Theor Appl Genet (2008) 116:407–415
DOI 10.1007/s00122-007-0678-y

ORIGINAL PAPER

Resistance to *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*: a case study for mapping two independent genes

Valérie Geffroy · Mireille Sévignac · Paul Billant · Michel Dron · Thierry Langin

Received: 19 February 2007 / Accepted: 13 November 2007 / Published online: 4 December 2007

Abstract Anthracnose, caused by the hemibiotrophic fungal pathogen *Colletotrichum lindemuthianum* is a devastating disease of common bean. Resistant cultivars are economical means for defense against this pathogen. In the present study, we mapped resistance specificities against 7 *C. lindemuthianum* strains of various geographical origins revealing differential reactions on BAT93 and JaloEEP558, two parents of a recombinant inbred lines (RILs) population, of Meso-american and Andean origin, respectively. Six strains revealed the segregation of two independent resistance genes. A specific numerical code calculating the LOD score in the case of two independent segregating genes (i.e. genes with duplicate effects) in a RILs population was developed in order to provide a recombination value (*r*) between each of the two resistance genes and the tested marker. We mapped two closely linked Andean resistance genes (*Co-x, Co-w*) at the end of linkage group (LG) B1 and mapped one Meso-american resistance genes (*Co-u*) at the end of LG B2. We also confirmed the complexity of the previously identified B4 resistance gene cluster, because four of the seven tested strains revealed a resistance specificity near *Co-y* from JaloEEP558 and two strains identified a resistance specificity near *Co-9* from BAT93. Resistance genes found within the same cluster confer resistance to different strains of a single pathogen such as the two anthracnose specificities *Co-x* and *Co-w* clustered at the end of LG B1. Clustering of resistance specificities to multiple pathogens such as fungi (*Co-u*) and viruses (*I*) was also observed at the end of LG B2.

Communicated by D. Mather.

V. Geffroy (✉) · M. Sévignac · M. Dron · T. Langin
Institut de Biotechnologie des Plantes (IBP), INRA,
Université Paris-Sud, UMR-CNRS 8618, bât. 630,
91405 Orsay Cedex, France
e-mail: valerie.geffroy@u-psud.fr

P. Billant
Ecole Polytechnique, LadHyX, UMR-CNRS 7646,
91128 Palaiseau Cedex, France

Introduction

Common bean (*Phaseolus vulgaris*) is an important source of protein in human diets in many parts of the world. Half the grain legumes consumed worldwide are common beans (Broughton et al. 2003). Unfortunately, bean diseases considerably reduce the yields of common beans. Anthracnose, caused by the specialized hemibiotrophic fungus *Colletotrichum lindemuthianum*, is one of the most important diseases of common beans throughout the world, especially in tropical bean-producing regions of Latin America and Eastern Africa where climatic conditions favour disease development (Pastor-Corrales and Tu 1989). The genetics of anthracnose resistance has been studied for a long time, since this host/pathogen interaction was the first report of race-cultivar specificity (Barrus 1911, 1915; McRostie 1919). Several dominant resistance genes which condition resistance against different races of the fungus have been described, suggesting the occurrence of a gene-for-gene-type resistance (Flor 1955). These specific resistance genes were renamed *Co-1* to *Co-10* (McRostie 1919; Mastenbroek 1960; Bannerot 1965; Bannerot et al. 1971; Fouilloux 1976, 1979; Pastor-Corrales et al. 1994; Young and Kelly 1996; Alzate-Marin et al. 1997; Young et al. 1998; Geffroy et al. 1999; Alzate Marin et al. 2003). Use of resistant genotypes appears to be a reliable control strategy

in developing countries where farmers cannot afford the purchase of pathogen-free seeds or fungicides.

At the molecular level, the recent addition of genomics to the more traditional experimental tools has strikingly increased our understanding of the molecular basis of gene-for-gene-type resistance (Michelmore 2003; Nobuta and Meyers 2005). It is now well recognized that the largest class of disease resistance (R) genes encode proteins presenting an amino-terminal nucleotide binding site plus a series of carboxy-terminal leucine-rich repeats (NBS-LRR) (Dangl and Jones 2001; Hammond Kosack and Parker 2003; McHale et al. 2006). Functional resistance genes belonging to this class have been identified in a large selection of plant species: monocots as well as dicots. They correspond to resistance genes effective against all types of pathogens and pests including fungi, bacteria, viruses, nematodes, oomycetes and insects (Dangl and Jones 2001, Hammond Kosack and Parker 2003; McHale et al. 2006). Consequently, the NBS-LRR sequences are now recognized as the "pillar" of plant disease resistance (Meyers et al. 2005; Chisholm et al. 2006).

Annotation of the *Arabidopsis thaliana* genomic sequence (Arabidopsis Genome Initiative 2000) has identified 149 NBS-LRR sequences (Meyers et al. 2003). The rice and *Populus trichocarpa* genomic sequences contain 297 and 480 NBS-LRR genes, respectively. These results confirm the abundance of this class of genes in any plant species genome (Zhou et al. 2004; Tuskan et al. 2006). However, only a small number of NBS-LRR sequences have been functionally characterized as resistance genes. For example, in *A. thaliana*, less than 15 NBS-LRR sequences are known to function as resistance genes (Hammond Kosack and Parker 2003; McHale et al. 2006). One of the main limitations to identify resistance genes is probably the lack of strains of a specific pathogen determining a disease resistance gene co-located with a "candidate" NBS-LRR sequence. This knowledge gap strongly reveals the necessity to increase the number of mapped disease resistance genes against specific strains of a pathogen. In common bean, an international consortium called "Phaseomics" has selected the Meso-american genotype BAT93 as a good candidate genotype to be sequenced (Broughton et al. 2003). Consequently, mapping disease resistance genes with this genotype as a parent is of great interest for future functional analyses.

In the present study, we used a strategy based on a hypothesis developed by Bennetzen and Hulbert (1992) stating that: "the number of resistance genes that can be detected is determined exclusively by the number of different isolates of the pathogen that have been identified and isolated". Based on this hypothesis, seven previously identified strains of *C. lindemuthianum* (Geffroy et al. 1999), revealing differential reaction on two representative genotypes of the Andean (JaloEEP558) and Mesoamerican (BAT93) gene pools, were inoculated on a recombinant inbred lines (RILs) population derived from an intercross of these two genotypes. We mapped three additional anthracnose disease resistance genes (*Co-x, Co-w*, *Co-u*) on the integrated linkage map of common bean (Freyre et al. 1998). We also confirmed the complexity of the B4 resistance gene cluster, because 4 and 2 of the 7 tested strains revealed resistance specificities in the vicinity of two previously identified resistance genes localized at the B4 resistance gene cluster, *Co-y* from JaloEEP558 and *Co-9* from BAT93, respectively. The *Co-x* resistance gene was easily mapped using traditional procedures, whereas other strains revealing digenic segregation required the implementation of a specific LOD score calculation in order to map the corresponding resistance genes (*Co-u, Co-w*).

Materials and methods

Plant and fungal material

An F9 recombinant inbred line (RIL) population, derived from the cross between Andean landrace JaloEEP558 and Mesoamerican breeding line BAT93 was used to map additional anthracnose disease resistance genes. These 77 RILs were used to set up an integrated linkage map of common bean (Freyre et al. 1998), and additional molecular markers, some of which correspond to genes of known function involved in resistance/defense have been subsequently added (Geffroy et al. 2000). BAT93 was developed at the Centro International de Agricultura Tropical (CIAT, Cali, Colombia) and was derived from a cross involving four Middle American genotypes (Veranic 2, PI207262, Jamapa, Great Northern Tara). JaloEEP558 is a selection from the Andean landrace Jalo obtained at the Estacao Experimental de Pato de Minas (Minas Gerais, Brazil). "La Victoire" is a French multi-susceptible common bean cultivar of Andean origin developed by the seed company "Tezier" (Valence-sur-Rhone, France).

A world collection of 177 *C. lindemuthianum* strains, from the Orsay (France) fungal library, was inoculated to the two parents BAT93 and JaloEEP558 (Geffroy et al. 1999). All these strains were known to attack the multi-susceptible cultivar "La Victoire". Eight strains, from those exhibiting avirulence towards JaloEEP558 (and virulence on BAT93) or exhibiting avirulence on BAT93 (and virulence on JaloEEP558) had already been inoculated onto the RIL population, and the corresponding resistance genes were mapped (Geffroy et al. 1999). In the present study, we present results of inoculation of seven additional *C. lindemuthianum* strains, giving differential reactions on BAT93 and JaloEEP558. The strains were isolated from

cultivated beans (the origin of the strain is indicated in parentheses): 100 (Costa Rica), E25 (Tanzania), 40 (Rwanda), 3616 (Rwanda), 82 (Costa Rica), E4 (Tanzania) and E42b (Tanzania).

Pathogenicity tests and disease scoring

Five seeds of each RIL were planted in individual pots filled with moist vermiculite. The parental lines BAT93 and JaloEEP558 and the highly susceptible cultivar « La Victoire » were included as controls. For each strain tested, three replicates of this experiment were carried, i.e. the resistance/susceptible phenotype was assessed on at least 15 seedlings for each RIL's genotype. Seedlings were grown for 8 days at 23°C, 75% relative humidity, with 8 h dark and 16 h light photoperiods under fluorescent tubes (166μE). The seedlings, at the two leaves stage, were infected by spray-inoculation 8 days after germination and incubated at 19°C under the light conditions described above with 90% relative humidity in a control growth chamber. The inoculum was prepared by harvesting conidia by flooding *C. lindemuthianum* plates with distilled sterile water. The spore concentration was measured using a haematocytometer and adjusted to 10^6 spores/ml.

Symptoms were scored 7 days after inoculation. Plants without visible symptoms or showing very limited necrotic lesions were scored as resistant. Plants with large, sporulating lesions and dead plants were scored as susceptible. That is, plant reactions were scored qualitatively. For resistance gene nomenclature (Kelly and Young 1996), putative new specificities were labelled *Co-* (*Colletotrichum*) plus a letter (for example *Co-x, Co-w, Co-u*). The mode of action of the identified R genes is not provided in the present publication.

Genetic mapping of the anthracnose resistance genes

Chi-squared (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios.

For the strains revealing a single resistance gene (1:1 segregation among RILs), the MAPMAKER software version 3.0 (Lander et al. 1987) was used to map the segregating resistance gene on the integrated linkage map of common bean. More precisely, we used the set of 142 makers in Geffroy et al. (2000). Linkage groups were established with an LOD threshold of 3.0 and a maximum recombination fraction of 0.3. Marker order was estimated with an LOD threshold of 2.0 based on multipoint "Compare", "Order" and "Ripple" analyses. Map distances were estimated by the Kosambi function (Kosambi 1944).

Table 1 Frequencies of genotypic classes in a recombinant inbred lines (RIL) population (with an infinite number of generations) when two loci are separated by recombination rate per meiosis *r*

	G1G1	g1g1
MM	$c_\infty = \frac{1}{2(1+2r)}$	$e_\infty = \frac{r}{1+2r}$
mm	$e_\infty = \frac{r}{1+2r}$	$c_\infty = \frac{1}{2(1+2r)}$

The M locus corresponds to a marker locus, the second locus corresponds to resistance gene (G1 = resistant, g1 = susceptible). Genotype of parent A is MM-G1G1, whereas genotype of parent B is mm-g1g1. Individuals lines with genotype MM-G1G1 or mm-g1g1 are "parental" types and individuals with genotype mm-G1G1 or MM-g1g1 are recombinants

For the strain revealing two independent resistance genes (3:1 segregation in RILs population), because the MAPMAKER software is not able to handle this case, a specific numerical code calculating the LOD score (Allard 1956) was written in FORTRAN. We developed a maximum likelihood procedure to evaluate the linkage relationships between each two independent genes (denoted G1 and G2) controlling a single phenotype and other segregating molecular markers (denoted *M*) in a single recombinant inbred population. Classically, the recombination rate per meiosis is denoted *r*, while the proportion of recombinant zygotes in RILs is denoted *R*. The relation between *r* and *R* for two loci *M* (marker) and G1 (resistance gene) in a RIL population was established in Haldane and Waddington (1931):

$$R = \frac{2r}{1+2r}$$

The frequencies of the different genotypic classes are given in Table 1. The formulas of Haldane and Waddington were the basis for linkage analysis in RILs, especially in the Mapmaker software (Lander et al. 1987), where RIL data are handled as if they were backcross data, i.e. produced by a single meiosis, except that *r* is replaced by *R* to account for multiple generation effects in RILs (Martin and Hospital 2006). When a 2nd resistance gene (referred to as G2), segregating independently of G1, is also present, the frequencies of the different genotypic classes are as given in Table 2. At the phenotypic level, it was impossible to know the genotype of a resistant RIL. Therefore, the frequencies of the different phenotypic classes were obtained by summing the frequencies of the genotypic classes giving a resistant phenotype (Table 3).

If the marker *M* and one of two independently segregating resistance genes, G1 or G2, are linked with recombination rate *r*, the probability of linkage is given by:

$$P(r) = \frac{(N)!}{a!b!c!d!} x^a y^b z^c t^d$$

where *a*, *b*, *c*, *d* are the observed numbers in each phenotypic class (Table 4), $N = a + b + c + d$, and *x*, *y*, *z*, *t* are

Table 2 Frequencies of genotypic classes for the case when two independent resistance genes (G1 and G2) are present in parent A, and the recombination frequency between G1 and marker locus M is *r*

	G1G1–G2G2	G1G1–g2g2	g1g1–G2G2	g1g1–g2g2
MM	$\frac{c_\infty}{2}$[a]	$\frac{c_\infty}{2}$	$\frac{e_\infty}{2}$	$\frac{e_\infty}{2}$
mm	$\frac{e_\infty}{2}$	$\frac{e_\infty}{2}$	$\frac{c_\infty}{2}$	$\frac{c_\infty}{2}$

Genotype of parent A is MM-G1G1-G2G2, genotype of parent B is mm-g1g1-g2g2. c_∞ and e_∞ are defined in Table 1

[a] The two resistance loci are assumed to be genetically independent, therefore, within G1G1 genotypes there is an equal chance of being G2G2 or g2g2

Table 3 Frequencies of phenotypic classes, when two independent resistance genes are present in parent A and when there is a recombination rate *r* between G1 and marker *M*

	Resistant	Susceptible
MM	$c_\infty + \frac{e_\infty}{2} = \frac{1+r}{2(1+2r)} = x$	$\frac{e_\infty}{2} = \frac{r}{2(1+2r)} = y$
mm	$e_\infty + \frac{c_\infty}{2} = \frac{4r+1}{4(1+2r)} = z$	$\frac{c_\infty}{2} = \frac{1}{4(1+2r)} = t$

Frequencies were obtained after summing the frequencies of genotypic classes with the same phenotype

Resistant phenotype; according to the Flor (1955) model, a resistant RIL at the phenotypic level can be G1G1–G2G2, G1G1–g2g2 or g1g1–G2G2 at the genotypic level

the expected frequencies of the different phenotypic classes under the hypothesis of linkage between the marker *M* and one of the two independently segregating resistance genes in the population (Table 3).

The LOD is defined by:

$$\begin{aligned}\mathrm{LOD} &\equiv \mathrm{Log}_{10}\frac{P(r)}{P\left(r=\frac{1}{2}\right)} \\ &= a.\log\left(\frac{4}{3}(1+r)\right) + b.\log(4r) \\ &\quad + c.\log\left(\frac{2}{3}(4r+1)\right) + d.\log 2 - N.\log(1+2r)\end{aligned} \tag{1}$$

where $P\left(r=\frac{1}{2}\right)$ is the probability of independence. The value of *r* maximizing the probability to have the observed numbers (*a, b, c, d*) is given by:

$$\frac{\mathrm{dLOD}}{\mathrm{d}r} = \frac{a}{1+r} + \frac{b}{r} + \frac{4c}{4r+1} - \frac{2N}{1+2r} = 0 \tag{2}$$

This is equivalent to finding the real roots in the interval [0; 0.5], of the following third-order polynomial:

$$\begin{aligned}&-8r^3d + r^2(6a+14b+12c-10N) \\ &\quad + r(a+7b+4c-2N) + b = 0.\end{aligned}$$

The recombination rate *r* is obtained numerically by a Newton Raphson method and the associated LOD value is calculated with formula (1). To declare a significant linkage between a marker and a resistance gene, we retained a minimal LOD value of 2.5. The case of two complementary resistance genes is similar and explained in Appendix.

Table 4 Abbreviations used for the maximum likelihood expression

	Resistant	Susceptible
MM	*a*	*b*
mm	*c*	*d*

The M locus corresponds to a marker locus. M: allele of the "resistant parent"; m: allele of the "susceptible parent". *a*, *b*, *c*, *d*: observed numbers for each phenotypic class. $N = a + b + c + d$

Results

Anthracnose resistance genes in BAT93 and JaloEEP558

Resistance specificities detected in the Andean parent JaloEEP558 will be referred to as Andean specificities. In the same way, resistance specificities identified in the Mesoamerican parent BAT93 will be named Mesoamerican specificities. For strain "100" the distribution of the RILs, conformed to a ratio of one homozygous parental type resistant to one homozygous parental type susceptible (Table 5), indicating that resistance of JaloEEP558 against this strain was controlled by a single gene, temporarily designated *Co-x*. *Co-x* was mapped straightforwardly using Mapmaker 3.0 on the bean genetic map at a distal position on linkage group B1, 12.4 cM from marker PROE8b (Fig. 1). For strains E25, 40, 3616, 82 (JaloEEP558 resistant) or strains E4, E42b (BAT93 resistant) the distribution of RILs, conformed to a ratio of three homozygous parental type resistant to one homozygous parental type susceptible (Table 5), indicating that resistance was controlled by two independent genes. Segregations towards strains 40, 3616 and 82 (resistance present in JaloEEP558) were identical. A few recombinations were detected between the resistance to strain E25 and the resistance to the latter three strains. Similarly, resistances to strains E4 and E42b showed perfect co-segregation.

LOD score calculation to map two independent resistance genes (i.e. genes with duplicate effects)

Mapmaker 3.0 is not adequate to map two independent resistance genes (digenic segregation). This situation corresponds to genes with duplicate effects. A specific numerical code calculating the LOD score, was developed as described in Materials and methods section.

Table 5 Name and origin of the *Colletotrichum lindemuthianum* strains inoculated on the RILs population

Strains	Origin of the strain	BAT93	JaloEEP558	1R:1S[a]		3R:1S	
				χ^2_{calc}	*P*	χ^2_{calc}	*P*
100	Costa Rica	S	R	0.06	0.80	11.36	0.0007
E25	Tanzania	S	R	19	0.0000	0.00	1.00
40	Rwanda	S	R	26.30	0.0000	0.73	0.39
3616	Rwanda	S	R	26.30	0.0000	0.73	0.39
82	Costa Rica	S	R	26.30	0.0000	0.73	0.39
E4	Tanzania	R	S	12.16	0.0005	0.88	0.34
E42b	Tanzania	R	S	12.16	0.0005	0.88	0.34

R resistant; *S* susceptible

[a] Goodness of fit to expected 1R:1S or 3R:1S ratios

Table 6 Linkage between markers and resistance genes detected by the LOD score calculation adapted for two independent resistance loci segregating in a recombinant inbred line population

Strain	Origin of the strain	BAT93	Jalo EEP558	Significant markers[a]	Linkage group	LOD score calculation		Assigned resistance gene name
						LOD	*R*	
E25	Tanzania	S	R	*Co-x*	B1	2.88	0.09	*Co-w*
				Co-y	B4	9.22	0.00	*Co-y*
40	Rwanda	S	R	*Co-x*	B1	5.99	0.00	*Co-x*
				Co-y	B4	7.62	0.00	*Co-y*
3616	Rwanda	S	R	*Co-x*	B1	5.99	0.00	*Co-x*
				Co-y	B4	7.62	0.00	*Co-y*
82	Costa Rica	S	R	*Co-x*	B1	5.99	0.00	*Co-x*
				Co-y	B4	7.62	0.00	*Co-y*
E4	Tanzania	R	S	*I*	B2	4.05	0.05	*Co-u*
				Co-9	B4	7.09	0.00	*Co-9*
E42b	Tanzania	R	S	*I*	B2	4.05	0.05	*Co-u*
				Co-9	B4	7.09	0.00	*Co-9*

[a] When several closely linked markers were significant, only the one presenting the highest LOD value is presented

For each of the six strains revealing a digenic segregation, each of the 142 markers of the bean map used in Geffroy et al. (2000) with addition to *Co-x* identified in the present study, was tested for linkage with one of the two segregating resistance genes. For the strains E4 and E42b, two significant regions of the common bean genome were detected (Table 6; Fig. 1): one resistance gene was mapped in the vicinity of the *I* gene, which confers resistance against various viruses (Vallejos et al. 2006), at a distal position on linkage group B2 ($r = 0.05$), and a second resistance gene was mapped to the *Co-9* locus on linkage group B4 ($r = 0.00$). This locus has been previously identified as a locus that controls resistance to *C. lindemuthianum* (Geffroy et al. 1999). For the latter, we assumed that it was *Co-9*, because it is impossible to determine if these two strains are recognized by *Co-9* itself or by resistance gene(s) closely linked to *Co-9*. The Meso-american resistance gene located in the vicinity of the *I* gene is referred to as *Co-u* (Table 6; Fig. 1).

For strains E25, 40, 3616 and 82, two significant regions of the common bean genome were detected: a first resistance gene was mapped in the vicinity of the *Co-x* anthracnose resistance gene at the end of linkage group B1, and a second gene was co-located with the *Co-y* anthracnose resistance gene on linkage group B4 ($r = 0.00$) (Geffroy et al. 1999). For strains, 40, 3616 and 82 (Table 6) one of the resistance genes co-located with the *Co-x* resistance gene ($r = 0.00$), whereas for strain E25 an *r* value of 0.09 separated one of the resistance genes and *Co-x*. For strain E25, three RILs were confirmed as susceptible to strain E25, but resistant to strain 100, which is recognized by the resistance gene *Co-x*. This result enabled us to infer that strain E25 is not recognized by the Andean resistance gene *Co-x*, but by a closely linked resistance gene. This gene was designated *Co-w*. We considered that the second resistance gene is *Co-y* (Table 6, Fig. 1) for the four strains E25, 40, 3616 and 82, because it is impossible to determine, due to the small size of the tested population, if they are recognized by *Co-y*, or by resistance gene(s) closely linked to *Co-y*.

Discussion

In the present study, three anthracnose resistance specificities were mapped on the integrated linkage map of common bean. *Co-u* from the Meso-american genotype

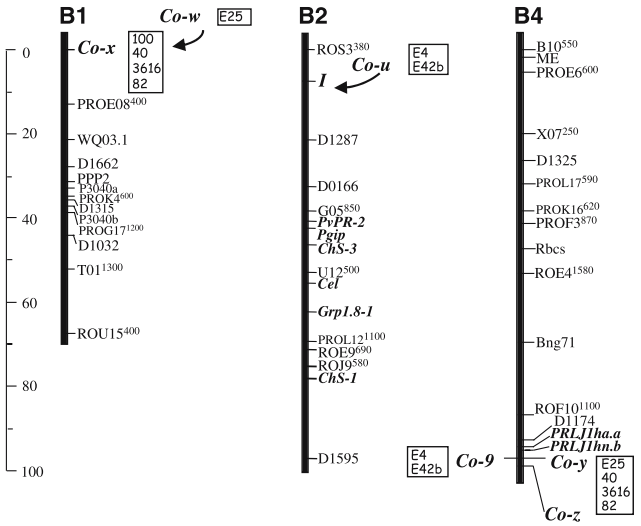

Fig. 1 Distribution of genes for resistance to anthracnose (*Co-*) on the BAT93 × JaloEEP558 recombinant inbred lines (RILs) linkage map, based on markers used in Geffroy et al. (2000). *Numbers in rectangles* adjacent to a *Co-* resistance gene represent strains, recognized by the considered resistance gene in the present study. B1, B2 and B4 are linkage groups relevant to this study. *Designations to the right* represent names of markers. A scale in centimorgans is shown on *left*. *Co-x* was mapped with Mapmaker 3.0 while *Co-w*, *Co-u* were mapped using an LOD score calculation for two independent genes as described in the present study

BAT93 mapped in the vicinity of the *I* resistance gene at the end of linkage group B2. *Co-x* and *Co-w* are two closely linked genes from the Andean JaloEEP558 genotype that are mapped at the end of linkage group B1. One of them (*Co-x*) segregating in a 1:1 ratio in the RIL population, was easily mapped using Mapmaker software (Lander et al. 1987). Strains E4 and E42b, and E25, 40, 3616 and 82 revealed segregation of two independent resistance genes. The development of an LOD score calculation for two independent segregating genes in a RIL population was made to map the latter genes. The problem of mapping two segregating genes on a genetic map was addressed by Caranta et al. (1996) by using χ^2 statistics to map corresponding resistance genes on the pepper (*Capsicum annuum*) map. Our method based on an LOD score calculation was the same as that used by Mapmaker (Lander et al. 1987) and has the advantage of providing the recombination value (r) between the resistance gene and the tested marker and the associated LOD score measuring its likelihood. Although, we developed this calculation for the case of two independent genes in a RIL population, it can be easily adapted to the case of two complementary genes (Appendix). Digenic segregations are often observed in disease resistance gene studies indicating that two avirulence gene products produced by a specific strain are recognized by two disease resistance genes. For fungal pathogens such as *C. lindemuthianum*, with no unequivocal sexual stage yet identified, it is impossible to obtain a strain with a single Avr gene by crossing.

Combination of Andean and Meso-american resistance genes was proposed as a way of achieving durable resistance against anthracnose in common bean. Indeed, adaptation of the *C. lindemuthianum* strains to plants of the same origin was observed in wild populations of common bean growing in the centre of diversity of *Phaseolus vulgaris* as well as with domesticated beans (Geffroy et al. 1999). The Andean *Co-x* resistance gene appears to be of particular interest for this strategy because it confers resistance to strain "100" which is virulent to G 2333, a germplasm accession of Mexican origin and resistant to a broad range of *C. lindemuthianum* strains (Pastor-Corrales et al. 1994). Therefore, combining the anthracnose Andean resistance gene *Co-x* from JaloEEP558 with the anthracnose resistance genes from G2333 of Meso-american origin has great potential. Combining resistance genes can be facilitated by the use of molecular markers linked to the relevant genes. Placing resistance genes on a map provides molecular markers that might be used directly in marker-assisted selection (MAS). In common bean, correlations have been established between several published maps (Vallejos et al. 1992; Adam-Blondon et al. 1994; Freyre et al. 1998; Blair et al. 2003), providing additional candidate markers for a target region. For example, in the case of *Co-x*, mapped at the end of linkage group B1, corresponding to linkage group H of the map from Vallejos et al. (1992), restriction fragment length polymorphism (RFLP) markers Bng173, Bng171, Bng122, are of particular interest. Some of these RFLP markers have been converted into PCR-based markers (Murray et al. 2002), facilitating their use in MAS strategies. Interestingly, these RFLP markers were used in soybean to increase the map density in syntenic regions of the soybean genome known to carry a soybean cyst nematode resistance (*rhg1*) (Concibido et al. 1996; Foster Hartnett et al. 2002). The syntenic region of *M. truncatula* has been sequenced but it does not seem to carry a *rhg1* homolog (Mudge et al. 2005). Synteny for disease resistance genes is an open issue in plant genome. Indeed, synteny for resistance genes (macro and/or micro) has been observed between several species especially in the Solanaceae family (Lefebvre 2004), while in cereal genome rapid re-organization of R-like sequences has been reported (Leister et al. 1998).

Allelism tests involving JaloEEP558 and the Andean genotype MDRK suggested that JaloEEP558 and MDRK possess the same allele at the *Co-1* locus (Kelly and Vallejo 2004). So far, the location of *Co-1* resistance at the end of linkage group B1 has only been tentative, as mapping involved molecular markers linked to *Co-1* (Kelly and Vallejo 2004). Consequently, assuming that the *C. lindemuthianum* strain mentioned in Kelly and Vallejo (2004)

Table 7 Frequencies of phenotypic classes, when two complementary resistance genes are present in parent A and that there is a recombination rate r between G1 and marker M

	Resistant	Susceptible
MM	$\frac{c_\infty}{2} = t$	$e_\infty + \frac{c_\infty}{2} = z$
mm	$\frac{e_\infty}{2} = y$	$c_\infty + \frac{e_\infty}{2} = x$

Resistant phenotype

Susceptible phenotype

and strain "100" used in the present study are recognized by the same resistance specificity from JaloEEP558, our results give the first precise genetic map position of *Co-1* locus (through the mapping of *Co-x*). Our results also provide evidence that both alleles (Melotto and Kelly 2000) and closely linked resistance genes occur at or near the *Co-1* locus, as we identified two different resistance specificities (*Co-w, Co-x*) with different *C. lindemuthianum* strains in JaloEEP558. The question of allelism versus linkage is difficult to solve with small segregating populations, due to the extreme difficulty of proving that any two putative "alleles" of a cluster of disease resistance genes are truly allelic (Bennetzen and Hulbert 1992). In common bean, sequencing of the B4 resistance gene cluster, located on linkage group B4, revealed that 11 NBS-LRR sequences were located within less than 150 kb (V. Geffroy, unpublished results). Therefore, the probability of identifying at least one recombinant in small segregating population is extremely low.

In the present study, an anthracnose resistance gene *Co-u* was mapped in the vicinity of the *I* locus which controls the development of four different phenotypes in response to inoculation with *Bean common mosaic virus*, 10 other related potyviruses, and 1 comovirus (Vallejos et al. 2006). Therefore, in the same region of the bean genome, resistance genes conditioning resistance against viral and fungal pathogens are clustered. Comparative mapping studies within several plant genera have shown that genes for resistance are often co-localized on maps (Hulbert et al. 2001; Ashfield et al. 2003; Hayes et al. 2004; Marczewski et al. 2006). The two resistance genes (*I* and *Co-u)* might have a common origin at the molecular level; it is now well documented that most resistance genes in plants encode NBS-LRR proteins that are involved in the recognition of diverse pathogens and pests, and that these NBS-LRR sequences are often clustered (Meyers et al. 2003; McHale et al. 2006). Recently, Vallejos et al. (2006) identified a complex family of TIR-NBS-LRR genes at the *I* locus; one of these sequences might condition resistance against a virus and another one against *C. lindemuthianum*.

Our results confirm the complexity of the B4 resistance gene cluster because of seven randomly chosen strains of *C. lindemuthianum* from various geographical origins, two identified a resistance specificity in the vicinity of the Meso-american *Co-9* gene and four identified an resistance specificity in the vicinity of the *Co-y* gene. The corresponding resistance genes referred to as *Co-9* and *Co-y*, should be considered as a minimum number of functional resistance genes identified by the numerous strains. We previously identified 3 and 4 strains revealing the existence of a resistance specificity in the vicinity of *Co-9* and *Co-y*, respectively (Geffroy et al. 1999). At the molecular level, we have confirmed the complexity of this cluster (Ferrier-Cana et al. 2003; Ferrier Cana et al. 2005), and we have now sequenced nearly 20 complete NBS-LRR sequences in the area of *Co-9* and *Co-y* (V. Geffroy; unpublished results), again confirming that the B4 clusters in both BAT93 and JaloEEP558 are complex cluster and composed of tightly-linked specific resistance genes.

Acknowledgment We thank Guy Fouilloux for helpful discussions. The research was supported by INRA-DGAP, CNRS and Ministère de la recherche.

Appendix

In the case of two complementary resistance genes (case of complementary epistasis), the same procedure as described previously for two independent genes can be used. The frequency of the different genotypic classes are given in Table 7, which is identical as in Table 4 except that the lines *MM*/*mm* and the columns Resistant/Susceptible are inverted, i.e. (x, y, z, t) change to (t, z, y, x). The probability of linkage is:

$$P(r) = \frac{(a+b+c+d)!}{a!b!c!d!} t^a z^b y^c x^d$$

The value of r maximizing the LOD score is given by substituting (d, c, b, a) for (a, b, c, d) in Eq. (2)

References

Adam-Blondon AF, Sevignac M, Dron M (1994) A genetic map of common bean to localize specific resistance genes against anthracnose. Genome 37:915–924

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Alzate-Marin AL, Baia GS, de Paula Junior TJ, de Carvalho GA, de Baros EG, Moreira MA (1997) Inheritance of anthracnose resistance in common bean differential cultivar AB 136. Plant Disease 81:996–998

Alzate Marin AL, Costa MR, Arruda KM, de Barros EG, Moreira MA (2003) Characterization of the anthracnose resistance gene present in Ouro Negro (Honduras 35) common bean cultivar. Euphytica 133:165–169

Ashfield T, Bocian A, Held D, Henk AD, Marek LF, Danesh D, Penuela S, Meksem K, Lightfoot DA, Young ND, Shoemaker RC, Innes RW (2003) Genetic and physical localization of the

soybean Rpg1-b disease resistance gene reveals a complex locus containing several tightly linked families of NBS-LRR genes. Mol Plant Microbe Interact 16:817–826

Bannerot H (1965) Résultat de l'infection d'une collection de haricots par six races physiologiques d'anthracnose. Ann Amélior Plantes 15:201–222

Bannerot H, Derieux M, Fouilloux G (1971) Mise en évidence d'un second gène de résistance totale à l'anthracnose chez le haricot. Ann Amélior Plantes 21:83–85

Barrus MF (1911) Variation of varieties of beans in their susceptibility to anthracnose. Phytopathology 1:190–195

Barrus MF (1915) An anthracnose-resistant red kidney bean. Phytopathology 5:303–311

Bennetzen JL, Hulbert SH (1992) Extramarital sex amongst the beets—organization, instability and evolution of plant-disease resistance genes. Plant Mol Biol 20:575–578

Blair MW, Pedraza F, Buendia HF, Gaitan Solis E, Beebe SE, Gepts P, Tohme J (2003) Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 107:1362–1374

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.)—model food legumes. Plant Soil 252:55–128

Caranta C, Palloix A, Gebre-Selassie K, Lefebvre V, Moury B, Daubèze AM (1996) A complementation of two genes originating from susceptible *Capsicum annum* lines confers a new and complete resistance to pepper veinal mottle virus. Phytopatholoy 86:739–743

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host–microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Concibido VC, Young ND, Lange DA, Denny RL, Danesh D, Orf JH (1996) Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor Appl Genet 93:234–241

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Ferrier-Cana E, Geffroy V, Macadre C, Creusot F, Imbert-Bollore P, Sevignac M, Langin T (2003) Characterization of expressed NBS-LRR resistance gene candidates from common bean. Theor Appl Genet 106:251–261

Ferrier Cana E, Macadre C, Sevignac M, David P, Langin T, Geffroy V (2005) Distinct post-transcriptional modifications result into seven alternative transcripts of the CC-NBS-LRR gene JA1tr of *Phaseolus vulgaris*. Theor Appl Genet 110:895–905

Flor HH (1955) Host–parasite interaction in flax rust. Its genetics and other implications. Phytopathology 45:680–685

Foster Hartnett D, Mudge J, Larsen D, Danesh D, Yan HH, Denny R, Penuela S, Young ND (2002) Comparative genomic analysis of sequences sampled from a small region on soybean (*Glycine max*) molecular linkage group G. Genome 45:634–645

Fouilloux G (1976) L'anthracnose du haricot (*Colletotrichum lindemuthianum*, Sacc et Magn.): nouvelles sources de résistance et nouvelles races physiologiques. Ann Amélior Plantes 26:443–453

Fouilloux G (1979) New races of bean anthracnose and consequences on our breeding programs. In: Maraitre H, Meyer JA (eds) Disease of tropical food crops. Université Catholique de Louvain la Neuve, Belgium, pp 221–235

Freyre R, Skroch PW, Geffroy V, AdamBlondon AF, Shirmohamadali A, Johnson WC, Llaca V, Nodari RO, Pereira PA, Tsai SM, Tohme J, Dron M, Nienhuis J, Vallejos CE, Gepts P (1998) Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor Appl Genet 97:847–856

Geffroy V, Sicard D, de Oliveira JCF, Sevignac M, Cohen S, Gepts P, Neema C, Langin T, Dron M (1999) Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. Mol Plant Microbe Interact 12:774–784

Geffroy V, Sevignac M, DeOliveira JCF, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M (2000) Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. Mol Plant Microbe Interact 13:287–296

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. Genetics 16:357–374

Hammond Kosack KE, Parker JE (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. Curr Opin Biotech 14:177–193

Hayes AJ, Jeong SC, Gore MA, Yu YG, Buss GR, Tolin SA, Maroof MAS (2004) Recombination within a nucleotide-binding-site/leucine-rich-repeat gene cluster produces new variants conditioning resistance to soybean mosaic virus in soybeans. Genetics 166:493–503

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol 39:285–312

Kelly JD, Vallejo VA (2004) A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. HortScience 39:1196–1207

Kelly JD, Young RA (1996) Proposed symbols for anthracnose resistance genes. Annu Rep Bean Improve Coop 39:20–24

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lefebvre V (2004) Molecular markers for genetics and breeding: development and use in pepper (*Capsicum* spp.). In: Lörz H, Wenze G (eds) Biotechnology in agriculture and forestry molecular marker systems. Springer, Heidelberg, pp 189–214

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, SchulzeLefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA 95:370–375

Marczewski W, Strzelczyk-Zyta D, Hennig J, Witek K, Gebhardt C (2006) Potato chromosomes IX and XI carry genes for resistance to potato virus M. Theor Appl Genet 112:1232–1238

Martin OC, Hospital F (2006) Two- and three-locus tests for linkage analysis using recombinant inbred lines. Genetics 173:451–459

Mastenbroek C (1960) A breeding programs for resistance to anthracnose in dry shell haricot beans, based on a new gene. Euphytica 9:177–184

McHale L, Tan XP, Koehl P, Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. Genome Biol 7:212

McRostie GP (1919) Inheritance of anthracnose resistance as indicated by a cross between a resistant and a susceptible bean. Phytopathology 9:141–148

Melotto M, Kelly JD (2000) An allelic series at the *Co-1* locus conditioning resistance to anthracnose in common bean of Andean origin. Euphytica 116:143–149

Meyers BC, Kozik A, Griego A, Kuang HH, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell 15:809–834

Meyers BC, Kaushik S, Nandety RS (2005) Evolving disease resistance genes. Curr Opin Plant Biol 8:129–134

Michelmore RW (2003) The impact zone: genomics and breeding for durable disease resistance. Curr Opin Plant Biol 6:397–404

Mudge J, Cannon SB, Kalo P, Oldroyd GE, Roe BA, Town CD, Young ND (2005) Highly syntenic regions in the genomes of soybean, *Medicago truncatula*, and *Arabidopsis thaliana*. BMC Plant Biol 5:15

Murray J, Larsen J, Michaels TE, Schaafsma A, Vallejos CE, Pauls KP (2002) Identification of putative genes in bean (*Phaseolus vulgaris*) genomic (Bng) RFLP clones and their conversion to STSs. Genome 45:1013–1024

Nobuta K, Meyers BC (2005) *Pseudomonas* versus *Arabidopsis*: models for genomic research into plant disease resistance. Bioscience 55:679–686

Pastor-Corrales MA, Tu JC (1989) Anthracnose. In: Schwartz HF, Pastor-Corrales MA (eds) Bean production problems in the tropics, 2nd edn. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, pp 77–104

Pastor-Corrales MA, Erazo OA, Estrada EI, Singh SP (1994) Inheritance of anthracnose resistance in common bean accession G2333. Plant Dis 78:959–962

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Dejardin A, Depamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam Faridi N, Jones S, Jones Rhoades M, Jorgensen R, Joshi C, Kangasjarvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens Mack J, Leple JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouze P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, de Peer YV, Rokhsar D (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. and Gray). Science 313:1596–1604

Vallejos CE, Sakiyama NS, Chase CD (1992) A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131:733–740

Vallejos CE, Astua Monge G, Jones V, Plyler TR, Sakiyama NS, Mackenzie SA (2006) Genetic and molecular characterization of the *I* locus of *Phaseolus vulgaris*. Genetics 172:1229–1242

Young RA, Kelly JD (1996) Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. Plant Dis 80:650–654

Young RA, Melotto M, Nodari RO, Kelly JD (1998) Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, "G2333". Theor Appl Genet 96:87–94

Zhou T, Wang Y, Chen JQ, Araki H, Jing Z, Jiang K, Shen J, Tian D (2004) Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol Gen Genomics 271:402–415