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ORIGINAL PAPER

Molecular mapping and intra-cluster recombination between anthracnose race-specific resistance genes in the common bean differential cultivars Mexico 222 and Widusa

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Abstract Resistance to races 19, 31, 38, 65, 73, 102, and 449, of the pathogenic fungus *Colletotrichum lindemuthianum* (anthracnose) was evaluated in F_3 families derived from the cross between the anthracnose differential bean cultivars Mexico 222 (resistant to races 19, 31, and 38) and Widusa (resistant to races 38, 65, 73, 102, and 449). Molecular marker analyses were carried out in the corresponding F_2 individuals in order to identify the genes for anthracnose resistance present in these two differential cultivars. The results of the combined segregation indicate that the resistance to anthracnose races 19, 31, and 38, present in Mexico 222, is conferred by single dominant race-specific genes organized in a cluster located in B4 linkage group, corresponding to the previously described *Co-3*/*Co-9* locus. The resistance to anthracnose races 65, 73, 102, and 449, present in Widusa, is conferred by a dominant gene (or genes) representing a different haplotype of the same *Co-3*/*Co-9* cluster. A single dominant gene located in a position independent from cluster *Co-3*/*Co-9* (probably at the *Co-1* locus) confers specific resistance to race 38 in Widusa. Recombinants for closely linked resistance specificities belonging to the *Co-3*/*Co-9* cluster have been detected. The possibility of pyramiding race-specific resistance genes by means of intra-cluster recombination, and its potential use in plant breeding, is indicated.

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Introduction

Anthracnose is the most serious disease worldwide of common bean (*Phaseolus vulgaris* L.) due to its seed-borne nature and to the pathogenic variability of its causal agent, *Colletotrichum lindemuthianum*. It causes severe economic losses in tropical, subtropical, and even temperate areas when favorable conditions for the fungus are present during the growing season (Pastor-Corrales and Tu 1994). More than 100 different races of the pathogen have been described (Rodríguez-Guerra et al. 2003). Currently, the different *C. lindemuthianum* races are characterized based on their phenotypic reaction on an anthracnose differential set formed by the 12 differential cultivars, Michelite, Michigan Dark Red Kidney (MDRK), Perry Marrow, Cornell 49242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136, and G2333, and named on the basis of a binary nomenclature system (Pastor-Corrales 1991).

Several genes, conferring dominant resistance to anthracnose, have been described and mapped to different linkage groups of the bean genome. Most of these genes are represented on the differential cultivars: The *Co-1* gene, mapped to linkage group B1, was described in the differential cultivar MDRK (Young and Kelly 1997), and three alleles of this gene were identified in the differential cultivars Perry Marrow, Kaboon, and Widusa, respectively (Melotto and Kelly 2000; Gonçalves-Vidigal and Kelly 2006). The *Co-2* gene is represented in the differential cultivar Cornell 49242 and was mapped to linkage group B11 (Freyre et al. 1998). The differential cultivars Mexico 222 and PI 207262 harbor the *Co-3*/*Co-9* gene, mapped to

linkage group B4 (Geffroy et al. 1999; Méndez-Vigo et al. 2005). Three different alleles of the *Co-4* gene, mapped to linkage group B8 (Melotto et al. 2004), have been reported as being present in the differential cultivars TO (Fouilloux 1976), G2333 (Young et al. 1998) and PI 207262 (Alzate-Marin et al. 2001), respectively. The *Co-5* gene was identified in the differential cultivars TU (Fouilloux 1976) and G2333 (Young and Kelly 1996; Young et al. 1998) and was mapped to linkage group B7 (Campa et al. 2005). Finally, the *Co-6* gene was described in the differential cultivar AB 136 and mapped to linkage group B7 (Schwartz et al. 1982; Young and Kelly 1996).

Even though the differential cultivars are used as an international reference, the anthracnose resistance systems of some of them remain unclear. These are the cases of the differential cultivars Widusa and Mexico 222. Allelism tests have been performed to identify the genes present in Widusa (Alzate-Marin et al. 2002; Ferreira et al. 2003; Gonçalves-Vidigal and Kelly 2006) and in Mexico 222 (Méndez-Vigo et al. 2005), but no attempts for mapping of resistances present in these two differential cultivars have been carried out.

Recent genetic analyses of joint segregations for resistance to different anthracnose races (Rodríguez-Suárez et al. 2007) indicated that *Co-2* and *Co-3*/*Co-9* genes, previously described as single major genes conferring resistance to several races, are organized as clusters of individual genes conferring race-specific resistance. This is in agreement with the cluster organization of families of resistance gene analogue sequences (RGAs) and/or resistance gene candidates (RGCs) mapping close to loci *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), as well as with the clusters of resistance genes to different pathogens or to different races of the same pathogen that have been described in many plant species (Michelmore and Meyers 1998).

The main objectives of this work were the characterization and mapping of the genes for specific resistance against seven races of anthracnose present in the differential cultivars Widusa and Mexico 222.

Materials and methods

Plant material

Molecular marker analyses were carried out using DNA extracted from 103 F_2 plants derived from the cross between the two anthracnose differential cultivars Widusa and Mexico 222. F_3 families (obtained by selfing individual F_2 plants) were used to characterize the corresponding F_2 plants for resistance to seven races of anthracnose (races 19, 31, 38, 65, 73, 102, and 449). In some cases, F_4 families were obtained in order to confirm the genotype assigned to the corresponding F_3 families. The resistance to each race was independently evaluated in 10–40 plants per F_3 or F_4 family. The remaining ten common bean anthracnose differential cultivars (Pastor-Corrales 1991) were used to confirm the identity of the *C. lindemuthianum* isolates.

Inoculation procedure and disease scoring

Seven different races of *C. lindemuthianum* were used: races 31, 65, 73, and 449, from the collection of the Crop and Soil Sciences Department (Michigan State University, USA), and races 19, 38, and 102, from the collection of the 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 19, 31, 38, 65, 102, and 449) or in Marthur's agar (Marthur et al. 1950) (race 73). 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, and F_3 families, were inoculated with a spore suspension of 1.2×10^6 spores/ml of the pathogen. Inoculations were carried out on 8–10 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 one is no visible symptoms and nine very severely diseased or dead (Van Schoonhoven and Pastor-Corrales 1987).

DNA extraction, PCR amplification and electrophoresis

Genomic DNA from Mexico 222, from Widusa and from the F_2 progeny derived from the cross Widusa × Mexico 222, was isolated from lyophilized young leaves using the Nucleon™ PhytoPure™ Genomic DNA Extraction Kit (Amersham Biosciences) following the supplier's instructions.

The following three molecular markers were used: the SCAR marker, SW12, linked to the BGMV resistance gene, *bgm-1* (Miklas et al. 2000) and to the anthracnose resistance gene, *Co-3*/*Co-9* (Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2007), the RAPD marker, OAH18, linked to the anthracnose-resistance gene *Co-3*/*Co-9* (Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2007), and the SSR marker PV-ctt001, previously mapped to linkage group B4 (Yu et al. 2000). The amplifications of

SW12 and OAH18 were carried out as described by Méndez-Vigo et al. (2005). The amplification of PV-ctt001 was carried out according to Yu et al. (2000).

PCR products amplified with SW12 and OAH18 primers were resolved on 2% agarose gels, stained with ethidium bromide and visualized under UV light. PCR products amplified with PV-ctt001 primers were resolved on 6% polyacrylamide gels, silver stained and were visualized in white light.

Statistical and linkage analysis

Chi-square was used to test goodness-of-fit of observed to expected ratios in the F_2 population Widusa × Mexico 222. The segregation analysis of the markers and the resistance genes was performed using MAPMAKER Macintosh version 2.0 (Lander et al. 1987). Distances between ordered loci (cM) were calculated using the Kosambi mapping function.

Results

Segregations of anthracnose resistance specificities

Parental line Widusa was resistant to races 38, 65, 73, 102, and 449 of *C. lindemuthianum*, whereas parental line Mexico 222 was resistant to races 19, 31, and 38.

For all races, 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 19, 31, 38, 65, 73, 102, and 449. Segregations for resistance to races 19, 31, 65, 73, 102, and 449 showed a good fit to the expected ratio for a single dominant gene (1 R: 2 R/S: 1 S). Segregations of resistance to race 38 showed a good fit to the expected ratio for two independent dominant genes (7 R: 8 R/S: 1 S).

Table 1 Segregation for resistance to races 19, 31, 38, 65, 73, 102, and 449 of *Colletotrichum lindemuthianum* in F_3 Widusa × Mexico 222 families

Race	Observed frequency[a]			Expected frequency[b]			χ^2	Probability
	R	R/S	S	R	R/S	S		
19	29	51	18	24.5	49.0	24.5	2.63	0.27
31	26	49	20	23.8	47.5	23.8	0.85	0.65
38	34	33	5	31.5	36.0	4.5	0.50	0.78
65	19	52	28	24.8	49.5	24.8	1.89	0.39
73	19	52	28	24.8	49.5	24.8	1.89	0.39
102	20	54	28	25.5	51.0	25.5	1.61	0.45
449	18	52	27	24.3	48.5	24.3	2.18	0.34

[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 19, 31, 65, 73, 102, and 449 the expected ratio was 1:2:1 (one dominant gene) and for race 38 the expected ratio was 7:8:1 (two independent dominant genes)

Molecular-marker analyses

Using the primer corresponding to the SSR marker PV-ctt001, a fragment of 170 bp was amplified in Widusa and a fragment of 165 bp was amplified in Mexico 222. The primer corresponding to the RAPD marker OAH18 amplified a fragment of 600 bp in Widusa and a fragment of 1,100 bp in Mexico 222. The two markers, PV-ctt001, and OAH18, behaved as codominant loci. Using the primer corresponding to the SCAR marker SW12 no amplification was obtained in Widusa, whereas two different fragments of 700 and 425 bp, respectively, were amplified in Mexico 222. Recombinant F_2 individuals (showing amplification of only one of these fragments) were found, and the two fragments were considered as belonging to different loci ($SW12_{700}$ and $SW12_{425}$, respectively). Table 2 shows the segregations for the molecular marker loci PV-ctt001, OAH18, $SW12_{700}$, and $SW12_{425}$, in the Widusa × Mexico 222 F_2 population. A good fit to the expected ratio for a single gene was obtained in all cases.

Linkage analysis

Table 3 shows the joint segregation for resistance to anthracnose races 19, 31, 65, 73, 102, and 449, in F_3 Widusa × Mexico 222 families, and for molecular marker loci PV-ctt001, OAH18, $SW12_{700}$, and $SW12_{425}$ in the corresponding F_2 plants. Recombination between resistance specificities was observed only in two F_3 families. In these families, the number of individuals scored for resistance to some races was increased in order to reduce the possibility of error. Table 4 shows the segregation for resistance to races 19, 31, 65, 73, 102, and 449, within these two recombinant F_3 families (F_3-94 and F_3-98). Resistances to races 19 (proceeding from Mexico 222) and 102 (proceeding from Widusa) were tested in the progenies (F_4 families) of seven individuals belonging to the F_3-94 family. Three of these F_4 families were homozygous resistant for both races, confirming their intra-cluster recombination origin. In sum, the results shown in Table 3 can be explained with a minimum of three closely linked resistance genes arranged in a cluster located in linkage group B4. Figure 1 shows a genetic map including all resistance specificities and molecular markers appearing in Table 3.

Given the previously deduced existence of two independent dominant genes conferring resistance to race 38

Table 2 Segregation for molecular marker loci PV-ctt001, OAH18, $SW12_{700}$, and $SW12_{425}$, in the Widusa × Mexico 222 F_2 population

Locus	Observed frequency[a]			Expected frequency[b]			χ^2	Probability
	W/W	W/M	M/M	W/W	W/M	M/M		
PV-ctt001	21	55	27	25.75	51.50	25. 75	1.17	0.56
OAH18	21	54	28	25. 75	51.50	25. 75	1.19	0.55
$SW12_{700}$	22	81		25. 75	77.25		0.73	0.39
$SW12_{425}$	23	80		25. 75	77. 25		0.39	0.53

[a] W/W homozygous for Widusa alleles; M/M homozygous for Mexico 222 alleles; W/M heterozygous

[b] For loci PV-ctt001 and OAH18 (codominant) the expected ratio was 1:2:1, and for loci $SW12_{700}$ and $SW12_{425}$ (dominant) the expected ratio was 1:3

Table 3 Joint segregation for resistance to races 19, 31, 65, 73, 102, and 449 of *Colletotrichum lindemuthianum* in F_3 Widusa × Mexico 222 families, and for molecular marker loci PV-ctt001, OAH18, $SW12_{700}$, and $SW12_{425}$ in the corresponding F_2 plants

Resistance spectrum of F_3 families[a]						Genotype of molecular marker loci of F2 plants[b]				Frequency
Race 19	Race 31	Race 65	Race 73	Race 102	Race 449	PV-ctt001	OAH18	$SW12_{700}$	$SW12_{425}$	
S	S	R	R	R	R	W/W	W/W	W/W	W/W	14
S	S	R	R	R	R	W/W	W/W	W/W	M/-	1
S	S	R	R	R	R	W/W	W/M	W/W	W/W	1
R	R	S	S	S	S	M/M	M/M	M/-	M/-	19
R	R	S	S	S	S	M/M	W/M	M/-	M/-	1
R	R	S	S	S	S	W/M	W/M	M/-	M/-	2
R/S	R/S	R/S	R/S	R/S	R/S	W/M	W/M	M/-	M/-	39
R/S	R/S	R/S	R/S	R/S	R/S	W/M	W/M	M/-	W/W	2
R/S	R/S	R/S	R/S	R/S	R/S	W/M	M/M	M/-	M/-	2
R/S	R/S	R/S	R/S	R/S	R/S	W/W	W/W	W/W	W/W	1
R/S	R/S	R/S	R/S	R/S	R/S	W/M	W/W	M/-	M/-	1
R	R/S	R/S	R/S	R/S	R/S	W/M	W/M	M/-	M/-	1[c]
R/S	S	R/S	R/S	R/S	R/S	W/M	W/M	W/W	W/W	1[c]

[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)

[b] W/W homozygous for the Widusa allele; W/- homozygous for the Widusa allele or heterozygous; M/M homozygous for the Mexico 222 allele; M/- homozygous for the Mexico 222 allele or heterozygous; W/M heterozygous

[c] Individuals showing evidence of recombination between resistance specificities

(Table 1), one of them proceeding from Widusa and the other from Mexico 222, the possibility of one of these genes being located in the cluster including the other resistance specificities can be considered. Table 5 shows the joint segregation for resistance to races 19, 31, 65, 73, 102, 449, and 38, compared to the expected frequencies under the assumption that the resistance gene to race 38 proceeding from Mexico 222 is the one located in the cluster. Differences between the observed and expected frequencies are not significant.

Discussion

Concerning Mexico 222, the results obtained indicate that its resistance to anthracnose races 19, 31, and 38, is conferred by single dominant race-specific genes organized in a cluster located in B4 linkage group, closely linked to molecular markers OAH18, SW12, and PV-ctt001. This cluster probably corresponds to the *Co-3* gene. The anthracnose resistance present in the genotype Mexico 222 was first studied by Bannerot (1965), the *Co-3* resistance gene, originally known as *Mexique 1*, being later described in this genotype (Bannerot et al. 1971). The resistance to race 31 (Kappa, Ebnet) present in Mexico 222 was analyzed by Fouilloux (1976) and assumed to be conferred by the *Co-3* (*Mexique 1*) gene. The cluster revealed in this work also corresponds to the *Co-9* gene, first described and located in B4 linkage group by Geffroy et al. (1999) in the genotype BAT 93, and later mapped between the markers OAH18 and SW12 (Méndez-Vigo et al. 2005), using a F_2 population proceeding from a cross including the genotype A493

Table 4 Segregation for resistance to races 19, 31, 65, 73, 102, and 449 within the two F_3 families proceeding from the cross Widusa × Mexico 222 that showed evidence of recombination

Race	F_3 Families			
	F_3-94		F_3-98	
	R	S	R	S
19	37	0	11	5
31	7	2	0	20
65	26	4	6	4
73	7	2	5	5
102	22	3	15	2
449	8	1	9	1

R resistant individuals, *S* susceptible individuals

(Alubia/BAT 93). The results obtained here confirm the allelism between *Co-3* and *Co-9*, found by Méndez-Vigo et al. (2005) using race 38.

Concerning Widusa, the results obtained indicate that its resistance to anthracnose races 65, 73, 102, and 449, is conferred by a dominant gene (or genes) representing a different haplotype of the same *Co-3*/*Co-9* cluster. In this material, specific resistance to race 38 is conferred by a single dominant gene located in a position independent from cluster *Co-3*/*Co-9*. The presence of two different anthracnose-resistance genes in Widusa, one of them conferring resistance to race beta (D_{10}) and the other conferring resistance to races beta and gamma ($D_{10} + E_8b$), was first indicated by Bannerot (1965). The correspondence between the gene conferring resistance to race 65 in Widusa and *Co-3*/*Co-9*, demonstrated in the current work, agrees with Alzate-Marin et al. (2007) who proposed the presence of an allele of the *Co-9* gene in Widusa from the observed lack of segregation, using race 65, in two F_2 populations derived from the crosses Widusa × BAT 93 and Widusa × PI 207262, respectively. The combined results of different allelism tests support the hypothesis that the anthracnose-differential cultivar PI 207262 carries resistance genes in clusters *Co-4* and *Co-3*/*Co-9* (Alzate-Marin et al. 2007). As BAT 93 was derived from PI 207262, it is assumed that these two genotypes share the same *Co-3*/*Co-9* haplotype. Concerning the resistance to race 73, Gonçalves-Vidigal and Kelly (2006) using this race, found a segregation ratio of 63 resistant:1 susceptible in a F_2 population derived from the cross Widusa × PI 207262, and a segregation ratio of 15 resistant:1 susceptible in a F_2 population derived from the cross Widusa × BAT 93. Assuming that PI 207262 carries two resistance genes to race 73, located in clusters *Co-4* and *Co-3*/*Co-9*, respectively, and that BAT 93 carries one resistance gene to race 73, located in cluster *Co-3*/*Co-9*, they concluded that the *Co-3*/*Co-9* cluster is not involved in the resistance to race 73 in Widusa. From the results of the current work concerning linkage between resistance genes and molecular markers (Table 3; Fig. 1), it can unequivocally be concluded that the gene conferring resistance to race 73 in Widusa is included in the *Co-3*/*Co-9* cluster. Then, the results of the allelism tests conducted by Gonçalves-Vidigal and Kelly (2006) would indicate that a second locus, different from *Co-3*/*Co-9*, confers resistance to race 73 in BAT 93, and a third locus, different from *Co-4* and from *Co-3*/*Co-9*, confers resistance to race 73 in PI 207262. Recently, Geffroy et al. (2007) demonstrated the presence in the genotype BAT 93 of two anthracnose resistance loci,

Table 5 Joint segregation of F_3 Widusa × Mexico 222 families for resistance to *C. lindemuthianum* races 19, 31, 65, 73, 102, 449 and 38, compared to the expected frequencies under the assumption of two dominant genes being responsible for the resistance to race 38; one of them, proceeding from Mexico 222, located in the cluster including the genes conferring resistance to the other races, and the other, proceeding from Widusa, not linked to this cluster

Resistance spectrum of F_3 families[a]							Observed frequency	Expected frequency
Race 19	Race 31	Race 65	Race 73	Race 102	Race 449	Race 38		
R	R	S	S	S	S	R	21	18.0 (4/16)
R	R	S	S	S	S	R/S	0	0
R	R	S	S	S	S	S	0	0
S	S	R	R	R	R	R	2	4.5 (1/16)
S	S	R	R	R	R	R/S	7	9.0 (2/16)
S	S	R	R	R	R	S	5	4.5 (1/16)
R/S	R/S	R/S	R/S	R/S	R/S	R	11	9.0 (2/16)
R/S	R/S	R/S	R/S	R/S	R/S	R/S	26	27.0 (6/16)
R/S	R/S	R/S	R/S	R/S	R/S	S	0	0
							$\chi^2 = 2.87$ ($p = 0.72$)	

In this assumption, intra-cluster recombination has not been considered

[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)

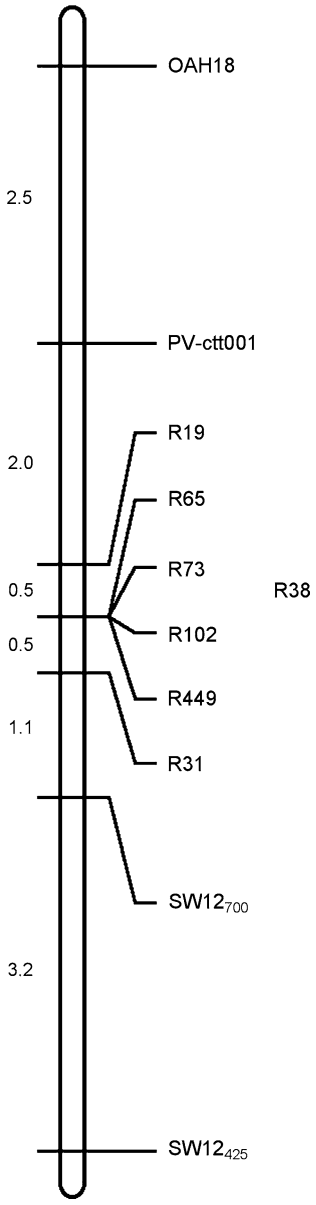

Fig. 1 Relative positions of markers OAH18, PV-ctt001, $SW12_{700}$, and $SW12_{425}$, and the genes conferring resistance to races 19 (R19), 31 (R31), 65 (R65), 73 (R73), 102 (R102), and 449 (R449), included in the *Co-3*/*Co-9* cluster. The relative position of the gene conferring resistance to race 38 in Mexico 222 (R38) is undetermined. Map distances, on the left, are expressed in centiMorgans, estimated using the Kosambi mapping function

located in linkage groups B4 (*Co-3*/*Co-9*) and B2 (*Co-u*), respectively. Alzate-Marin et al. (2007) using race 23, found a 63:1 segregation ratio, corresponding to three independent dominant genes, in a F_2 population derived from the cross PI 207262 × Mexico 222. They assumed that PI 207262 carries only the two resistance genes *Co-4* and *Co-3*/*Co-9*, and concluded that the resistance to race 23 present in Mexico 222 should be conferred by a gene different from *Co-3*/*Co-9*. There are some evidences indicating that Mexico 222 possesses two genes conferring resistance against race 7 (unpublished data, cited by Kelly and Vallejo 2004). The existence of a third resistance locus in PI 207262 conferring resistance to race 23 could be an alternative explanation.

The results of the current work concerning resistance to race 38 in Widusa differ from those obtained in a previous allelism study involving resistance to race 38 (Ferreira et al. 2003), in which 13 resistant:3 susceptible ratio was observed in a F_2 population proceeding from a cross between Widusa (resistant) and Xana (susceptible). This was interpreted under the assumption of two independent genes, one dominant and the other recessive, being present in Widusa. In that study, the segregation ratios observed in the F_2 populations from crosses between Widusa and other materials carrying a single dominant gene conferring resistance to race 38 (PI 207262, A1183, Mexico 222, and TU), showed a good fit to the expected values under the hypothesis of three genes, two dominant and one recessive, being involved (61:3). However, the reexamination of these data indicates that they show also a good fit to the expected values under the hypothesis of two dominant genes (a single dominant gene in each parent) being involved (15:1). The excess of resistant F_2 individuals proceeding from the cross Widusa × Xana observed by Ferreira et al. (2003), if not artifactual, could be due to gene interaction affecting resistance to race 38 in this specific cross. Although recessive resistance to anthracnose has been reported in other cases in the literature (Cardenas et al. 1964; Muhalet et al. 1981), it has not been detected in the current work. Gonçalves-Vidigal and Kelly (2006), using a race 65 (65b) overcoming BAT 93, different from the one used by Alzate-Marin et al. (2007), and from the one used in the current work, found no segregation in F_2 populations derived from the crosses Widusa × MDRK and Widusa × Kaboon, concluding that the resistance to race 65b present in Widusa is conferred by the *Co-1* locus. The resistance-specificity to race 38 present in Widusa could be also located at this locus.

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). In a previous work (Rodríguez-Suárez et al. 2007), it was suggested that anthracnose-resistance *Co-* loci of common bean, previously described as single major gene conferring resistance to several anthracnose races (Kelly and Vallejo 2004), could be organized as clusters of several genes, each giving resistance to one, or at most a few, races. The results obtained in this work confirm this model. The recombination found between resistance specificities (Table 3) indicate that resistance to races 19 and 31 present in Mexico 222 are determined by individual race-specific genes. The absence of recombination found between resistance to races 65, 73, 102, and 449, present in

Widusa can be explained by a tighter linkage between the individual genes conferring resistance to these races. In this case, the possibility of resistance to more than one race being conferred by a single gene cannot be excluded.

The existence of intra-cluster recombination can be of considerable practical importance. Race-specific resistance genes present in different haplotypes of the same cluster could be pyramided by genetic recombination within the cluster. After accumulation of the resistance specificities, the enhanced new recombinant haplotype could be introgressed into susceptible varieties, practically as a single locus.

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