Genetic Analysis of the Resistance to Eight Anthracnose Races in the Common Bean Differential Cultivar Kaboon

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ABSTRACT

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Resistance to the eight races (3, 7, 19, 31, 81, 449, 453, and 1545) of the pathogenic fungus *Colletotrichum lindemuthianum* (anthracnose) was evaluated in F₃ families derived from the cross between the anthracnose differential bean cultivars Kaboon and Michelite. Molecular marker analyses were carried out in the F₂ individuals in order to map and characterize the anthracnose resistance genes or gene clusters present in Kaboon. The analysis of the combined segregations indicates that the resistance present in Kaboon against these eight anthracnose races is determined by 13 different race-specific genes grouped in three clusters.

One of these clusters, corresponding to locus *Co-1* in linkage group (LG) 1, carries two dominant genes conferring specific resistance to races 81 and 1545, respectively, and a gene necessary (dominant complementary gene) for the specific resistance to race 31. A second cluster, corresponding to locus *Co-3/9* in LG 4, carries six dominant genes conferring specific resistance to races 3, 7, 19, 449, 453, and 1545, respectively, and the second dominant complementary gene for the specific resistance to race 31. A third cluster of unknown location carries three dominant genes conferring specific resistance to races 449, 453, and 1545, respectively. This is the first time that two anthracnose resistance genes with a complementary mode of action have been mapped in common bean and their relationship with previously known *Co*-resistance genes established.

Anthracnose, caused by *Colletotrichum lindemuthianum*, is one of the most destructive diseases of common bean (*Phaseolus vulgaris* L.) worldwide. The fungus is seedborne and has highly pathogenic variability. More than 100 different races of this pathogen have been described (5,15,33,41,49). Identification of anthracnose races has been internationally standardized based on the disease reaction of the 12 differential common bean cultivars—Michelite, Michigan Dark Red Kidney (MDRK), Perry Marrow, Cornell 49242, Widusa, Kaboon, Mexico 222, PI207262, TO, TU, AB136 and G2333—and named based on a binary nomenclature system (46).

Up to now, 18 anthracnose resistance loci (designated as *Co*- followed by a number or a letter), at first considered as single genes conferring resistance to several anthracnose races, have been reported in common bean. Some of these loci have been located in the integrated linkage map (18,31): *Co-1*, probably allelic to *Co-x* and *Co-w*, on linkage group (LG) 1 (7,19,51); *Co-2* on LG 11 (1,34); *Co-3* (*Co-3/9*), allelic to *Co-9*, *Co-y*, and *Co-z*, on LG 4 (6,14,20,22,40,50,51); *Co-4* on LG 8 (16,36,39); *Co-5* on LG 7 (10,16); *Co-6*, probably allelic to *Co-v*, on LG 7 (19,30,59); *Co-10* on LG 4 (3,30); and *Co-u*, on LG 2 (21).

The characterization of the loci involved in the resistance of most anthracnose differential cultivars was at first interpreted based on the combined results of different allelism tests. Only in some cases have such resistance loci been mapped using molecular markers. From the results of allelism tests, a single dominant gene (*Co-11*) conferring resistance to race 64 has been described in the differential cultivar Michelite (25). The recessive resistance

gene described in Michelite by Cardenas et al. (12) has been recently reinterpreted as a reverse of dominance of the *Co-11* gene due to epistatic interaction with other genes (25). *Co-1* was assumed to be the only anthracnose resistance locus present in the differential cultivar MDRK (35). However, using direct mapping, Campa et al. (10) demonstrated that MDRK carries two independent anthracnose resistance loci. One of them, located in LG 1 (corresponding to the *Co-1* locus), confers resistance to race 1545, and the other, located in LG 4 (corresponding to the *Co-3/9* locus), is a cluster of at least two different genes conferring specific resistance to races 1545 and 449, respectively. From the results of allelism tests, the *Co-1* and other unspecified complementary loci have been reported to be present in Perry Marrow and Kaboon (12,38,44). The *Co-2* locus is present in the differential cultivar Cornell 49242, in which it was first described as a single major gene conferring resistance to races α , β , γ , and Δ (34). From the results of allelism tests, it was assumed that *Co-1* was the only anthracnose resistance locus present in the differential cultivar Widusa (24). Later, Alzate-Marín et al. (4), using allelism tests conducted with other anthracnose races, proposed the presence of an allele of *Co-9* in Widusa. Rodríguez-Suárez et al. (50), using direct mapping, demonstrated the presence of two independent loci in Widusa. One of them, located in LG 4 (corresponding to the *Co-3/9* locus), is a cluster formed by different genes conferring specific resistance to races 65, 73, 102, and 449, respectively, and the other, located in a position independent from the *Co-3/9* cluster (probably corresponding to *Co-1*), confers resistance to race 38. The *Co-3* gene (*Co-3/9* locus) was first described as the only resistance locus present in the differential cultivar Mexico 222 (6) but the presence of a second unidentified dominant resistance locus was later reported (54). The *Co-3/9* locus present in Mexico 222 was directly mapped (LG 4), and its organization as a cluster of at least three different genes conferring specific resistance to races 19, 31, and 38, respectively, was demonstrated (50). The presence of *Co-3/9* (40), as well as

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that of *Co-4* and other undefined complementary genes (4), has been described in PI207262 from the results of different allelism tests. In the differential cultivar TO, the *Co-4* locus was first described as a single major gene conferring resistance to anthracnose pathotypes Δ and γ -2 (16). The *Co-5* locus was first described in the differential cultivar TU (16) and, to date, it was assumed to be the only anthracnose resistance locus present in this cultivar. However, a recent work of Campa et al. (10) demonstrated by direct mapping that TU carries at least three independent loci, one of which, located in LG 7 (corresponding to the *Co-5* locus), is formed by a cluster of at least eight different genes conferring specific resistance to races 3, 6, 7, 31, 38, 39, 102, and 449, respectively; a second locus confers resistance to races 31 and 102, and a third locus confers specific resistance to race 102, the location of these two loci remaining unknown. In the differential cultivar AB136, the *Co-6* locus (59), the recessive gene *co-8* (2), and a second unspecified independent dominant resistance gene (11) have been reported. Using allelism tests, three independent loci (*Co-4*, *Co-5*, and *Co-7*) were reported to be present in the differential cultivar G2333 (61).

Kaboon is among the anthracnose differential cultivars showing the highest resistance spectrum to most of the *C. lindemuthianum* races (5,33). Although there is importance of the anthracnose resistance system present in Kaboon, it has not been fully elucidated and no attempts have been made to map the resistance loci. The main objective of this work was to characterize and map the genes conferring specific resistance against eight anthracnose races in this differential cultivar.

MATERIALS AND METHODS

Plant material. Molecular marker analyses were carried out using DNA extracted from 99 F₂ plants derived from the cross between the anthracnose differential cultivars Kaboon and Michelite. The corresponding 99 F₃ families were used to characterize the F₂ plants for resistance to eight anthracnose races (races 3, 7, 19, 31, 81, 453, 449, and 1545). Based on the combined segregations of anthracnose resistance specificities and molecular markers, a specific F₃ family (F₃-28) was chosen in order to perform a genetic analysis of the resistance to races 449, 453, and

1545. The characterization for resistance to anthracnose races 449, 453, and 1545 was carried out in 46 F₄ families proceeding from family F₃-28. Molecular marker analyses were also carried out using DNA extracted from the corresponding 46 F₃-28 plants.

DNA extraction, polymerase chain reaction amplification, and electrophoresis. Genomic DNA was isolated from young leaves using the FastDNA kit (MP Biomedicals, Illkirch, France) following the supplier's instructions. DNA concentrations were quantified photometrically (absorbance measurements at 260 and 280 nm) with Biomate 3 UV-visible spectrophotometer (Thermo Scientific, MA). DNA markers analyzed in F₂ Kaboon $\times$ Michelite are shown in Table 1. Another 55 DNA markers, microsatellite (simple-sequence repeat [SSR]), and sequence-characterized amplified region (SCAR) markers were analyzed in the F₃-28 family. Data concerning these markers are available upon request. The polymerase chain reaction (PCR) amplifications of these molecular markers were carried out in a 20- μ l solution containing 25 ng of DNA, 100 mM Tris-HCl, 100 mM KCl (pH 8.3), 4 mM MgCl₂, 0.2 mM each dNTP (Bioline, London), 0.2 μ M each primer, and 1.25 U of Biotaq DNA polymerase (Bioline). Amplifications were performed in a Veriti thermal cycler (Applied Biosystems, Life Technologies, Foster City, CA) programmed according to the corresponding author. The SCAR PCR products were resolved on 2% agarose gels. The SSR PCR products were resolved on 8% polyacrylamide gels, except for marker BM172, in which infrared dye labeled forward primer (IRD-700) and an automated sequencer with fluorometric detection (LiCor 4300 DNA analyzer) was used. Agarose and polyacrylamide gels were stained with SYBR safe (Invitrogen, Life Technologies, CA) and visualized under UV light.

Inoculation procedure and disease scoring. Eight different races of *C. lindemuthianum* were used: races 7, 31, 81, 453, 449, and 1545 from the collection of the Crop and Soil Sciences Department (Michigan State University), and races 3 and 19 from the collection of the SERIDA (Villaviciosa, Asturias, Spain). The virulence and avirulence patterns of the *C. lindemuthianum* races used in this work on the standard set of 12 common bean differential cultivars are shown in Table 2. Isolates of each race were obtained from monospore cultures maintained in fungus-colonized filter paper at -20°C for long-term storage. To obtain

TABLE 1. Molecular markers analyzed in the Kaboon $\times$ Michelite F₂ population^a

Marker name	Type ^b	LG	Linked to ^c	Reference	Parental phenotypes ^d		χ^2	Probability
					Kaboon	Michelite		
OF10 ₅₃₀	RAPD	1	<i>Co-1</i>	51,60	...	530	0.46	0.50
AT003	SSR	1	...	8	125	135	4.62	0.10
BMd45-AIA	SSR	1	<i>fin</i>	9,45	250	200	6.61	0.04
BM200	SSR	1	...	9	221	...	5.60	0.02
BM167	SSR	2	...	9	165	...	0.06	0.81
BM172	SSR	3	...	9	112	77	3.69	0.16
BMd26	SSR	4	...	9	135	140	7.36	0.03
ATA20	SSR	4	...	8	...	150	1.38	0.24
BM140	SSR	4	...	9	180	160	2.89	0.24
OF10 ₁₁₀₀	RAPD	4	<i>Co-10</i>	3,30	1,100	...	0.19	0.66
SW12	SCAR	4	<i>Co-3/9</i>	40,43	700	...	1.52	0.22
SB10	SCAR	4	HBB	17	525	...	0.97	0.32
PV-ctt001	SSR	4	<i>Co-3/9</i>	9,51	162	175	0.42	0.81
SI19	SCAR	4	<i>Ur-5</i>	37,43	460	...	0.06	0.81
BM210	SSR	7	<i>Co-5</i>	9,10	...	170	2.64	0.10
Phs	SCAR	7	<i>Co-5</i>	10,29	300	...	0.06	0.81
SC08	SCAR	8	<i>Co-4</i>	9,48	910	...	0.24	0.62
PV-ag001	SSR	11	<i>Co-2</i>	9,47	160	150	3.77	0.15

^a Marker type, linkage group (LG) in which the marker was located, and gene to which the marker has been described to be linked are indicated. Results of the comparison between the segregations of the molecular markers and the corresponding expected monogenic segregations are shown (the detailed results can be obtained upon request).

^b RAPD = random amplified polymorphic DNA, SSR = simple-sequence repeat, and SCAR = sequence-characterized amplified region.

^c *Co*- = anthracnose resistance gene, HBB = halo bacterial blight (*Pseudomonas syringae* pv. *phaseolicola*), *Ur-5* = rust (*Uromyces appendiculatus*) resistance gene, and *fin* = determinate growth habit gene.

^d For DNA molecular markers, the parental phenotype refers to the size of the amplified products, expressed in base pairs.

abundant sporulation, all races were grown at 19 to 21°C in darkness for ≈10 days in potato dextrose agar (Difco, Becton Dickinson and Company, Sparks, MD). Spore suspensions were prepared by flooding the plates with 5 ml of 0.01% Tween 20 (Sigma-Aldrich, St. Louis) in sterile distilled water and scraping the surface of the culture with a spatula. Differential cultivars and F₃ and F₄ families were inoculated with a spore suspension of the pathogen at 1.2 × 10⁶ spores/ml. 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 to 22°C, 95 to 100% humidity, and a 12-h photoperiod. The responses of the plants were evaluated after 7 to 9 days using a 1-to-9 scale (56). Seedlings with no visible symptoms (severity value 1) or showing limited necrotic lesions (severity values 2 to 3) were considered resistant. Seedlings with large sporulating lesions (severity values 4 to 8) or dead (severity value 9) were considered susceptible. Resistance to each race was evaluated in separate experiments. Each experiment consisted on one evaluation including the parental differential cultivars Kaboon and Michelite, 10 to 20 plants per F₃ family or F₄ family, and the remaining 10 common bean anthracnose differential cultivars used to confirm the identity of the *C. lindemuthianum* isolates.

Statistical and linkage analyses. To test goodness-of-fit of observed/expected ratios in the F₂ population Kaboon × Michelite and in the corresponding F₃ progenies, χ^2 was used. Linkage analyses between the markers and the resistance genes were performed using MAPMAKER (Macintosh version 2.0) (32). LGs were established with a log of the likelihood ratio (LOD) threshold of 3.0 and a recombination fraction of 0.25. Marker order was estimated based on multipoint compare, order, and ripple analyses. Distances between ordered loci (in centimorgans) were calculated using the Kosambi mapping function.

RESULTS

Segregations of anthracnose resistance specificities. Parental Kaboon was resistant to races 3, 7, 19, 31, 81, 449, 453, and 1545 of *C. lindemuthianum*, whereas parental Michelite was susceptible to all these races. For each anthracnose race, the F₃ Kaboon × Michelite families were classified as R, having all individuals resistant (all individuals resistant to race X = R^X); R/S, having individuals resistant and susceptible (individuals resistant and susceptible to race X = R/S^X); and S, having all individuals susceptible (all individuals susceptible to race X = S^X). Segregations of F₃ families for resistance to races, 3, 7, 19, 31, 81, 449, 453, and 1545 are shown in Table 3. Segregations for resistance to races 3, 7, 19, and 81 showed a good fit to the expected ratio for a single dominant gene (1:2:1, R:R/S:S). Segregations for resistance to races 449, 453, and 1545 showed no significant differences when compared with the expected ratio for two independent dominant genes (7:8:1, R:R/S:S). Segregation for resistance to race 31 showed a good fit to the expected ratio for resistance being determined by the simultaneous presence of two dominant independently located genes (two complementary genes = 1:8:7, R:R/S:S). In Table 3, the observed segregation for resistance to race 1545 is also compared with the expected ratio for three independent dominant genes (37:26:1, R:R/S:S). In this case, the highly significant difference found is mainly due to the very low expected number (1.3) of F₃ families having all individuals susceptible (S), a good fit being attained in the other two classes (R and R/S). Notwithstanding, there are some reasons that support the possibility that resistance to race 1545 is determined by three independent dominant genes (see below).

Segregations of molecular markers. The results of the comparison between the segregations of the molecular markers and the corresponding expected monogenic segregations are shown in

TABLE 2. Virulence and avirulence patterns of the *Colletotrichum lindemuthianum* races used in this work (S = susceptible and R = resistant)

Differential cultivar	Binary value	Race							
		3	7	19	31	81	449	453	1545
Michelite	1	S	S	S	S	S	S	S	S
MDRK	2	S	S	S	S	R	R	R	R
Perry Marrow	4	R	S	R	S	R	R	S	R
Cornell 49242	8	R	R	R	S	R	R	R	S
Widusa	16	R	R	S	S	S	R	R	R
Kaboon	32	R	R	R	R	R	R	R	R
Mex222	64	R	R	R	R	S	S	S	R
PI207262	128	R	R	R	R	R	S	S	R
TO	256	R	R	R	R	R	S	S	R
TU	512	R	R	R	R	R	R	R	S
AB136	1,024	R	R	R	R	R	R	R	S
G2333	2,048	R	R	R	R	R	R	R	R

TABLE 3. Segregation for resistance to races 3, 7, 19, 31, 81, 449, 453, and 1545 of *Colletotrichum lindemuthianum* in the F₃ Kaboon × Michelite families

Race ^a	Parental phenotypes ^b		F ₃ Kaboon × Michelite families ^c			Each segregation tested against	χ^2	P
	Kaboon	Michelite	R	R/S	S			
3	R	S	17	52	22	One dominant gene	2.41	0.30
7	R	S	15	53	22	One dominant gene	3.93	0.14
19	R	S	17	56	23	One dominant gene	3.42	0.18
31	R	S	4	28	35	Two complementary genes	2.01	0.37
81	R	S	16	29	24	One dominant gene	3.61	0.16
449	R	S	41	41	10	Two independent dominant genes	3.70	0.16
453	R	S	38	41	10	Two independent dominant genes	3.84	0.15
1545	R	S	45	32	7	Two independent dominant genes	4.82	0.09
1545	R	S	45	32	7	Three independent dominant genes	25.04	0.00

^a For races 3, 7, 19, and 81, the expected ratio was 1:2:1 R:R/S:S (one dominant gene); for races 449 and 453, the expected ratio was 7:8:1 R:R/S:S (two independent dominant genes); and for race 31 the expected ratio was 1:8:7 R:R/S:S (two independent complementary dominant genes). Segregation of resistance to race 1545 is compared with expected ratios 7:8:1 R:R/S:S (two independent dominant genes) and 37:26:1 R:R/S:S (three independent dominant genes).

^b R = resistant and S = susceptible.

^c Number observed for R = F₃ families having all individuals resistant, R/S = F₃ families having individuals resistant and susceptible, and S = F₃ families having all individuals susceptible.

Table 1 (the detailed results can be obtained upon request). A good fit to the expected ratio for a single locus was obtained in all cases, except for the microsatellites Bmd45-AIA, BM200, and Bmd26, in which a significant excess of the alleles corresponding to parental Michelite was observed.

Linkage analyses. Resistance to races 3, 7, 19, and 81 (showing monogenic segregation). Resistance to race 81 segregated independently from resistance to races 3, 7, and 19 (the values of the contingency χ^2 tests are 6.07, 4.74, and 5.97, respectively, $P > 0.05$ in the three cases). The results of the linkage analysis between the locus conferring specific resistance to race 81 and each one of the DNA markers indicated in Table 1 are shown in Table 4. This resistance locus is significantly linked (LOD >3) to the molecular markers previously located in LG 1, OF10₅₃₀ (linked to *Co-1*), and AT003 (8,51,60). From these results, it can be concluded that resistance to race 81 present in Kaboon is conferred by a gene corresponding to the *Co-1* locus.

Resistance to the three races 3, 7, and 19 was evaluated in 88 F₃ families (the remaining 11 F₃ families could only be evaluated for one or two of these races). Co-segregation for resistance to the three races was observed in 85 of these F₃ families (14 F₃ families = R³ R⁷ R¹⁹, 49 F₃ families = R/S³ R/S⁷ R/S¹⁹, and 22 F₃ families = S³ S⁷ S¹⁹) and only in three cases was evidence of recombination found (two F₃ families = R³ R/S⁷ R¹⁹ and one F₃ family = R³ R⁷ R/S¹⁹). The recombinant nature of these F₃ families was confirmed by increasing the number of F₃ individuals evaluated for resistance. These results indicate that three different race-specific, closely linked genes determine the resistance to races 3, 7, and 19, respectively. The results of the linkage analysis between the locus conferring specific resistance to race 3 and each one of the DNA markers indicated in Table 1 are shown in Table 5 (values almost identical to the ones shown in Table 5 were obtained for loci conferring specific resistance to races 7 and 19). These resistance loci are significantly linked (LOD >3) to the molecular markers located in LG 4, OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19, most of them previously linked to *Co-3/9* (17,40,43,50,51,62). This indicates that the three clustered genes conferring specific resistance to races 3, 7, and 19, respectively, correspond to the *Co-3/9* locus.

The genetic map of LG 1 and LG 4 obtained from the segregation data is shown in Figure 1.

Resistance to races 449, 453, and 1545. The results shown in Table 3 indicate that resistance to race 449 and resistance to race 453 are conferred by two independent dominant genes each. Resistance to these two races was evaluated in 88 F₃ families (the remaining 11 F₃ families could not be evaluated for both races). Co-segregation for resistance to races 449 and 453 was observed in 83 of these F₃ families (35 F₃ families = R⁴⁴⁹ R⁴⁵³, 38 F₃ families = R/S⁴⁴⁹ R/S⁴⁵³, and 10 F₃ families = S⁴⁴⁹ S⁴⁵³) and only in five cases was evidence of recombination found (3 F₃ families = R⁴⁴⁹ R/S⁴⁵³ and 2 F₃ families = R/S⁴⁴⁹ R⁴⁵³). The recombinant nature of these F₃ families was confirmed by increasing the number of F₃ individuals evaluated for resistance. This result agrees with the assumption that resistance to races 449 and 453 in Kaboon is determined by two independent clusters, each one containing two closely linked race-specific genes, one of these genes conferring resistance to race 449 and the other conferring resistance to race 453.

Contingency χ^2 tests corresponding to the joint segregation of resistance to races 449 and 453, respectively, with each one of the resistances to monogenic races 3, 7, 19, and 81 and with the molecular markers appearing in Table 1 are shown in Table 6. For both races, the χ^2 values deviate significantly from the expectation of random segregation when monogenic races 3, 7, and 19 and markers linked to the *Co-3/9* locus (OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19) are considered. These results indicate that one of the two independent clusters including specific resistance genes against races 449 and 453 corresponds to the *Co-3/9* locus.

Contingency χ^2 tests corresponding to the joint segregation of resistance to race 1545 with each one of the resistances to monogenic races 3, 7, 19, and 81 and with the markers appearing in Table 1 are shown in Table 6. The χ^2 values deviate significantly from the expectation of random segregation when monogenic races 3, 7, 19, and 81 and markers linked to the *Co-1* (OF10₅₃₀) and the *Co-3/9* loci (OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19) are considered. These results indicate that one of the genes conferring resistance to race 1545 corresponds to locus *Co-1*, and that a second gene corresponds to locus *Co-3/9*.

A specific F₃ family (F₃-28) was analyzed in more detail. The F₃-28 family had the resistance spectrum S³ S⁷ S¹⁹ S⁸¹ R/S⁴⁴⁹ R/S⁴⁵³ R/S¹⁵⁴⁵ and was homozygous for the alleles corresponding to parental Michelite for the markers closely linked to cluster

TABLE 4. Linkage analysis between the gene conferring resistance to race 81 and each one of the DNA markers indicated in Table 1^a

		F ₃ Kaboon × Michelite families																
Markers		R ⁸¹					R/S ⁸¹					S ⁸¹						
Name	LG ^b	K/K	K/–	K/M	M/–	M/M	K/K	K/–	K/M	M/–	M/M	K/K	K/–	K/M	M/–	M/M	RF	LOD
OF10 ₅₃₀	1	10	...	...	3	...	3	...	...	23	...	0	...	...	23	...	0.11	6.71
AT003	1	7	...	5	...	2	0	...	20	...	4	2	...	7	...	13	0.23	4.63
BMd45-AIA	1	4	...	7	...	3	1	...	18	...	7	1	...	9	...	13	0.30	2.54
BM200	1	...	10	...	...	4	...	18	...	...	7	...	11	...	...	12	0.37	0.89
BM167	2	...	10	...	...	4	...	18	...	...	8	...	18	...	...	5	0.50	0.00
BM172	3	1	...	9	...	4	11	...	8	...	7	6	...	8	...	8	0.50	0.00
BMd26	4	3	...	4	...	7	3	...	9	...	14	8	...	11	...	4	0.50	0.00
ATA20	4	2	...	...	12	...	4	...	...	22	...	7	...	...	16	...	0.50	0.00
BM140	4	2	...	9	...	3	4	...	13	...	9	5	...	10	...	7	0.48	0.01
OF10 ₁₁₀₀	4	...	11	...	...	2	...	20	...	...	6	...	16	...	...	7	0.42	0.20
SW12	4	...	12	...	...	2	...	18	...	...	8	...	13	...	...	10	0.36	0.92
SB10	4	...	12	...	...	2	...	18	...	...	8	...	14	...	...	9	0.38	0.63
PV-ctt001	4	6	...	6	...	2	4	...	17	...	5	6	...	11	...	6	0.43	0.24
SI19	4	...	13	...	...	1	...	20	...	...	6	...	17	...	...	6	0.41	0.25
BM210	7	3	...	...	8	...	7	...	...	16	...	7	...	...	13	...	0.50	0.00
Phs	7	...	9	...	...	5	...	21	...	...	5	...	15	...	...	8	0.47	0.02
SC08	8	...	9	...	...	5	...	22	...	...	4	...	17	...	...	6	0.50	0.00
PV-ag001	11	5	...	5	...	4	6	...	12	...	8	3	...	9	...	10	0.40	0.73

^a R⁸¹ = F₃ families having all individuals resistant to race 81, R/S⁸¹ = F₃ families having individuals resistant and susceptible to race 81, and S⁸¹ = F₃ families having all individuals susceptible to race 81. K/K = homozygous for the Kaboon alleles of the corresponding marker, K/– = homozygous for the Kaboon alleles or heterozygous, K/M = heterozygous, M/– = homozygous for the Michelite alleles or heterozygous, and M/M = homozygous for the Michelite alleles. Linkage values are expressed as recombination fraction (RF) and log of the likelihood ratio (LOD).

^b Linkage group in which the marker is located.

Co-1 (OF10₅₃₀ and AT003) and for the markers flanking the *Co-3/9* cluster (OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19). Then, most probably, the Kaboon haplotype of cluster *Co-1*, bearing the genes conferring resistance to races 81 and 1545, and the Kaboon haplotype of cluster *Co-3/9*, bearing the genes conferring resistance to races 3, 7, 19, 449, 453, and 1545, were lacking in the F₃-28 family, the segregation for resistance to races 449, 453, and 1545 shown by this family being due to heterozygosity in the second cluster carrying resistance to races 449 and 453, indicated before, and to a third gene conferring resistance to race 1545. In total, 46 individuals of the F₃-28 family were selfed and their progenies (F₄-28 families) were analyzed for resistance to races 449, 453, and 1545. Monogenic segregations were observed for the three resistance specificities (race 449: 10 R, 28 R/S, 8 S, $\chi^2 = 2.35$, $P = 0.31$; race 453: 6 R, 27 R/S, 6 S, $\chi^2 = 5.77$, $P = 0.06$; and race 1545: 6 R, 22 R/S, 8 S, $\chi^2 = 2.00$, $P = 0.37$), and the analysis of the recombination fractions (RFs) between the corresponding three resistance loci indicated that they were significantly linked (RF₄₄₉₋₄₅₃ = 0.10, LOD = 6.73; RF₄₄₉₋₁₅₄₅ = 0.17, LOD = 3.46; RF₄₅₃₋₁₅₄₅ = 0.20, LOD = 2.05). These results support the idea that, in addition to *Co-1* and *Co-3/9*, Kaboon has a third cluster bearing genes for specific resistance to races 449, 453, and 1545, with the gene conferring resistance to race 449 occupying the central position.

In order to map this cluster, its linkage relationship to 55 molecular markers (different from those indicated in Table 1), previously mapped in all LGs, was tested in the F₃-28 family. Parental Kaboon and Michelite were polymorphic for 33 of these markers, 19 of them being monomorphic in the F₃-28 family and the remaining 14 being not linked to the resistance cluster (more detailed data concerning these analyses are available upon request).

Resistance to race 31. The results shown in Table 3 indicate that two independent complementary genes are necessary for resistance to race 31 in Kaboon. Contingency χ^2 tests corresponding to the joint segregation of resistance to race 31 with each one of the resistances to monogenic races, 3, 7, 19, and 81 and with the markers appearing in Table 1 are shown in Table 6. The χ^2 values deviate significantly from the expectation of random segregation when monogenic races 3, 7, 19, and 81, and markers linked to the *Co-1* (OF10₅₃₀, AT003, and BMd45) and the

Co-3/9 loci (BM140, OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19) are considered. From these results, it can be inferred that one of the complementary genes conferring resistance to race 31 corresponds to locus *Co-1* and the other corresponds to locus *Co-3/9*.

The resistance spectrum of 13 of the 99 F₃ families analyzed showed evidence of intracluster recombination between either the genes conferring resistance to races 31 and 81 in cluster *Co-1* (for instance, S⁸¹ R³ R⁷ R¹⁹ R/S³¹) or between the gene conferring resistance to race 31 and the genes conferring resistance to races 3, 7, and 19, in cluster *Co-3/9* (for instance, R⁸¹ S³ S⁷ S¹⁹ R/S³¹). These results indicate that the complementary genes that determine resistance to race 31 are different from the genes that determine resistance to races 81, 3, 7, and 19.

In order to confirm the complementary mode of action of the two loci conferring resistance to race 31, the F₃ families F₃-64 and F₃-105 were used. The F₃ family F₃-64 had the resistance spectrum S³¹ R⁸¹ S³ S⁷ S¹⁹ and was homozygous for the alleles

Fig. 1. Linkage map of the genes conferring specific resistance to anthracnose races 3 (R³), 7 (R⁷), 19 (R¹⁹), 31 (R³¹), 81 (R⁸¹), 449 (R⁴⁴⁹), 453 (R⁴⁵³), and 1545 (R¹⁵⁴⁵) in linkage groups 1 (corresponding to cluster *Co-1*) and 4 (corresponding to cluster *Co-3/9*). Relative positions of the genes R³¹ and R¹⁵⁴⁵ within the *Co-1* cluster, as well as those of the genes R³¹, R⁴⁴⁹, R⁴⁵³, and R¹⁵⁴⁵ within the *Co-3/9* cluster, are undetermined. Map distances, on the left, are expressed in centimorgans, estimated using the Kosambi mapping function.

TABLE 5. Linkage analysis between the gene conferring resistance to race 3 and each one of the DNA markers indicated in Table 1^a

		F ₃ Kaboon × Michelite families																
Markers		R ³					R/S ³					S ³						
Name	LG ^b	K/K	K/–	K/M	M/–	M/M	K/K	K/–	K/M	M/–	M/M	K/K	K/–	K/M	M/–	M/M	RF	LOD
OF10 ₅₃₀	1	4	...	...	12	...	12	...	...	38	...	3	...	...	17	...	0.43	0.13
AT003	1	2	...	5	...	8	9	...	29	...	12	3	...	9	...	7	0.50	0.00
BMd45-AIA	1	3	...	4	...	9	7	...	30	...	14	3	...	11	...	6	0.50	0.00
BM200	1	...	7	...	...	9	...	34	...	...	16	...	14	...	...	5	0.50	0.00
BM167	2	...	12	...	...	4	...	39	...	...	12	...	13	...	...	7	0.44	0.12
BM172	3	3	...	8	...	4	19	...	20	...	12	6	...	7	...	7	0.50	0.00
BMd26	4	4	...	8	...	4	13	...	19	...	19	2	...	8	...	10	0.42	0.56
ATA20	4	5	...	...	11	...	11	...	...	39	...	1	...	...	19	...	0.34	0.92
BM140	4	5	...	7	...	4	11	...	27	...	12	1	...	7	...	12	0.34	1.83
OF10 ₁₁₀₀	4	...	16	...	...	0	...	47	...	...	3	...	3	...	...	17	0.07	11.64
SW12	4	...	14	...	...	2	...	46	...	...	5	...	1	...	...	19	0.11	9.66
SB10	4	...	16	...	...	0	...	45	...	...	6	...	0	...	...	20	0.06	13.55
PV-ctt001	4	15	...	1	...	0	3	...	44	...	2	2	...	0	...	18	0.06	21.49
SI19	4	...	16	...	...	0	...	46	...	...	5	...	4	...	...	16	0.10	9.36
BM210	7	0	...	...	13	...	21	...	...	27	...	5	...	...	13	...	0.50	0.00
Phs	7	...	11	...	...	5	...	44	...	...	7	...	11	...	...	9	0.38	0.36
SC08	8	...	8	...	...	8	...	40	...	...	11	...	15	...	...	5	0.50	0.00
PV-ag001	11	3	...	6	...	5	12	...	21	...	18	6	...	8	...	6	0.50	0.00

^a R³ = F₃ families having all individuals resistant to race 3, R/S³ = F₃ families having individuals resistant and susceptible to race 3, and S³ = F₃ families having all individuals susceptible to race 3. K/K = homozygous for the Kaboon alleles of the corresponding marker, K/- = homozygous for the Kaboon alleles or heterozygous, K/M = heterozygous, M/- = homozygous for the Michelite alleles or heterozygous, and M/M = homozygous for the Michelite alleles. Linkage values are expressed as recombination fraction (RF) and log of the likelihood ratio (LOD).

^b Linkage group in which the marker is located.

corresponding to parental Kaboon for the markers closely linked to cluster *Co-1* (OF10₅₃₀ and AT003) and for the alleles corresponding to parental Michelite for the markers flanking the *Co-3/9* cluster (OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19). It can be expected that, in this family, the complementary gene conferring resistance to race 31 located in cluster *Co-1* is present in homozygous condition, and the one located in cluster *Co-3/9* is lacking. The F₃ family F₃-105 had the resistance spectrum S³¹ S⁸¹ R³ R⁷ R¹⁹ and was homozygous for the alleles corresponding to parental Michelite for the markers closely linked to cluster *Co-1* (OF10₅₃₀ and AT003) and for the alleles corresponding to parental Kaboon for the markers flanking the *Co-3/9* cluster (OF10₁₁₀₀, SW12, SB10, PV-ctt001, and SI19). In this family, the opposite situation to that of F₃-64 can be expected: the complementary gene conferring resistance to race 31 located in cluster *Co-3/9* would be present in the homozygous condition whereas the one located in cluster *Co-1* would be lacking. Then, the offspring of the cross F₃-46 × F₃-105 would be heterozygous for the two complementary genes conferring resistance to race 31 located in clusters *Co-1* and *Co-3/9*, respectively, and would be resistant to this race. The results obtained confirmed this hypothesis. The offspring obtained by self-pollination of individual plants of families F₃-64 and F₃-105 were susceptible to race 31, whereas the offspring obtained from crosses between such plants (F₃-64 × F₃-105) were resistant to this race.

DISCUSSION

Our results indicate that resistance to the eight *C. lindemuthianum* races 3, 7, 19, 31, 81, 449, 453, and 1545, present in Kaboon, is determined by 13 different race-specific genes grouped in three clusters. One of these clusters, located in LG 1, corresponding to locus *Co-1*, carries two dominant genes conferring specific resistance to races 81 and 1545, respectively, and a gene necessary (dominant complementary gene) for the specific resistance to race 31. A second cluster, located in LG 4, corresponding to locus *Co-3/9*, carries six dominant genes conferring specific resistance to races 3, 7, 19, 449, 453, and 1545, respectively, and the second dominant complementary gene for the specific resistance to race 31. A third cluster of unknown location, segregating independently from molecular markers

linked to *Co-2* (PV-ag001) (47), *Co-4* (SC08) (48), and *Co-5* (BM210 and Phs) (10), carries three dominant genes conferring specific resistance to races 449, 453, and 1545, respectively. Disease resistance loci in plants are frequently organized as clusters of genes, each one conferring resistance to a different pathogen or to a specific race of the same pathogen (13,42,53,57). The results obtained in the present work agree with those obtained in other common bean genotypes (10,50,51) and suggest that *Co*-loci, contrary to the previous assumption of being formed by single major genes conferring resistance to several anthracnose races (31), are also organized as clusters of race-specific resistance genes.

Among all described anthracnose resistance loci, *Co-1* and, recently, the unmapped *Co-12* and *Co-13* genes, have been considered the only loci originating in the Andean gene pool (23,31). However, these are not the only anthracnose resistance loci present in Andean materials. In previous works, Geffroy (19) and Geffroy et al. (22), using the Jalo EEP558 × BAT93 recombinant inbred line population, located Andean resistance genes (*Co-x* and *Co-w*) at the end of LG 1 (coinciding with *Co-1*), and both Andean (Jalo EEP558) and Mesoamerican (BAT93) resistance specificities at LG 4 (*Co-y*, *Co-z*, and *Co-9*), corresponding to the *Co-3/9* locus. They concluded that this locus existed prior to the separation of the two major gene pools of *P. vulgaris*. In agreement with the results of Geffroy et al. (22), Campa et al. (10) demonstrated that the Andean cultivar MDRK carries different anthracnose race-specific genes at both the *Co-1* and *Co-3/9* loci. The results obtained in this work provide additional evidence supporting the presence of Andean anthracnose resistance genes at these loci.

The resistance of Kaboon to anthracnose races 7 and 73 was studied by Melotto and Kelly (38). After inoculating the F₂ populations MDRK × Kaboon and Cardinal × Kaboon (susceptible × resistant crosses, respectively) with race 7, a segregation ratio of 3:1 R:S was observed in both cases, indicating that a single dominant gene in Kaboon confers resistance to race 7. After inoculating the F₂ population Cardinal × Kaboon (susceptible × resistant cross) with a mixture of races 7 and 73, the same 3:1 R:S ratio was observed, indicating that the same gene in Kaboon confers resistance to races 7 and 73. A lack of segregation was observed after inoculating the F₂ population MDRK × Kaboon

TABLE 6. Contingency χ^2 tests corresponding to the joint segregations for each one of the resistances to races 449, 453, 1545, and 31 with each one of the monogenic resistance to races 3, 7, 19, and 81 and with each one of the different markers^a

Locus or marker	LG	Race 449		Race 453		Race 1545		Race 31	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Race 3	4	61.87	**	60.41	**	39.14	**	10.46	*
Race 7	4	59.20	**	60.41	**	39.09	**	10.65	*
Race 19	4	61.23	**	59.71	**	38.47	**	16.04	**
Race 81	1	4.76	n.s.	3.02	n.s.	20.78	**	28.13	**
OF10 ₅₃₀	1	2.68	n.s.	2.69	n.s.	15.35	**	8.32	*
AT003	1	3.39	n.s.	2.30	n.s.	9.38	n.s.	15.70	**
BMd45-AIA	1	3.56	n.s.	1.49	n.s.	3.25	n.s.	13.69	**
BM200	1	0.97	n.s.	0.48	n.s.	1.34	n.s.	2.45	n.s.
BM167	2	0.16	n.s.	0.12	n.s.	0.15	n.s.	3.11	n.s.
BM172	3	8.57	n.s.	8.07	n.s.	9.46	n.s.	2.16	n.s.
BMd26	4	2.62	n.s.	2.91	n.s.	1.19	n.s.	6.62	n.s.
ATA20	4	3.21	n.s.	2.99	n.s.	0.30	n.s.	3.98	n.s.
BM140	4	7.25	n.s.	7.12	n.s.	2.23	n.s.	24.21	**
OF10 ₁₁₀₀	4	28.61	**	27.03	**	17.54	**	8.59	*
SW12	4	26.40	**	30.08	**	14.75	**	8.79	*
SB10	4	35.51	**	33.33	**	24.53	**	7.58	*
PV-ctt001	4	43.67	**	41.77	**	36.56	**	10.29	*
SI19	4	23.64	**	21.92	**	10.90	**	6.61	*
BM210	7	5.55	n.s.	3.10	n.s.	2.66	n.s.	0.62	n.s.
Phs	7	4.12	n.s.	3.89	n.s.	5.41	n.s.	2.17	n.s.
SC08	8	1.77	n.s.	1.47	n.s.	0.53	n.s.	0.66	n.s.
PV-ag001	11	5.36	n.s.	2.89	n.s.	1.04	n.s.	4.16	n.s.

^a LG = linkage group in which the locus or marker is located, n.s. = not significant, * indicates 0.05 > P > 0.01, and ** indicates 0.01 > P.

(resistant $\times$ resistant cross) with race 73. Under the assumption that *Co-1* was the only anthracnose resistance locus present in MDRK, Melotto and Kelly (36) concluded that resistance against races 7 and 73 in Kaboon is conferred by an allele of the *Co-1* locus. In the present work, the gene conferring resistance to race 7 of Kaboon has been directly mapped at the *Co-3/9* cluster, in LG 4. The results of Melotto and Kelly (38) could be reinterpreted by assuming that the genes conferring resistance to race 73 in MDRK and in Kaboon, respectively, are located at the *Co-3/9* locus not at the *Co-1* locus. The presence of two anthracnose resistance genes against races 449 and 1545 at the *Co-3/9* cluster in MDRK was demonstrated by Campa et al. (10). The lack of segregation of resistance to race 73 in the F_2 population Jalo EEP558 $\times$ MDRK (resistant $\times$ resistant cross), observed by Vallejo et al. (55), can also be interpreted under the assumption that resistance to this race is located at the *Co-3/9* locus in both genotypes. Geffroy et al. (21) demonstrated the presence of several anthracnose resistance specificities at the *Co-3/9* locus in Jalo EEP558. The confirmation of the *Co-3/9* location of resistance to race 73 in MDRK is of great interest because new *Co-* genes have been described on some occasions (3,23) based on the results of allelism tests in which it was assumed that resistance to race 73 in MDRK was due to the *Co-1* gene.

The results of different segregation analyses for resistance to races β (130), γ (102), and Δ (23), carried out by Muhalet et al. (44), suggested that Kaboon carries two dominant complementary genes conferring resistance to race β , one dominant complementary gene to race γ (a second complementary gene being present in the genotype Cornell 49242 as well as in the genotype Tuscola), and two dominant complementary genes conferring resistance to race Δ . Also, the segregations 57:7 R:S obtained by Melotto and Kelly (38) in the F_2 from the crosses Perry Marrow (S) $\times$ Kaboon (R) and Cardinal (S) $\times$ Kaboon (R) suggested that resistance to race 5 in Kaboon is conferred by three dominant independent genes, two of them having a complementary mode of action. The presence in common bean of anthracnose resistance genes with a complementary mode of action has been also suggested in other instances (31) but no attempts were made for mapping these genes, and their relationship with previously known *Co-* resistance genes was not established. The results obtained in this work confirm the presence in Kaboon of at least two anthracnose resistance genes with a complementary mode of action. One of these genes has been mapped at the *Co-3/9* anthracnose resistance cluster in LG 4 and the other has been located at the *Co-1* anthracnose resistance cluster in LG 1. Complementary resistance genes required for resistance have been described in common bean (against the bean rust pathogen *Uromyces appendiculatus*) (28), tomato (26,52), *Arabidopsis* (58), and barley (27).

The 1:8:7 R:R/S:S segregation observed in this work for resistance to a specific anthracnose race is evidence of the presence of two dominant, independent complementary loci being responsible for that anthracnose resistance. Nevertheless, the 1:2:1 R:R/S:S segregation found in other cases does not preclude the possibility for a two-gene system to be of general occurrence in the anthracnose resistance response in common bean. A segregation corresponding to a single gene (1:2:1 R:R/S:S) would be observed if the parental cultivars differed in only one of the two genes or, alternatively, if, although differing in the two genes, they are closely linked in the same cluster.

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