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ORIGINAL PAPER

# A genetic linkage map of *Phaseolus vulgaris* L. and localization of genes for specific resistance to six races of anthracnose (*Colletotrichum lindemuthianum*)

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**Abstract** A genetic map of common bean was constructed using 197 markers including 152 RAPDs, 32 RFLPs, 12 SCARs, and 1 morphological marker. The map was established by using a $F_2$ population of 85 individuals from the cross between a line derived from the Spanish landrace Andecha (Andean origin) and the Mesoamerican genotype A252. The resulting map covers about 1,401.9 cM, with an average marker distance of 7.1 cM and includes molecular markers linked to disease resistance genes for anthracnose, bean common mosaic virus, bean golden yellow mosaic virus, common bacterial blight, and rust. Resistance to races 6, 31, 38, 39, 65, and 357 of the pathogenic fungus *Colletotrichum lindemuthianum* (anthracnose) was evaluated in $F_3$ families derived from the corresponding $F_2$ individuals. The intermediate resistance to race 65 proceeding from Andecha can be explained by a single dominant gene located on linkage group B1, corresponding to the *Co-1* gene. The recombination between the resistance specificities proceeding from A252 agrees with the assumption that total resistance to races 6, 31, 38, 39, 65, and 357, is organized in two clusters. One cluster, located on B4 linkage group, includes individual genes for specific resistance to races 6, 38, 39, and 357. The second cluster is located on linkage group B11 and includes individual genes for specific resistance to races 6, 31, 38, 39, and 65. These two clusters correspond to genes *Co-3/Co-9* and *Co-2*, respectively. It is concluded that most anthracnose resistance *Co-* genes, previously described as single major genes conferring resistance to several races, could be organized as clusters of different genes conferring race-specific resistance.

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## Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption worldwide (Schoonhoven and Voysest 1991). It is a diploid legume ($2n = 22$) with a relatively small genome (0.65 pg per haploid genome; Aramuganathan and Earle 1991). Several genetic maps have been developed in common bean (Vallejos et al. 1992; Nodari et al. 1993; Adam-Blondon et al. 1994) including different economic traits of interest, such as domestication syndrome, crop quality, tolerance to abiotic stresses, and disease resistances. An integrated linkage map was established in order to align these previous maps (Freyre et al. 1998), new microsatellite markers have been described and located on the integrated linkage map (Blair et al. 2003), and the correspondence between linkage groups (B1–B11) and chromosomes has been established (Pedrosa et al. 2003).

Anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum*, is the most serious disease of common bean worldwide due to its seed-borne

nature and pathogenic variability (Pastor-Corrales and Tu 1994). Currently, races of *C. lindemuthianum* are classified by inoculating isolates onto a universal set of 12 differential cultivars and named based on a binary nomenclature system (Pastor-Corrales 1991). Genetic resistance is regarded as the most effective disease management strategy for the control of anthracnose. Several genes conferring dominant resistance to different sets of pathogenic races have been described and located to the integrated linkage map, either by direct mapping or indirectly, using linked molecular markers: *Co-1* on linkage group B1 (Barrus 1915; Méndez de Vigo 2001), *Co-2* on B11 (Mastenbroek 1960; Adam-Blondon et al. 1994), *Co-3* on B4 (Bannerot 1965; Méndez-Vigo et al. 2005), *Co-4* on B8 (Fouilloux 1976; Melotto et al. 2004; Méndez de Vigo 2001), *Co-5* on B7 (Fouilloux 1976; Campa et al. 2005), *Co-6* on B7 (Young and Kelly 1996a; Kelly et al. 2003), *Co-9* on B4 (Geffroy et al. 1999), and *Co-10* on B4 (Alzate-Marin et al. 2003). All these resistance loci segregate independently from one another except *Co-3* and *Co-9*, which are probably allelic (Méndez-Vigo et al. 2005).

Clusters of resistance genes to different pathogens or to different races of the same pathogen have been widely described in many plant species (Michelmore and Meyers 1998). In *P. vulgaris*, it has been reported the existence of genomic regions that concentrate resistance to several pathogens (Kelly et al. 2003). Recent studies have provided information on the genomic organization of regions harbouring resistance to anthracnose in common bean. These reports revealed the existence of families of resistance gene analogue sequences (RGAs) and/or resistance gene candidates arranged in clusters in the vicinity of *Co-2* (Geffroy et al. 1998; Creusot et al. 1999), *Co-4* (Melotto and Kelly 2001; Melotto et al. 2004), and *Co-3/Co-9* (Geffroy et al. 1999; Ferrier-Cana et al. 2003, 2005).

The main objectives of this work were the construction of a linkage map using the $F_2$ population derived from the cross between the two bean lines, Andecha and A252, and the characterization and mapping of the genes for specific resistance against six races of anthracnose present in these two lines. We discuss the possibility that clusters of different race-specific genes form most of the previously described anthracnose resistance loci.

## Materials and methods

### Plant material

Andecha is a very valuable large white seeded cultivar originating from a selection of landraces of Asturias (Spain). A252 is a breeding line derived from the cross Carioca × Guanajuato 31, developed in CIAT (Cali, Colombia). To construct the linkage map, molecular marker analyses were carried out using DNA extracted from 85 $F_2$ plants from the cross Andecha × A252. Seventy-six $F_3$ families were used to characterize the corresponding $F_2$ plants for resistance to six races of anthracnose (races 6, 31, 38, 39, 65, and 357). The resistance to each race was independently evaluated in 10–40 plants per $F_3$ family. The 12 common bean differential cultivars (Pastor-Corrales 1991) were used to confirm the identity of the *C. lindemuthianum* isolates.

### DNA extraction

Genomic DNA from the parental lines Andecha and A252 and from the $F_2$ progeny derived from the cross Andecha × A252, was isolated from lyophilized young leaves using the Nucleon™ PhytoPure™ Genomic DNA Extraction Kit (Amersham Biosciences) following the supplier's instructions.

### PCR analysis

A total of 240 decamer primers obtained from Operon Technologies (Alameda, CA, USA) were used to screen the parents for polymorphism, and those generating repeatable and distinct fragments were selected. Polymerase chain reactions (PCRs) were performed in a 25-µl total reaction mixture containing 30 ng of total DNA, 100 mM Tris–HCl, 100 mM KCl pH 8.3, 4 mM $MgCl_2$, 0.2 mM of each dNTP (Roche), 0.2 µM of primer and 1.25 U Stoffel DNA polymerase (Applied Biosystems). Amplification reactions were performed in a PCR System 9600 (Applied Biosystems) programmed as follows: 94°C for 5 min, 35 cycles of 94°C for 35 s, 40°C for 2 min, and 72°C for 2 min with a ramp of 1 s per cycle, followed by a final step of 72°C for 7 min. The name of each RAPD marker is derived from an 'O' prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate size (bp) of the marker (subscript).

Polymerase chain reactions of the SCAR markers, BAC6 (Ariyaranthe et al. 1999), Phs (Kami et al. 1995), SAP6 (Miklas et al. 2000a), SAS13 (Young et al. 1998), SB8 (Rivkin et al. 1999), SB12 (Méndez-Vigo et al. 2005), SBD5 (Miklas et al. 2000b), ROC11 (Johnson et al. 1997), SI19 (Melotto and Kelly 1998), and SW13 (Melotto et al. 1996), were carried out as described by their corresponding authors. The amplification of SCAreoli$_{500}$ was performed as described by Geffroy et al. (1998) modifying the annealing temperature (50°C instead of 58°C). The amplification of the SCAR SW12

(Miklas et al. 2000c) was carried out in a 25-µl solution containing 25 ng of DNA, 100 mM Tris–HCl, 100 mM KCl pH 8.3, 5 mM $MgCl_2$, 0.2 mM of each dNTP (Roche), 0.2 µM of each primer, and 1.25 U of Stoffel DNA polymerase (Applied Biosystems).

Polymerase chain reaction products amplified with RAPD, and SCAR primers were resolved on 2% agarose gels (except the SCAR Phs which was resolved on 8% polyacrylamide gels), stained with ethidium bromide and visualized under UV light. A 100-bp ladder (Amersham Biosciences) was used to measure the sizes of the amplification products.

### RFLP analysis

RFLP analyses were performed with 33 probes designated as Bng, which are derived from a *Pst*I genomic library (Chase et al. 1991). These probes correspond to RFLP markers that were previously included in the University of Florida genetic map (Vallejos et al. 1992) and in the bean integrated linkage map (Freyre et al. 1998). The amplification products of the SCARs, SAS13 and SB8, were also used as probes for RFLP analysis after purification with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Genomic DNA samples were digested with restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Taq*I, and *Hae*III (Amersham Biosciences). Restriction fragments were separated on 0.8% agarose gels and transferred to nylon membranes (Hybond-N; Amersham Biosciences) by vacuum blotting (VacuGene XL; Amersham Biosciences). Hybridizations of Southern blots with $^{32}$P-labelled probes were carried out as described by Vallejos et al. (1992).

### Seed colour (P locus)

The *P* locus controls the pigmentation on the seed coat; the dominant allele is present in A252 (coloured), and absent in Andecha (white). The segregation at this locus was followed by recording the presence or absence of pigmentation on the seeds formed by the $F_2$ plants.

### Inoculation procedure and disease scoring

Six races of *C. lindemuthianum* were used: races 31, 39, 65, and 357, from the collection of the Crop and Soil Sciences Department (Michigan State University, US), and races 6 and 38 from the collection of SERIDA (Villaviciosa, Asturias, Spain). Isolates of each race were obtained from monosporic cultures maintained in fungus-colonized filter paper at −20°C for long-term storage. The identity of each isolate was confirmed with the anthracnose differential set (Pastor-Corrales 1991). To obtain abundant sporulation, all races were grown at 19–21°C in darkness for about 10 days in Potato Dextrose Agar (Difco) (races 6, 31, 38, and 357) or in Marthur's agar (Marthur et al. 1950) (races 39 and 65). Spore suspensions were prepared by flooding the plates with 5 ml of 0.01% Tween 20 (Sigma) in sterile distilled water and scraping the surface of the culture with a spatula. Differential cultivars, parental lines, and $F_3$ families, were inoculated with a spore suspension of $1.2 \times 10^6$ spores/ml of the pathogen. Inoculations were carried out on 8 to 10-day-old seedlings in a climate chamber. The seedlings were sprayed with the aqueous conidial suspension and maintained at 20–22°C, 95–100% humidity, and 12 h photoperiod. The responses of the plants were evaluated after 7–9 days using a 1–9 scale where 1 is no visible symptoms and 9 very severely diseased or dead (Van Schoonhoven and Pastor-Corrales 1987).

### Statistical analysis

Chi-square was used to test goodness-of-fit of observed to expected ratios in the $F_2$. The segregation analysis of the markers and the resistance genes was performed using MAPMAKER Macintosh version 2.0 (Lander et al. 1987). A LOD score of 3.0 was used as a linkage threshold with 0.25 as the maximum recombination fraction for linkage groups. Distances between ordered loci (cM) were calculated using the Kosambi mapping function.

## Results

### Construction of the genetic map

After screening the parental lines, Andecha and A252, 97 RAPD decamer primers were selected for their analysis in the $F_2$ population. These primers generated 164 dominant, and 8 codominant RAPD loci. Chi-square analyses indicated that the segregation of 11 of these RAPD markers (6.4%) deviated from the expected 3:1 ratio and were not included in the linkage analysis. All codominant RAPDs fitted the 1:2:1 expected segregation.

The Bng probes used constituted 28 codominant and 5 dominant RFLP markers that, in all cases, fitted the expected 1:2:1 and 3:1 segregations, respectively.

Ten out of the 12 SCARs analysed in the $F_2$ population segregated as dominant loci and fitted the 3:1 expectation. The remaining two SCARs, SAS13 (Young et al. 1998) and SB8 (Rivkin et al. 1999), produced no

differences in amplification pattern between the parental lines and did not segregate in the $F_2$. The amplification products of these two SCARs were used as probes in RFLP analyses and segregations corresponding to codominant loci (1:2:1) were observed in both cases.

The seed colour trait (gene *P,p* ) fitted a 3:1 monogenic dominant mode of inheritance.

The linkage analysis was performed on 85 $F_2$ individuals and 207 segregating markers (169 dominant and 38 codominant), using the multipoint linkage analysis software Mapmaker version 2.0 (Lander et al. 1987). The markers were sorted into distinct linkage groups using a LOD score of 3.0 as a linkage threshold with 0.25 as the maximum recombination fraction. This procedure resulted in the formation of 16 linkage groups with a number of markers ranging from 2 to 26, and 10 isolated markers (9 RAPDs and 1 Bng). The loci order of each linkage group was established following the full multipoint analysis mapping method. Later, correspondences of 16 of the linkage groups obtained with the 11 linkage groups of the bean integrated linkage map (Freyre et al. 1998) were established using Bng markers as anchor points. The resulting map (Fig. 1) includes 197 markers and covers 1,401.9 cM with an average marker distance of 7.1 cM.

### Characterization of race-specific anthracnose resistance genes

Parental line Andecha was susceptible to races 6, 31, 38, 39, and 357 of *C. lindemuthianum* and showed an intermediate resistance phenotype to race 65, whereas parental line A252 was resistant to the six races.

For each of the races 6, 31, 38, 39, and 357, $F_3$ families were classified as having all individuals resistant (R), individuals resistant and susceptible (R/S), and all individuals susceptible (S). Table 1 shows the segregations of $F_3$ families for resistance to races 6, 31, 38, 39, and 357. Segregations for resistance to races 31 and 357 showed a good fit to the expected ratio for a single dominant gene (1 R:2 R/S:1 S), and were mapped on linkage groups B11 and B4, respectively (see Fig. 1). Segregations of resistance to races 6, 38, and 39 showed a good fit to the expected ratio for two independent dominant genes (7 R:8 R/S:1 S).

Table 2 shows the joint segregation of $F_3$ families for resistance to races 6, 31, 38, 39, and 357. Only 12 types of $F_3$ families (I–XII), considering their resistance spectra to these five races, were found. Types I–IX can be explained under the assumption of only two dominant genes being responsible for the different resistances, one of them conferring resistance to races 6, 38, 39, and 31 (located on B11) and the other conferring resistance to races 6, 38, 39, and 357 (located on B4). However, types X, XI, and XII, indicate the occurrence of recombination between resistance specificities not supported by the two-single-genes hypothesis.

Table 3 shows the segregation of resistance to races 6, 38, 39, 31, and 357 within the six $F_3$ families constituting types X, XI, and XII. In these six families, the number of $F_3$ individuals scored for resistance to some races was increased in order to reduce the possibility of error.

The results indicated in Table 2 can be explained if different specific genes for resistance to races 6, 38, 39, 31, and 357, were arranged in two independent clusters on linkage groups B4 and B11. The B4 cluster would include the specific resistance genes $R_1^6$ $R_1^{38}$, $R_1^{39}$, and $R^{357}$, and the B11 cluster would include the specific resistance genes $R_2^6$ $R_2^{38}$, $R_2^{39}$ and $R^{31}$. Figure 2 illustrates this possibility.

Concerning resistance to race 65, all possible combinations of plants with total resistance ($R_T$), plants with intermediate resistance ($R_I$), and/or susceptible plants (S), were observed in the $F_3$ families. Table 4 shows the segregation of $F_3$ families for resistance to race 65, compared to the expected frequencies under the assumption of two independent dominant genes being responsible for the resistance to this race; one of them conferring total resistance in A252 ($R_T^{65}$, $r_T^{65}$), and the other conferring intermediate resistance in Andecha ($R_I^{65}$, $r_I^{65}$). Differences between the observed and expected frequencies are not significant.

The genotypes for gene $R_T^{65}$, $r_T^{65}$ can be deduced from Table 4: $F_3$ families type I are homozygous resistant, $R_T^{65}$ $R_T^{65}$, $F_3$ families types IV, V, and VII are heterozygous $R_T^{65}$ $r_T^{65}$, and $F_3$ families types II, III, and VI are homozygous susceptible, $r_T^{65}$ $r_T^{65}$. The segregation of this gene fits a 1:2:1 ratio ($\chi^2 = 3.98$; $P = 0.14$) and it was mapped at 10.2 cM (LOD = 13.99) of the $R^{31}$ gene, in linkage group B11.

The genotypes for gene $R_I^{65}r_I^{65}$ can also be deduced in most $F_3$ families: types II and IV are homozygous for intermediate resistance, $R_I^{65}R_I^{65}$, types VI and VII are heterozygous $R_I^{65}r_I^{65}$, and types III and V are homozygous susceptible, $r_I^{65}r_I^{65}$. The segregation of this gene fitted the 1:2:1 ratio ($\chi^2 = 3.73$; $P = 0.15$) and it was mapped in linkage group B1.

## Discussion

### Localization of molecular markers linked to traits of interest

In regard to anthracnose resistance, the present map includes RAPD markers $OF10_{500}$ on LG B1 (probably

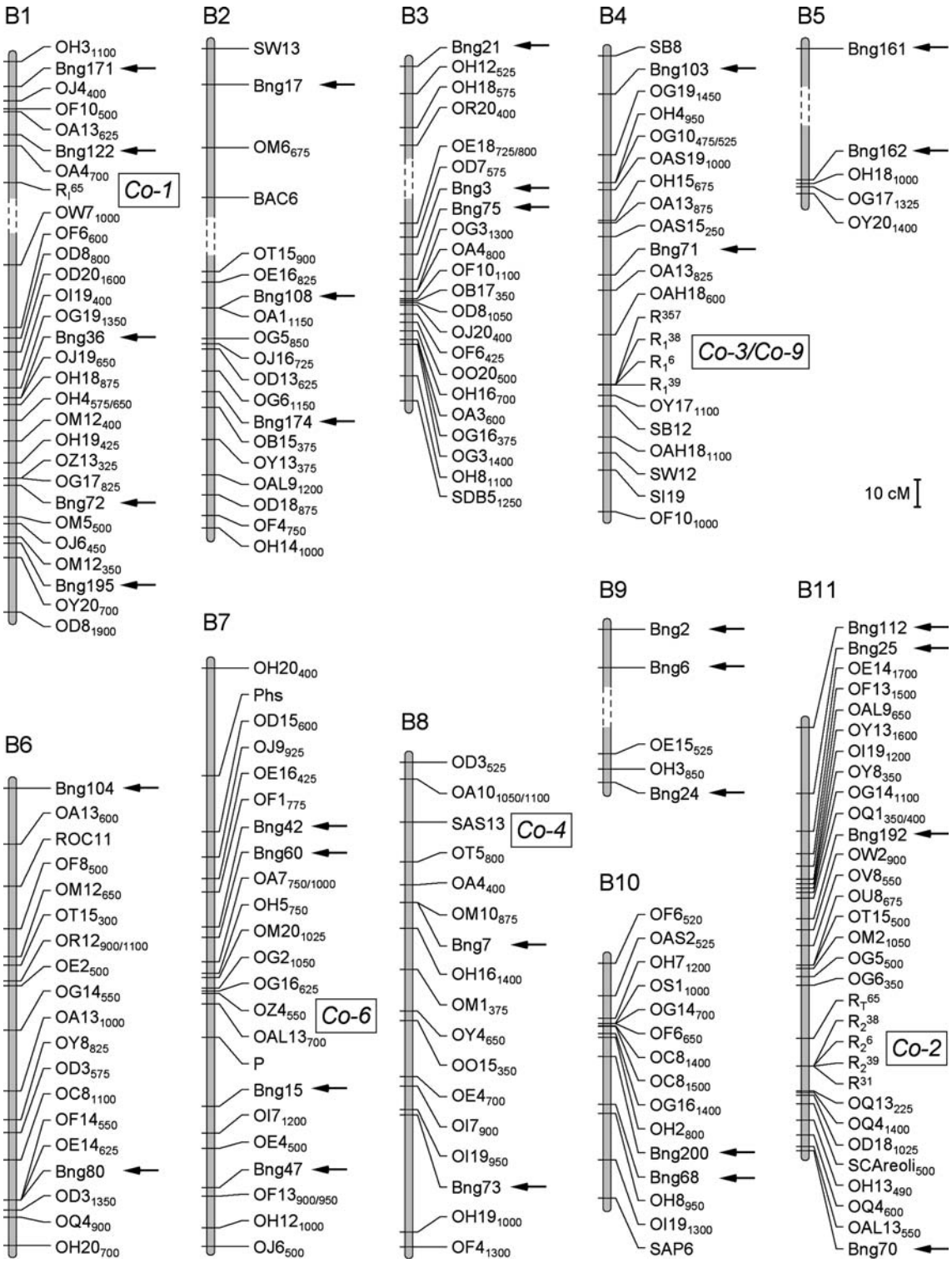


**Fig. 1** Genetic linkage map of common bean in the $F_2$ population Andecha × A252. RAPD and SCAR markers, and race-specific resistance genes are indicated with designations described in the text. *P* is a seed color gene. *Arrows* indicate common RFLP markers to the reference map published by Vallejos et al. (1992)

corresponding to $OF10_{530}$ linked to *Co-1*; Young and Kelly 1997), $OQ04_{1400}$ on B11 (probably equivalent to $OQ04_{1440}$ linked to *Co-2*; Young and Kelly 1996b), and $OZ04_{550}$ on B7, previously described as linked to *Co-6* (Alzate-Marin et al. 1999). The amplification product of SCAreoli obtained in the present work (500 bp), was different from that described by Geffroy et al. (1998), but might identify close regions as both are

**Table 1** Segregation for resistance to races 6, 31, 38, 39, and 357 of *Colletotrichum lindemuthianum* in $F_3$ Andecha × A252 families

| Race | Observed frequency[a] | | | Expected frequency[b] | | | $\chi^2$ | Probability |
|---|---|---|---|---|---|---|---|---|
| | R | R/S | S | R | R/S | S | | |
| 6 | 28 | 39 | 2 | 30.2 | 34.5 | 4.3 | 0.745 | 0.39 |
| 31 | 20 | 32 | 15 | 16.8 | 33.5 | 16.8 | 0.881 | 0.64 |
| 38 | 32 | 42 | 2 | 33.3 | 38.0 | 4.8 | 0.468 | 0.49 |
| 39 | 29 | 38 | 2 | 30.2 | 34.5 | 4.3 | 0.402 | 0.53 |
| 357 | 16 | 35 | 17 | 17.0 | 34.0 | 17.0 | 0.088 | 0.96 |

[a] $F_3$ families were classified as having all individuals resistant (R), individuals resistant and susceptible (R/S), and all individuals susceptible (S)

[b] For races 6, 38, and 39 the expected ratio was 7:8:1 (two independent dominant genes) and for races 31 and 357 the expected ratio was 1:2:1 (one dominant gene)

**Table 2** Joint segregation for resistance to races 6, 31, 38, 39, and 357 of *Colletotrichum lindemuthianum* in $F_3$ Andecha × A252 families

| Type | Resistance spectrum of $F_3$ families[a] | | | | | |
|---|---|---|---|---|---|---|
| | Race 6 | Race 38 | Race 39 | Race 31 | Race 357 | Frequency |
| I | R/S | R/S | R/S | R/S | R/S | 11 |
| II | R/S | R/S | R/S | R/S | S | 13 |
| III | R/S | R/S | R/S | S | R/S | 10 |
| IV | R | R | R | R | R | 10 |
| V | R | R | R | R/S | R | 2 |
| VI | R | R | R | R | R/S | 8 |
| VII | R | R | R | R | S | 2 |
| VIII | R | R | R | S | R | 3 |
| IX | S | S | S | S | S | 2 |
| X | R/S | R/S | R | R/S | R/S | 1 |
| XI | R/S | R | R/S | R/S | R/S | 3 |
| XII | R | R | R | R/S | R/S | 2 |

[a] $F_3$ families were classified for each race as having all individuals resistant (R), individuals resistant and susceptible (R/S), and all individuals susceptible (S)

**Table 3** Segregation of resistance to races 6, 38, 39, 31, and 357 within the six $F_3$ families proceeding from the cross Andecha × A252, corresponding to types X, XI, and XII of Table 2

| Race | $F_3$ families | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_3$-75 (type X) | | $F_3$-15 (type XI) | | $F_3$-52 (type XI) | | $F_3$-53 (type XI) | | $F_3$-56 (type XII) | | $F_3$-68 (type XII) | |
| | R | S | R | S | R | S | R | S | R | S | R | S |
| 6 | 13 | 1 | 12 | 1 | 13 | 1 | 21 | 1 | 14 | 0 | 14 | 0 |
| 38 | 10 | 1 | 25 | 0 | 30 | 0 | 27 | 0 | 16 | 0 | 16 | 0 |
| 39 | 27 | 0 | 9 | 3 | 12 | 1 | 10 | 2 | 13 | 0 | 14 | 0 |
| 31 | 33 | 5 | 4 | 2 | 12 | 2 | 8 | 2 | 12 | 1 | 11 | 2 |
| 357 | 10 | 2 | 9 | 4 | 11 | 3 | 7 | 4 | 12 | 1 | 13 | 1 |

*R* resistant individuals, *S* susceptible individuals

located at the end of B11. SCAR markers SB12, SI19 and SW12, and RAPDs $OAH18_{1100}$ and $OY17_{1100}$ described as located in the vicinity of *Co-3/Co-9* resistance locus (Méndez-Vigo et al. 2005), were also mapped to B4 linkage group. Markers SI19 and $OY17_{1100}$ had been previously described to be linked to the rust resistance gene *Ur-5* and SW12 linked to a QTL of resistance to bean golden yellow mosaic virus (Miklas et al. 2002). The use of the amplification product of SAS13 (Young et al. 1998) as a probe in RFLP analysis (as polymorphism was not found between parents by direct amplification), allowed the indirect localization of *Co-4* locus on linkage group B8. This result agrees with the recent physical mapping of *Co-4* to chromosome 3 (which corresponds to linkage group B8; Melotto et al. 2004).

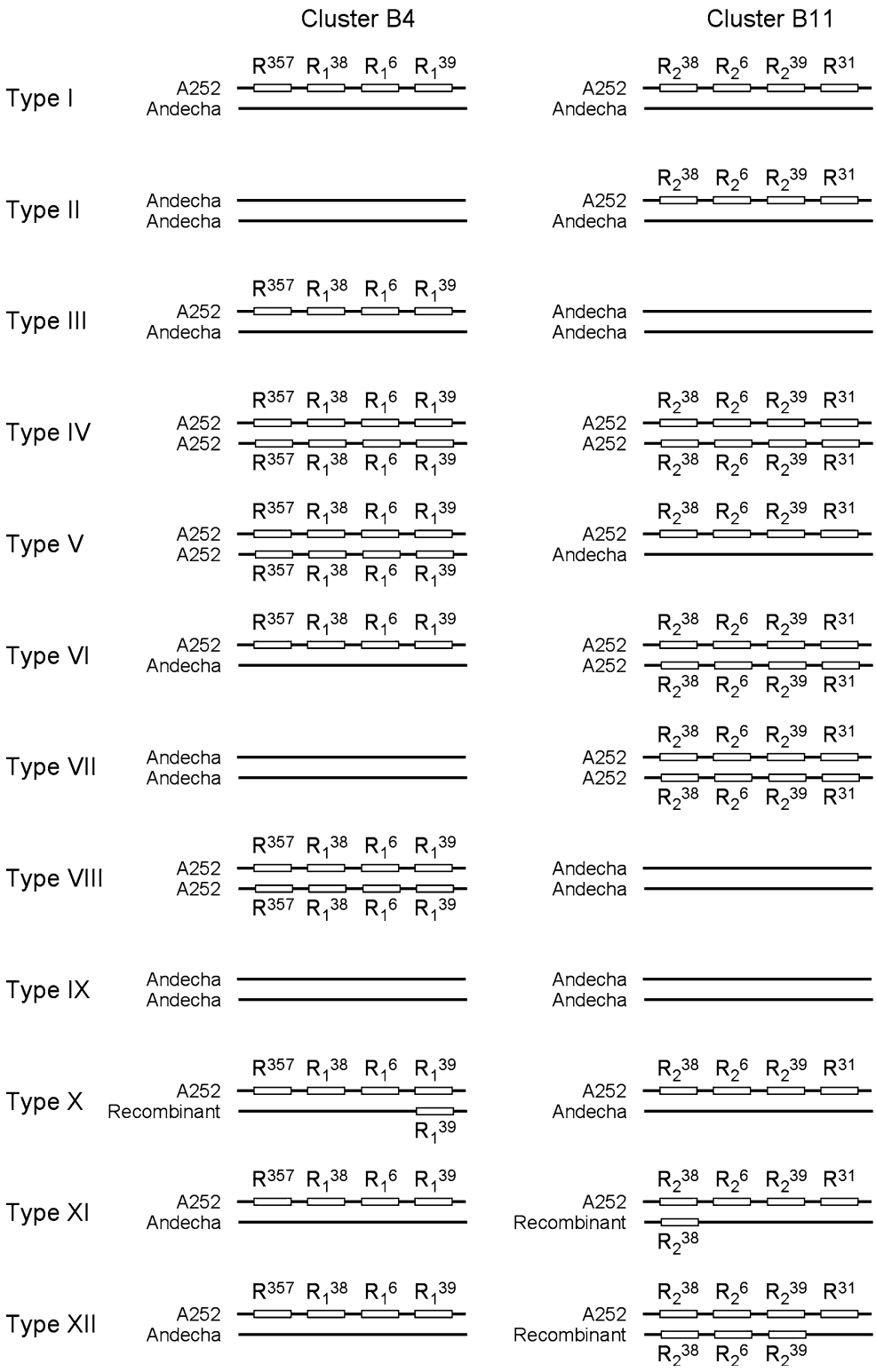


**Fig. 2** An explanation for the results shown in Table 2, concerning the different $F_3$ family types observed (I–XII) for their combined resistance to races 6, 31, 38, 39, and 357. The genes proceeding from A252 providing resistance to these races are arranged in two clusters: the *B4* cluster includes the race-specific resistance genes $R_1^6$ , $R_1^{38}$ , $R_1^{39}$ , and $R^{357}$, and the *B11* cluster includes the race-specific resistance genes $R_2^6$ , $R_2^{38}$ , $R_2^{39}$ , and $R^{31}$. The corresponding *Andecha* haplotypes do not confer resistance to these races. *Types I–IX* are the different possible genotypes constituted by parental haplotypes. *Types X*, *XI*, and *XII* can only be explained with intra-cluster recombination. The relative positions of the different race-specific genes within each cluster is only tentative. The indicated positions explain types X–XII with a single recombination event

The present map also includes molecular markers linked to bean common mosaic virus resistance loci: SW13 (Melotto et al. 1996) linked to *I* gene on B2 linkage group, ROC11 (Johnson et al. 1997) linked to *bc-3* gene on B6 LG, and SBD5 linked to *bc-1*$^3$ (Miklas et al. 2000b) on B3 LG. Two SCARs linked to common bacterial blight resistance were also mapped: SAP6 (Miklas et al. 2000a) on B10 and BAC6 on B2, although this location is not coincident with that given by Ariyaranthe et al. (1999) on B10.

**Table 4** Segregation of $F_3$ Andecha × A252 families for resistance to race 65, compared to the expected frequencies under the assumption of two independent dominant genes being responsible for the resistance to this race; one of them conferring total resistance ($R_T^{65}$, $r_T^{65}$), and the other conferring intermediate resistance ($R_I^{65}$, $r_I^{65}$)

| $F_3$ families | | | Genotypes of the corresponding $F_2$ plants | Expected frequency |
|---|---|---|---|---|
| Type | Phenotypes[a] for resistance | Frequency | | |
| I | $R_T$ | 22 | $R_T^{65}R_T^{65}$– – | 15.8 (1/4) |
| II | $R_I$ | 5 | $r_T^{65}r_T^{65}R_I^{65}R_I^{65}$ | 3.9 (1/16) |
| III | S | 6 | $r_T^{65}r_T^{65}r_I^{65}r_I^{65}$ | 3.9 (1/16) |
| IV | $R_T/R_I$ | 6 | $R_T^{65}r_T^{65}R_I^{65}R_I^{65}$ | 7.9 (1/8) |
| V | $R_T$/S | 9 | $R_T^{65}r_T^{65}r_I^{65}r_I^{65}$ | 7.9 (1/8) |
| VI | $R_I$/S | 6 | $r_T^{65}r_T^{65}R_I^{65}r_I^{65}$ | 7.9 (1/8) |
| VII | $R_T/R_I$/S | 9 | $R_T^{65}r_T^{65}R_I^{65}r_I^{65}$ | 15.8 (1/4) |
| | | | $\chi^2 = 7.79$ ($P = 0.25$) | |

[a] $F_3$ families were classified as having all individuals with total resistance ($R_T$); all individuals with intermediate resistance ($R_I$); all individuals susceptible (S); individuals with total resistance and intermediate resistance ($R_T/R_I$); individuals with total resistance and susceptible ($R_T$/S); individuals with intermediate resistance and susceptible ($R_I$/S); individuals with total resistance, intermediate resistance and susceptible ($R_T/R_I$/S)

SB8 marker, a resistance analogue sequence, was located on linkage group B4 as described by Rivkin et al. (1999). The positions of genes *P* (seed colour) and *Phs* (Phaseolin: seed storage protein) on linkage group B7 agree with those of previous maps (Nodari et al. 1993; Freyre et al. 1998).

## Characterization of race-specific anthracnose resistance genes

The intermediate resistance to race 65 present in Andecha can be explained by a single dominant gene ($R_I^{65}$) located on linkage group B1, linked to molecular markers Bng122 (17.6 cM) and $OF10_{500}$ (27.2 cM) previously described as linked to the *Co-1* gene (Young and Kelly 1997; Kelly et al. 2003). Among all characterized anthracnose resistance loci, *Co-1* is the only one originating in the Andean gene pool of common bean (Kelly and Vallejo 2004). As described before, landrace Andecha is considered to be Andean origin due to its morphological and biochemical characteristics.

The recombination between resistance specificities found in this work agrees with the assumption that total resistance to races 6, 31, 38, 39, 65, and 357, present in A252, is organized in two clusters. One of them, located on B4 linkage group, includes the race-specific resistance genes $R_1^6$ $R_1^{38}$, $R_1^{39}$, and $R^{357}$. The linkage between the $R^{357}$ gene and the molecular markers $OY17_{1100}$ (3.9 cM), SB12 (7.8 cM), $OAH18_{1100}$ (19.2 cM), and SW12 (25 cM), indicates that this cluster corresponds to the *Co-3/Co-9* gene to which these markers were previously linked (Méndez-Vigo et al. 2005). The second cluster is located on linkage group B11 and includes the race-specific resistance genes $R_2^6$ $R^{31}$, $R_2^{38}$, $R_2^{39}$, and $R_T^{65}$. In this case, the genetic distance between genes $R^{31}$ and $R_T^{65}$could be estimated as 10.2 cM. The linkage between the $R^{31}$ gene and the molecular markers $OQ04_{1400}$ (9.7 cM) and SCAreoli (14 cM), indicates that this cluster corresponds to the *Co-2* gene to which these markers were previously linked (Young and Kelly 1996b; Geffroy et al. 1998).

Genes providing resistance to different pathogens as well as to different races of the same pathogen have been reported to be clustered in chromosomal regions in many plant species (Crute and Pink 1996; Vear et al. 1997; Sharma et al. 2004). The results obtained in this work reveal that resistance genes *Co-2* and *Co-3/Co-9*, previously described as single major genes conferring resistance to several anthracnose races (Kelly and Vallejo 2004), should be considered as clusters of different genes conferring anthracnose race-specific resistance. The alleles of the *Co-3/Co-9* gene identified to date (Fouilloux 1976; Geffroy et al. 1999; Méndez-Vigo et al. 2005) could actually be haplotypes carrying different combinations of race-specific resistance genes. The tight linkage between the anthracnose resistance specificities *Co-y*, *Co-z*, and *Co-9* found by Geffroy et al. (1999) in the BAT93/JaloEEP558 RIL population, could also be interpreted as intra-cluster recombination of race-specific resistance genes. Also in the B4 cluster, recombination between race-specific genes for resistance to rust (Stavely 1984) and halo blight (Fourie et al. 2004) has been reported.

The *Co-2* locus had been only identified in the differential cultivar Cornell 49242 and to date no `alleles' had been reported (Kelly and Vallejo 2004). The *Co-2* haplotype present in A252 includes a specific gene for resistance to race 31 that would be absent at the *Co-2* cluster of Cornell 49242 (susceptible to race 31).

As found in other resistance loci in plants (Hulbert et al. 2001), the analysis of molecular organization of genomic DNA fragments corresponding to loci *Co-2* (Geffroy et al. 1998; Creusot et al. 1999), *Co-3/Co-9* (Geffroy et al. 1999; Ferrier-Cana et al. 2003, 2005), and *Co-4* (Melotto and Kelly 2001; Melotto et al. 2004) revealed the presence of clustered RGA sequences, suggesting a functional correspondence with the race-specific resistance genes described here.

Clusters of race-specific resistance genes could also constitute each of the remaining anthracnose resistance *Co-* loci of common bean. Segregation studies, using different anthracnose races could be carried out to determine whether it is possible to separate closely linked resistance specificities through recombination.

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