al.*, 1995). Second, indirect evidence for the role of a RIN4 homolog in the recognition of AvrRpm1 exists in common bean, as observed in soybean (Ashfield *et al.*, 2004). However, two major differences exist between common bean and soybean in terms of specific resistance to AvrB or AvrRpm1

Common bean lacks an AvrB-specific resistance gene

A first difference between common bean and soybean is that while resistance to AvrB is encoded by *Rpg1-b* in soybean (Ashfield *et al.*, 2004), we failed to identify any specific resistance after inoculation of large set of common bean genotypes of various geographical origins with *Psp(avrB)* (Table 1), suggesting that common bean is unable to specifically recognize AvrB. Therefore, the ability to recognize AvrB presumably emerged in soybean after *Phaseolus* and *Glycine* diverged ~20 Mya (Lavin *et al.*, 2005), or alternatively, was lost in common bean after this time point. Interestingly, the common bean region collinear to *Rpg1-b* is devoid of CNL genes (Fig. 2b). Thus, the absence of AvrB specificity is hypothetically due to complete absence of an *Rpg1-b* homolog in common bean, suggesting that the birth and death process observed between common bean and soybean at the *Rpg1* locus could be responsible for this polymorphism. Although *avrB* was originally cloned in a *P. syringae* strain attacking soybean (Tamaki *et al.*, 1988), the majority of *avrB* homologs found to date were isolated from *P. syringae* strains attacking common bean (<http://pseudomonas-syringae.org/>) (Mansfield, 2009). More precisely, three of these *avrB* homologs (*avrB2*, *avrB4-1*, and *avrB4-2*) are present in the genome of the *Psp* 1448AN strain used in our experiments. This strain was shown to promote susceptibility in a panel of 37 common bean genotypes of diverse geographical origins (Table 1), in agreement with previous inoculation on eight additional bean genotypes (Taylor *et al.*, 1996), suggesting that common bean cannot recognize any of those *avrB* homologs. Existence of multiple *avrB* homologs in strains attacking common bean while common bean cannot recognize AvrB suggests an adaptation of the pathogen to its host, as previously shown for the interaction between common bean and *C. lindemuthianum* (Geffroy *et al.*, 1999). In absence of resistance, AvrB was shown to promote virulence in soybean (Ashfield *et al.*, 1995; Ong & Innes, 2006), and more hypothetically in *Arabidopsis* (Nimchuk *et al.*, 2000). Thus, it is tempting to speculate that absence of selection pressure could have favored *avrB* diversification to promote virulence in *P. syringae* strains attacking common bean. Total absence of an *R* gene against AvrB in common bean is intriguing. Why would such a resistance be entirely absent from a plant species that is confronted with a pathogen carrying *avrB* homologs? Riely and Martin (2001) proposed that a genetic bottleneck during the

course of domestication of wild *Lycopersicon esculentum* could explain the total absence of *Pto* orthologs in *Lycopersicon esculentum*. Here, we can rule out a genetic bottleneck due to domestication because we used a large set of common bean genotypes including wild accessions from various geographical origins, suggesting that AvrB resistance specificity is absent in both wild and cultivated genotypes (Table 1). Another explanation could be that common bean has not been infected by *P. syringae* strains carrying *avrB* before its relatively recent domestication (*c.* 5000 yr ago), however presence of *avrB* homologs in the chromosome of different strains attacking common bean (<http://pseudomonas-syringae.org/>) suggests a more ancient origin of *avrB* in these strains, arguing against this later hypothesis. Alternatively, AvrB resistance specificity could exist in common bean, but with a frequency not sufficiently high to be identified in the set of genotypes screened.

AvrRpm1 resistance specificity is encoded by two genes in common bean

A second striking difference is that in common bean two different *R* genes (*Rpsar-1* and *Rpsar-2*) can independently be responsible for resistance to AvrRpm1, while in soybean a single gene, *Rpg1-r*, was previously identified in two tested soybean genotypes (Ashfield *et al.*, 1995). These results are supported by previous observations that an AvrRpm1 homolog from *Ps pv pisi* (AvrPpiA1) with amino acid sequence nearly identical to AvrRpm1, induced a resistance phenotype with digenic segregation in an F₂ progeny of common bean (Dangl *et al.*, 1992; Fillingham *et al.*, 1992). Intriguingly, AvrRpm1 induced an intermediate phenotype in P12R, which is a putative *rpsar-1-Rpsar-2* genotype (Fig. 3), while *rpsar-1-Rpsar-2* RILs from the BAT93xJaloEEP558 progeny displayed full resistance phenotypes. This phenotypic polymorphism suggests that different functional alleles may exist in common bean for *Rpsar-1* and *Rpsar-2*, or alternatively, that a more complex effect of genetic background modulates *avrRpm1*-induced responses. Our results mirror recent work from Eitas *et al.*, (2008) showing that *TAOI* mediates weak resistance phenotype to AvrB independently from *RPM1*. Interestingly, they proposed that presence of more than one *R* gene may provide greater evolutionary capacity, by allowing *R* proteins to continually fine-tune their responses to a single pathogen effector, while maintaining an intermediate level of disease resistance.

We mapped *Rpsar-1* at one end of LG B11 in the region syntenic to *Rpg1-r*, suggesting that this resistance specificity appeared before *Phaseolus* and *Glycine* diverged ~20 Mya, and was maintained in both genera. This, to our knowledge, would be the first example of specific resistance conservation between species at such a time scale. Fine mapping of *Rpsar-1* in comparison to CNL1 and CNL3/4 subfamilies in both RILs and NILs, however, shows that *Rpsar-1* co-localizes with the CNL1 subfamily, suggesting that it is encoded by a CNL1 sequence (Figs 1, 3c), while *Rpg1-r* belongs to the CNL4 subfamily (T. Ashfield & R. W. Innes, unpublished). This result suggests that the ability to recognize AvrRpm1 was maintained in different *Rpg1*-related CNL subfamilies through a recombination event between a CNL1 sequence and a CNL4 sequence after the divergence between common bean and soybean (Fig. 6. models B1 and B2) or alternatively, that AvrRpm1-recognition ability arose independently in these two lineages (Fig. 6 model A). Resolving these alternative hypotheses will require identification and analysis of the functional *Rpsar-1* and *Rpg1-r* genes.

It is noteworthy that both *Rpsar-1* and *Rpsar-2* map in the vicinity of *R* genes that confer resistance to the fungus *C. lindemuthianum* (Geffroy *et al.*, 1998; Melotto *et al.*, 2004). Tight linkage between *R* genes conferring resistance against fungal and bacterial pathogens has also been observed in other species such as *Arabidopsis* (Narusaka *et al.* 2009). In common bean, these two loci are located at one end of LG B11 (*Co-2*) and LG B8 (*Co-4*) (Fig. 1). Furthermore, we have mapped the *Co-2* homoeologous region (H3) containing one *RPM1*-related CNL at the end of LG B5, (Fig. 1, Fig. 2). Altogether, these results reinforce

the previously proposed idea that chromosome ends are favorable niches for *R* gene proliferation in the common bean genome (Geffroy *et al.*, 2008, 2009; David *et al.*, 2009).

Potential involvement of a RIN4 homolog in the recognition of AvrRpm1 in common bean. In *Arabidopsis*, Mackey *et al.*, (2003) demonstrated that AvrRpt2 suppresses RPM1 function by inducing RIN4 disappearance. AvrRpt2 also suppresses Rpg1-b function in soybean (Ashfield *et al.*, 2004), as well as Rpsar-1 and Rpsar-2 functions in common bean (Fig. 4), indicating that AvrRpt2 interferes with the recognition pathways induced by AvrB and AvrRpm1 in both *Arabidopsis* and legumes. Interestingly, two RIN4 homologues are expressed in common bean, and four in soybean (Fig. 5). Alignment of AtRIN4 with its putative orthologs in various plant species, including common bean and soybean, revealed highly conserved domains (Fig. 5), including the two previously identified AvrRpt2 cleavage sites (RCS) and the AvrB binding site (BBS) (Chisholm *et al.*, 2005; Kim, HS *et al.*, 2005), indicating that RIN4 orthologs (and particularly PvRIN4s and GmRIN4s) are putative virulence targets of both AvrRpt2 and AvrB. This supports previous suggestions that a RIN4 homolog is involved in Rpg1-b-mediated AvrB recognition in soybean (Ashfield *et al.*, 2004; Ong & Innes, 2006). Mackey *et al.*, (2003) showed that AvrRpt2-induced RIN4 disappearance is time-dependent in *Arabidopsis*, and that high levels of RIN4 inhibit RPM1 loss of function induced by AvrRpt2. Our observations that AvrRpt2 prevents the HR phenotype induced by Rpsar-1 or Rpsar-2 in a time-dependent manner (Fig. 4) is consistent with a model in which RIN4 must be degraded to prevent Rpsar-1 and Rpsar-2 activation. However, the RCS and BBS domains are widely distributed in evolutionarily distant plant species, and many RIN4-unrelated proteins contain both RCS and BBS domains in *Arabidopsis* (Chisholm *et al.*, 2005; Kim, HS *et al.*, 2005; Desveaux *et al.*, 2007). It is thus possible that Rpsar-1, Rpsar-2, Rpg1-r, and RPM1 monitor the status of different targets, but all of which are targeted by both AvrRpm1 and AvrRpt2.

Models for the evolution of AvrB and AvrRpm1-specificity

Ashfield *et al.*, (2004) have demonstrated that *Rpg1-b* and *RPM1* belong to distinct evolutionary lineages of CNL that diverged before the monocot-dicot split (reviewed by McDowell, 2004). They proposed that the ability to recognize AvrB and AvrRpm1 appeared independently in *Arabidopsis* and soybean through convergent evolution. In *Arabidopsis*, the TAO1-mediated resistance response to AvrB is RIN4 independent, and is encoded by an *RPM1*-unrelated sequence from the TNL family (Eitas *et al.*, 2008). Therefore, convergent evolution was likely responsible for the emergence of *TAO1* and *RPM1* in *Arabidopsis*. Similarly convergent evolution could also be responsible for the emergence of *Rpg1-r* in soybean and *Rpsar-1* and *Rpsar-2* in common bean. Interestingly, our genomic and phylogenetic data show that *RPM1*- and *Rpg1*-related CNL are physically close in both *Arabidopsis* and soybean (Fig. 2). Moreover, the regions containing *RPM1*- and *Rpg1*-related CNL in *Arabidopsis*, common bean and soybean share partial syntenic relationships (Fig. 2), suggesting that *RPM1*- and *Rpg1*-related CNL were present at the same locus in a common ancestor of *Arabidopsis* and legumes. Under this assumption, a scenario of convergent evolution would imply that the recognition of AvrB and/or AvrRpm1 arose three times independently in the same genomic region in *Arabidopsis* (*RPM1*), common bean (*Rpsar-1*) and soybean (*Rpg1-b* plus *Rpg1-r*).

The repeated evolution of similar specificities in the same CNL cluster suggests that these CNL may be predisposed to evolve AvrB and/or AvrRpm1 recognition ability. Because recognition of these effectors by RPM1 is known to require a physical association between RPM1 and RIN4 (Mackey *et al.*, 2002), and because these effectors induce modification of RIN4, we propose that physical association with unmodified RIN4 predisposes CNL proteins to evolve the ability to detect modifications to RIN4. In this model, evolution of specificity is a two-step process. First, the CNL protein must evolve the ability to associate

with an unmodified effector target (e.g. RIN4), and second they must evolve the ability to detect modification of that target. Since effector targets are likely to be important regulators of disease resistance (Block *et al.*, 2008), they are likely to be well conserved during plant evolution, and there will be selection on CNL proteins to maintain the ability to interact with effector targets. Thus, once association with an effector target has evolved, this may be an evolutionarily stable trait. However, there will be strong selection on pathogens to evolve novel ways of modifying these effector targets in order to escape recognition. Such novelty would then drive diversification of the domain(s) of the CNL involved in detecting modifications, while maintaining the domain(s) required for interaction with the effector target. Following this model, *Rpg1-b*, *Rpg1-r* and *Rpsar-1* are derived from a common ancestral CNL with the ability to interact with RIN4, but have evolved the ability to detect specific modifications of RIN4 independently (Fig 6. model A).

The physical proximity of *RPM1*- and *Rpg1*-related CNL in both *Arabidopsis* and soybean could suggest an alternative model in which the ability to recognize AvrB and/or AvrRpm1 arose in a common ancestor of *Arabidopsis* and legumes, and was further maintained in different CNL lineages through an ancient recombination event between *RPM1*- and *Rpg1*-related CNL sequences (Fig. 6. models B1 and B2). Recombination between NBS-LRR sequences are thought to have played a major role in their evolution, leading to changes in copy number via unequal crossing-over in tandem arrays, as well as changes in specificity (Wicker *et al.*, 2007; Nagy & Bennetzen, 2008).

The convergent evolution model and evolution by recombination model differ from a simple evolution model (Fig. 6. model C) in that the latter model would require maintaining duplicated genes with dual AvrB/AvrRpm1 specificity for tens of millions of years, which seems unlikely given the rapid evolution of *R* gene specificity. Distinguishing between the convergent evolution model and recombination model will require identification of *Rpsar-1* so that it can be compared directly to *Rpg1-r*. If the NBS regions of these two genes do indeed belong to different CNL clades, as our mapping data predict, but their LRR domains belong to the same clade, it would favor the recombination model. On the other hand, if no evidence of recombination is found, then the convergent evolution model would be favored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Genetic mapping of *RpgI* syntenic regions, *RpgI*-related CNL, and *Rpsar-1* and *Rpsar-2* resistance genes in common bean (*Phaseolus vulgaris*). (a) Linkage groups (LG) according to Hougaard et al. (2008). Green vertical bars indicate regions magnified in (b) and (c). Green text indicates markers used in (b) and (c). The genomic location of homoeologous region 3 (H3) is indicated by an arrow at the end of LG B5. (b) Genetic mapping of the sequenced common bean contigs (Innes et al., 2008) and of CNL from the *RpgI-b* syntenic region to LG B11, and mapping of the *Rpsar-1* gene. Vertical black lines on the left represent sequenced BAC contigs. Arrows represent predicted CNL genes and their orientation. Fill and text colors correspond to the colors of the CNL clades defined in Innes et al. (2008) (CNL1, blue; CNL2, absent; CNL3, red; CNL4, purple). The LOD score values, which indicate the most probable genomic location of *Rpsar-1*, are presented on the right of B11. (c) Mapping of the *Rpsar-2* gene to the end of LG B8. SAS13 and Dj1kSCAR are two SCAR markers linked to the anthracnose *Co-4 R* gene (Adam-Blondon, 1994; Young et al., 1998). Oval symbols correspond to *R* genes. Genetic distances between each marker are indicated on the left in Kosambi cM for (b) and (c).

Fig. 2. Phylogenetic analysis and physical map of CNL genes. (a) Bayesian tree of CNL genes from the *Rpg1* syntenic region in comparison to all *Arabidopsis* CNL genes. This tree was constructed using just the NB domains (from the P-loop to the MHD motif). Clades of CNL from the *Rpg1* locus are colored according to Innes *et al.* (2008). Green text indicates CNL from the RPM1 clade. Gene names are abbreviated as follows: At, *Arabidopsis thaliana*; Gm, *Glycine max*; Mt, *Medicago truncatula*; Pv, *Phaseolus vulgaris*. (b) Physical map of CNL genes of the *Rpg1-b* syntenic region. Vertical black lines represent sequenced BAC contigs from common bean, or chromosome regions from soybean or *Arabidopsis* with the following coordinates (from top to bottom): AtChr3 (from 4880000bp to 2145000bp), AtChr5 (from 3010000bp to 275000bp), GmChr13 (from 28650000bp to 31385000bp), and GmChr6 (from 50600000bp to 47865000bp). Arrows represent predicted CNL genes and their orientation. Filled colors correspond to the colors of the clades defined in (a). White CNL are pseudogenes with partial or total deletion of the NB region, that were not included in the phylogenetic analysis (Innes *et al.*, 2008). Horizontal lines represent low copy genes

conserved between species or homoeologues (in black for genes from side-by-side regions in the Figure (e.g. PvChr11 and GmChr13) and in grey for genes from two non-side-by-side regions (e.g. GmChr6 and AtChr3). The *Rpg1-b* co-linear region in PvChr11 is indicated by a black horizontal arrow. In common bean, LG B11 and LG B5 corresponds to PvChr11 and PvChr5, respectively (Pedrosa-Harand *et al.*, 2009; Fonsêca *et al.* 2010).

Fig. 3.

(a, b) *Psp* 1448AN race 6 strains carrying *avrRpm1* or not, were inoculated into near-isogenic lines (NILs) diverging for the *Co-2* gene (P12S and P12R), and into Cornell49242 (abbreviated as Cornell), the *Co-2* donor parent. (a) Phenotypes in leaves 3 d after inoculation. (b) Bacterial titres determined immediately after inoculation (dark grey bars) and 3 d later (light grey bars). Each bar represents the average of three independent samples, ± 1 SD. This experiment was performed twice with similar results. (c) Hybridization of CNL1 or CNL3/4 probes on restricted DNA with *EcoRI* and *HaeIII* of P12R, P12S, and Cornell49242. CNL1 bands that differ between P12R and Cornell49242 are indicated by a red asterisk. Molecular weights in kilobases are indicated to the left.

Fig. 4.

Reaction phenotypes in pods of common bean (*Phaseolus vulgaris*) genotypes after inoculation with *Psp* 1448AN race 6 strains expressing *avrRpm1* or *avrRpt2* type III effectors. B76 and B87 are two recombinant inbred lines (RILs; derived from a cross between BAT93 and JaloEEP558) displaying an intermediate and a full resistance phenotype after inoculation with *Psp(avrRpm1)* in leaves, respectively (data not shown). +, a 1:1 mixture of two strains was inoculated simultaneously. →, the second strain was inoculated 2 h after the first strain: 1, *Psp*; 2, *Psp(avrRpm1)*; 3, *Psp(avrRpt2::Ω)*; 4, *Psp(avrRpt2)*; 5, 1:1 mix of *Psp(avrRpm1)* + *Psp(avrRpt2::Ω)*; 6, 1:1 mix of *Psp(avrRpt2)* + *Psp(avrRpt2::Ω)*; 7, 1:1 mix of *Psp(avrRpm1)* + *Psp(avrRpt2)*; 8, *Psp(avrRpt2)* followed by *Psp(avrRpm1)* 2 h later; 9, *Psp(avrRpt2::Ω)* followed by *Psp(avrRpm1)* 2 h later.

Fig. 5. Phylogenetic analysis and alignment of RIN4 homologs from various plant species. Species names are abbreviated as follows: Al, *Arabidopsis lyrata*; At, *Arabidopsis thaliana*; Bd, *Brachypodium distachyon*; Gm, *Glycine max*; Os, *Oryza sativa*; Pt, *Populus trichocarpa*; Pv, *Phaseolus vulgaris*; Sb, *Sorghum bicolor*; Vv, *Vitis vinifera*. (a) Bayesian tree of RIN4 homologs. The first polyploidy event ($K_s = 0.64 \pm 0.06$) is indicated by an open circle, and the second polyploidy event ($K_s = 0.16 \pm 0.06$) is indicated by closed circles. (b) Alignment of complete amino acid sequences of RIN4 homologs. Two highly conserved domains (1 and 2) are indicated. The two AvrRpt2 cleavage sites (RCS1 and RCS2), and the AvrB binding site (BBS) are boxed (Kim *et al.*, 2005). The region that has been co-crystallized with AvrB (RIN4₁₄₂₋₁₇₆) is underlined (Desveaux *et al.*, 2007).

Fig. 6. Hypothetical models for the evolution of AvrB and AvrRpm1 recognition specificities in plants. *Leg*, legumes; *At*, *Arabidopsis thaliana*. Only models implying the most parcimonious series of events are shown. In the recombination and classical models, the ability to interact with RIN4 is included with the ability to recognize AvrRpm1 and/or AvrB. Time is not drawn to scale. Mya, million yr ago.

Table 1

Distribution of AvrB or AvrRpm1 resistance specificities in wild and cultivated common bean (*Phaseolus vulgaris*) accessions from various geographical origins

Accession	Species	Origin ^a	Status ^b	P _{sp}	P _{sp(avrB)}	P _{sp(avrRpm1)}
Pi 207262	<i>P. vulgaris</i>	M	Cul	S	S	R
Eo2	<i>P. vulgaris</i>	M	Cul	S	S	S
To	<i>P. vulgaris</i>	M	Cul	S	S	I
BAT93	<i>P. vulgaris</i>	M	Cul	S	S	S
Cornell49242	<i>P. vulgaris</i>	M	Cul	S	S	S
My	<i>P. vulgaris</i>	M	Cul	S	S	S
Mz	<i>P. vulgaris</i>	M	Cul	S	S	S
A55	<i>P. vulgaris</i>	M	Cul	S	S	S
Tu	<i>P. vulgaris</i>	M	Cul	S	S	I
M222	<i>P. vulgaris</i>	M	Cul	S	S	I
Michelite	<i>P. vulgaris</i>	M	Cul	S	S	S
AB136	<i>P. vulgaris</i>	M	Cul	S	S	R
G11051	<i>P. vulgaris</i>	M	Wild	S	S	I
G19908	<i>P. vulgaris</i>	M	Wild	S	S	R
G23416	<i>P. vulgaris</i>	M	Wild	S	S	S
G21227	<i>P. vulgaris</i>	C	Cul	S	S	R
G21242	<i>P. vulgaris</i>	C	Cul	S	S	S
G24404	<i>P. vulgaris</i>	C	Wild	S	S	S
G122	<i>P. vulgaris</i>	A	Cul	S	S	R
AFN	<i>P. vulgaris</i>	A	Cul	S	S	S
La Victoire	<i>P. vulgaris</i>	A	Cul	S	S	I
Aiguille verte	<i>P. vulgaris</i>	A	Cul	S	S	R
JaloEEP558	<i>P. vulgaris</i>	A	Cul	S	S	R
Mdrk	<i>P. vulgaris</i>	A	Cul	S	S	R
Widusa	<i>P. vulgaris</i>	A	Cul	S	S	I
P12S	<i>P. vulgaris</i>	A	Cul	S	S	R
G19833	<i>P. vulgaris</i>	A	Cul	S	S	R
G23582	<i>P. vulgaris</i>	NA	Wild	S	S	R

Accession	Species	Origin ^a	Status ^b	Psp	Psp(arrB)	Psp(arrRpmI)
G23585	<i>P. vulgaris</i>	NA	Wild	S	S	S
G19889	<i>P. vulgaris</i>	SA	Wild	S	S	R
G19897	<i>P. vulgaris</i>	SA	Wild	S	S	R
G19898	<i>P. vulgaris</i>	SA	Wild	S	S	S
G19898A	<i>P. vulgaris</i>	SA	Wild	S	S	R
G23422	<i>P. vulgaris</i>	SA	Wild	S	S	R
G23442	<i>P. vulgaris</i>	SA	Wild	S	S	R
G23419	<i>P. vulgaris</i>	SA	Wild	S	S	R

^a A, Andean; SA, South-Andean; NA, North-Andean; M, Mesoamerican; C, Columbian.

^b Cul, cultivated.
S, Susceptible; R, Resistant; I, Intermediate.