Theor Appl Genet (2009) 119:1–11
DOI 10.1007/s00122-009-1011-8

ORIGINAL PAPER

Genetic dissection of the resistance to nine anthracnose races in the common bean differential cultivars MDRK and TU

Ana Campa · Ramón Giraldez · Juan José Ferreira

Received: 22 July 2008 / Accepted: 8 March 2009 / Published online: 25 March 2009
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Abstract Resistance to nine races of the pathogenic fungus *Colletotrichum lindemuthianum*, causal agent of anthracnose, was evaluated in F_3 families derived from the cross between the anthracnose differential bean cultivars TU (resistant to races, 3, 6, 7, 31, 38, 39, 102, and 449) and MDRK (resistant to races, 449, and 1545). Molecular marker analyses were carried out in the F_2 individuals in order to map and characterize the anthracnose resistance genes or gene clusters present in these two differential cultivars. The results of the combined segregation indicate that at least three independent loci conferring resistance to anthracnose are present in TU. One of them, corresponding to the previously described anthracnose resistance locus *Co*-5, is located in linkage group B7, and is formed by a cluster of different genes conferring specific resistance to races, 3, 6, 7, 31, 38, 39, 102, and 449. Evidence of intra-cluster recombination between these specific resistance genes was found. The second locus present in TU confers specific resistance to races 31 and 102, and the third locus confers specific resistance to race 102, the location of these two loci remains unknown. The resistance to race 1545 present in MDRK is due to two independent dominant genes. The results of the combined segregation of two F_4 families showing monogenic segregation for resistance to race 1545 indicates that one of these two genes is linked to marker $OF10_{530}$, located in linkage group B1, and corresponds to the previously described anthracnose resistance locus *Co*-1. The second gene conferring resistance to race 1545 in MDRK is linked to marker Pv-ctt001, located in linkage group B4, and corresponds to the *Co*-3/*Co*-9 cluster. The resistance to race 449 present in MDRK is conferred by a single gene, located in linkage group B4, probably included in the same *Co*-3/*Co*-9 cluster.

Communicated by D. Hoisington.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1011-8) contains supplementary material, which is available to authorized users.

A. Campa · J. J. Ferreira
Area de Cultivos Hortofrutícolas y Forestales,
SERIDA, Apdo. 13, 33300 Villaviciosa, Asturias, Spain

R. Giraldez (✉)
Department of Biología Funcional, University of Oviedo,
33006 Oviedo, Spain
e-mail: giraldez@uniovi.es

Introduction

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is one of the most widespread and economically important diseases of common bean (*Phaseolus vulgaris* L.). Currently, 13 anthracnose resistance genes, designated as *Co*- (*Co*-1 to *Co*-13), have been described in this species. Some of these genes have been located in the integrated linkage map (Freyre et al. 1998; Kelly and Vallejo 2004), either by direct mapping or indirectly using molecular markers: *Co*-1 on linkage group B1 (Barrus 1915; Méndez de Vigo 2001; Rodríguez-Suárez et al. 2007; Geffroy et al. 2008; Vallejo and Kelly 2008), *Co*-2 on B11 (Mastenbroek 1960; Adam-Blondon et al. 1994), *Co*-3 and *Co*-9, demonstrated to be allelic (*Co*-3/*Co*-9), located on B4 (Bannerot 1965; Geffroy et al. 1999; Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2007, 2008), *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 1996; Kelly et al. 2003), and *Co*-10 on B4 (Alzate-Marin et al. 2003; Kelly

et al. 2003). Recently, an anthracnose resistance gene provisionally designed as *Co-u* has been mapped on B2 (Geffroy et al. 2008).

Broad genetic variability for *C. lindemuthianum* has been described worldwide, with more than 100 different races of the pathogen identified (Menezes and Dianese 1988; Sicard et al. 1997; Balardin et al. 1997; Rodríguez-Guerra et al. 2003; Mahuku and Riascos 2004; Ferreira et al. 2008). Identification of *C. lindemuthianum* 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, Cornell49242, Widusa, Kaboon, Mexico222, PI207262, TO, TU, AB136, and G2333, and named based on a binary nomenclature system (Pastor-Corrales 1991).

Although the genetic characterization of the anthracnose resistance present in these differential cultivars is of major importance, it has not been fully elucidated in all cases. *Co*-1 has been reported to be the only anthracnose resistance gene present in the differential cultivar MDRK (Melotto et al. 2000); *Co*-1 and other unspecified complementary genes have been reported to be present in Perry Marrow and Kaboon (Cardenas et al. 1964; Muhalet et al. 1981; Melotto and Kelly 2000); *Co*-1 and *Co*-3/*Co*-9 have been reported to be present in Widusa (Gonçalves-Vidigal and Kelly 2006; Rodríguez-Suárez et al. 2008) in which the presence of two dominant genes was previously suggested by Bannerot (1965); the *Co*-2 gene was first described in Cornell49242 (Mastenbroek 1960); *Co*-3 (*Co*-3/*Co*-9) was first described in Mexico 222 (Bannerot 1965; Rodríguez-Suárez et al. 2008), in which the presence of a second unidentified dominant resistance gene has been reported (Vallejo and Kelly 2005); the presence of *Co*-3/*Co*-9 (Méndez-Vigo et al. 2005), as well as that of *Co*-4, and other undefined complementary genes (Alzate-Marín et al. 2007) have been described in PI207262; *Co*-4 and *Co*-5 were first described in the differential cultivars TO and TU, respectively (Fouilloux 1976); *Co*-4, *Co*-5, and *Co*-7 were reported to be present in G2333 (Young et al. 1998); *Co*-6 (Young and Kelly 1996), the recessive gene *co*-8 (Alzate-Marin et al. 1997), and a second unspecified independent dominant resistance gene (Campa et al. 2007) have been reported in AB136; finally, *Co*-11 has been described as a new independent resistance gene present in Michelite (Gonçalves-Vidigal et al. 2007), in which at least a recessive resistance gene was previously reported (Cardenas et al. 1964).

The characterization of the genes involved in the anthracnose resistance of most differential cultivars has been studied based on the combined results of different allelism tests (Kelly and Vallejo 2004). In many cases, allelism tests involving the same differential cultivars, conducted by different authors, have not been carried out with the same pathogenic races. This increases the difficulty of the comparison between the different results obtained, since the different races used could overcome different resistance genes present in the same host. These difficulties can be avoided if the characterization of the resistance genes is carried out by direct mapping, using molecular markers. Only some of the resistance genes present in the cultivars, Cornell 49242, G2333, Mexico 222, and Widusa, have been identified using this procedure (Adam-Blondon et al. 1994; Young et al. 1998; Rodríguez-Suárez et al. 2008).

Most of the *Co*-genes were described as single genes conferring dominant resistance (except *co*-8) to several anthracnose races. However, in agreement with the cluster organization of families of resistance gene analogue sequences (RGAs) and/or resistance gene candidates (RGCs), mapping close to some of these genes (Geffroy et al. 1998, 1999; Creusot et al. 1999; Melotto and Kelly 2001; Ferrier-Cana et al. 2003, 2005; Melotto et al. 2004), genetic analyses of joint segregations for resistance to different anthracnose races demonstrated that some of the *Co*-genes are organized as clusters of individual genes conferring race-specific resistance (Geffroy et al. 1999; Rodríguez-Suárez et al. 2007, 2008).

The main objectives of this work were to characterize and to map the genes conferring specific resistance against nine races of anthracnose present in the differential cultivars TU and MDRK, as well as investigating the possibility of these resistance genes being organized as clusters.

Materials and methods

Plant material

Molecular marker analyses were carried out using DNA extracted from 86 F_2 plants derived from the cross between the two anthracnose differential cultivars TU and MDRK. F_3 families were used to characterize the corresponding F_2 plants for resistance to nine races of anthracnose (races, 3, 6, 7, 31, 38, 39, 102, 449, and 1545). In order to characterize the genes conferring resistance to anthracnose race 1545, two F_4 families proceeding from specific F_3 families were analyzed. The resistance to race 1545 was evaluated in 10–20 plants per F_3 or F_4 family.

The remaining 10 common bean anthracnose differential cultivars (Pastor-Corrales 1991) were used to confirm the identity of the *C. lindemuthianum* isolates.

Inoculation procedure and disease scoring

Nine different races of *C. lindemuthianum* were used: races, 7, 31, 39, 449, and 1545, from the collection of the

Crop and Soil Sciences Department (Michigan State University, USA), and races, 3, 6, 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). 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, F_3 families, and F_4 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 1 is no visible symptoms and 9 very severely diseased or dead (van Schoonhoven and Pastor-Corrales 1987).

DNA extraction, PCR amplification, and electrophoresis

Genomic DNA from the cultivars TU and MDRK, from the F_2 TU × MDRK plants, and from the corresponding F_3 plants, was isolated from lyophilized young leaves using the Nucleon™ PhytoPure™ Genomic DNA Extraction Kit (Amersham Biosciences) following the supplier's instructions.

The parental lines, TU and MDRK, were polymorphic for the amplification products obtained from the primers corresponding to SCAR markers, SAB3 (linked to the *Co*-5 gene, Vallejo and Kelly 2001; located in linkage group B7 Campa et al. 2005), SW12 (located in linkage group B4, Miklas et al. 2000; linked to the *Co*-3/*Co*-9 gene, Méndez-Vigo et al. 2005), SCAreoli (located in linkage group B11, linked to the *Co*-2 gene; Geffroy et al. 1998), SCARAZ20 (linked to the *Co*-6 gene, Queiroz et al. 2004; located in linkage group B7, Campa et al. 2007), SBB14 (linked to the *Co*-4 gene, Awale and Kelly 2001; located in linkage group B8, Rodríguez-Suárez et al. 2007), and SAS8 (located in linkage group B7, linked to the *Bct* gene; Larsen and Miklas 2004); to microsatellite markers BM183, BM210 (located in linkage group B7, Blair et al. 2003), and PV-ctt001 (located in linkage group B4, Blair et al. 2003; linked to the *Co*-3/*Co*-9 gene, Rodríguez-Suárez et al. 2008); to RAPDs $OF10_{530}$ (linked to the *Co*-1 gene, Young and Kelly 1997; located in linkage group B1, Rodríguez-Suárez et al. 2007), $OF10_{1000}$ (linked to *Co*-10; Alzate-Marin et al. 2003; located in linkage group B4, Kelly et al. 2003), and $OAK20_{890}$ (linked to *Co*-6; Young and Kelly 1997). The PCR amplifications of these molecular markers were carried out as described by the corresponding authors. The SCAR PCR products were resolved on 2% agarose gels. The microsatellite PCR products were resolved on 8% polyacrylamide gels. Agarose and polyacrylamide gels were stained with ethidium bromide and visualized under UV light.

Seed protein analyses

The parental lines, TU and MDRK, were polymorphic for the seed protein loci, phaseolin (Pha), SpB, and SpI, located in linkage group B7 (Campa 2006). The analyses of these seed proteins were carried out in the F_2 TU × MDRK, using the SDS-PAGE procedure as modified by Ferreira et al. (2000).

Statistical and linkage analyses

Chi-square was used to test goodness-of-fit of observed to expected ratios in the F_2 population TU × MDRK and in the corresponding F_3 progenies. The segregation analyses of the markers and the resistance genes were 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

The differential cultivar TU was resistant to races 3, 6, 7, 31, 38, 39, 102, and 449, and susceptible to race 1545 of *C. lindemuthianum*, whereas the differential cultivar MDRK was resistant to races 449, and 1545, and susceptible to races 3, 6, 7, 31, 38, 39, and 102. For each anthracnose race, the F_3 TU × MDRK 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 3, 6, 7, 31, 38, 39, 102, 449, and 1545. Segregations for resistance to races 3, 6, 7, 38, and 39 showed a good fit to the expected ratio for a single dominant gene (1 R:2 R/S:1 S). Segregations for resistance to races 31, 449, and 1545 showed a good fit to the expected ratio for two independent dominant genes (7 R:8 R/S:1 S). Segregation for resistance to race 102 showed a good fit to the expected ratio for three independent dominant genes (37 R:26 R/S:1 S).

Table 1 Segregation for resistance to races, 3, 6, 7, 31, 38, 39, 102, 449, and 1545 of *Colletotrichum lindemuthianum* in the F_3 TU × MDRK families

Race	Parental phenotypes[a]		F_3 TU × MDRK families[b]						χ^2	Probability
			Observed			Expected[c]				
	TU	MDRK	R	R/S	S	R	R/S	S		
3	R	S	22	36	21	19.8	39.5	19.8	0.65	0.72
6	R	S	23	36	20	19.8	39.5	19.8	0.85	0.65
7	R	S	25	31	19	18.8	37.5	18.8	3.21	0.20
31	R	S	28	33	8	30.2	34.5	4.3	3.38	0.18
38	R	S	22	38	19	19.8	39.5	19.8	0.34	0.84
39	R	S	24	31	20	18.8	37.5	18.8	2.68	0.26
102	R	S	47	21	2	40.5	28.4	1.1	3.75	0.15
449	R	R	34	25	2	26.7	30.5	3.8	3.86	0.15
1545	S	R	32	36	2	30.6	35.0	4.4	1.38	0.50

[a] *R* Resistant, *S* susceptible

[b] *R* F_3 families having all individuals resistant, *R/S* F_3 families having individuals resistant and susceptible, *S* F_3 families having all individuals susceptible

[c] For races, 3, 6, 7, 38, and 39 the expected ratio was 1:2:1 (one dominant gene); for races 31, 449, and 1545 the expected ratio was 7:8:1 (two independent dominant genes); and for race 102 the expected ratio was 37:26:1 (three independent dominant genes)

Segregations of markers

Table 2 shows the F_2 TU × MDRK segregations for molecular markers $OF10_{530}$, $OF10_{1000}$, SW12, PV-ctt001, BM183, SAS8, SCARAZ20, SAB3, BM210, SBB14, SCAreoli, and $OAK20_{890}$, and for seed proteins Pha, SpB, and SpI. A good fit to the expected ratio for a single locus was obtained in all cases, except for the marker SCAreoli in which a significant excess of the genotype corresponding to parental MDRK was observed.

Linkage analyses

Resistance specificities showing monogenic segregation (races 3, 6, 7, 38, and 39)

Table 3 shows the joint segregation for resistance to races, 3, 6, 7, 38, and 39, in the F_3 TU × MDRK families. Evidence of recombination between resistance specificities was obtained only in three F_3 families (F_3-11, F_3-83, and F_3-86). In these three recombinant families, the number of F_3 individuals scored for resistance to some races was increased in order to reduce the possibility of error. The progenies (F_4 families) of 22 individuals belonging to the F_3-86 family were analyzed for resistance to races, 7, 38, and 39. As expected from the results indicated in Table 3, these F_4 families were homozygous susceptible to races 7 and 39, and showed segregation for resistance to race 38 (8 F_4 families homozygous resistant, 10 heterozygous, and 2 homozygous susceptible; $\chi^2_{1:2:1} = 3.60$; $P > 0.05$). These results agree with the assumption that the loci conferring specific resistance against races, 3, 6, 7, 38, and 39, are closely linked, arranged in a cluster. Table 4 shows the linkage relationships between the resistance loci included in this cluster and the markers appearing in Table 2. The cluster is significantly linked (LOD >3.0) to the six markers, SpI, Pha, SCARAZ20, SAB3, BM210, and SpB, previously located in linkage group B7 (Campa 2006; Freyre et al. 1998; Campa et al. 2007; Campa et al. 2005; Blair et al. 2003). Figure 1 shows the genetic map of linkage group B7 derived from the segregation data obtained.

Resistance specificities showing two-loci segregations (races, 31, 449, and 1545)

The results shown in Table 1 indicate that two independent dominant loci conferring resistance to race 31 are present in the parental cultivar TU. Table 5 shows the contingency χ^2 tests corresponding to the joint segregations of the resistance to race 31 and each one of the different markers analyzed in the F_2 TU × MDRK population. These results indicate that one of the two loci conferring resistance to race 31 is located in linkage group B7. The possibility of this locus being located in the B7 cluster, which includes the resistance specificities showing monogenic segregation, can be considered. Table 6 shows the joint segregations for resistances to races, 31, 3, 6, 7, 38, and 39, compared to the expected frequencies under the assumptions that one of the loci conferring resistance to race 31 is located in the B7 cluster and no intra-cluster recombination is present. The observed fit supports the location of a locus conferring resistance to race 31 in the B7 cluster. On the other hand, a F_3 family showing evidence of intra-cluster recombination was observed. This recombinant F_3 family was homozygous resistant for races, 3, 6, 7, 38, and 39, and heterozygous for resistance to race 31 (Table 6). In respect to the markers flanking the resistance cluster, it was homozygous for the S type phaseolin allele and heterozygous for SCARAZ20. This suggests that the resistance to race 31 locus is located within the cluster in a proximal position with respect to SCARAZ20 (see Fig. 1).

Concerning resistance to race 449, the results shown in Table 1 indicate that a dominant locus conferring resistance to this race is present in the parental TU, and a second independent dominant locus is present in the parental MDRK. The contingency χ^2 tests corresponding to the joint segregations of the resistance to race 449 and each one of the different markers analyzed in the F_2 TU × MDRK population are shown in Table 5. These results indicate that the locus conferring resistance to race

Table 2 Segregation of molecular markers, $OF10_{530}$, $OF10_{1000}$, SW12, PV-ctt001, BM183, SAS8, SCARAZ20, SAB3, BM210, SBB14, SCAreoli, and $OAK20_{890}$, and seed protein markers, Pha, SpB, and SpI, in the TU × MDRK F_2 population. The linkage groups (LG) of the different markers are indicated

Marker	LG	Parental phenotypes[a]		F_2 TU × MDRK plants[b]										χ^2	Probability
				Observed					Expected[c]						
		TU	MDRK	T/T	T/–	T/M	M/–	M/M	T/T	T/–	T/M	M/–	M/M		
$OF10_{530}$	B1	530	–	–	66	–	–	19	–	63.7	–	–	21.3	0.32	0.57
$OF10_{1000}$	B4	1,000	–	–	54	–	–	28	–	61.5	–	–	20.5	3.66	0.06
SW12	B4	725	–	–	59	–	–	25	–	63.0	–	–	21.0	1.02	0.31
PV-ctt001	B4	162	175	15		44	–	25	21.0		42.0	–	21.0	2.57	0.28
BM183	B7	149	–	–	54	–	–	23	–	57.7	–	–	19.3	0.97	0.32
SAS8	B7	1,550	–	–	54	–	–	22	–	57.0	–	–	19.0	0.63	0.43
SpI	B7	28.5	–	–	50	–	–	22	–	54.0	–	–	18.0	1.19	0.28
Pha	B7	S	T	23	–	30	–	19	18.0	–	36.0	–	18.0	2.44	0.29
SCARAZ20	B7	900	850	20	–	36	–	20	19.0	–	38.0	–	19.0	0.21	0.90
SAB3	B7	400	–	–	64	–	–	21	–	63.7	–	–	21.2	0.00	0.95
BM210	B7	180	166	19	–	37	–	22	19.5	–	39.0	–	19.5	0.44	0.80
SpB	B7	–	63.1	22	–	–	50	–	18.0	–	–	54.0	–	1.19	0.28
SBB14	B8	1,150	1,050	25	–	32	–	21	19.5	–	39.0	–	19.5	2.92	0.23
SCAreoli	B11	1,100	–	–	51	–	–	35	–	64.5	–	–	21.5	11.3	0.00
$OAK20_{890}$	?	–	890	15			56		17.7			53.2		0.57	0.45

[a] For DNA molecular markers, the parental phenotype refers to the molecular weight of the amplification products, and is expressed in base pairs (bp); for phaseolin, it refers to the corresponding types S and T; and for the two other seed protein markers, it refers to the molecular weight of the polypeptides, and is expressed in kdaltons (kD)

[b] *T/T* Homozygous for the TU alleles, *T/–* homozygous for the TU alleles or heterozygous, *T/M* heterozygous, *M/–* homozygous for the MDRK alleles or heterozygous, *MM* homozygous for the MDRK alleles

[c] For loci SAB3, SW12, SCAreoli, $OF10_{530}$, $OF10_{1000}$, SAS8, BM183, and SpI (dominant) the expected ratio was 3:1; for loci $OAK20_{890}$ and SpB (dominant) the expected ratio was 1:3; and for loci PV-ctt001, SCARAZ20, SBB14, BM210, and Pha (codominant) the expected ratio was 1:2:1

449 present in the parental TU is located in linkage group B7, and the one present in the parental MDRK is located in linkage group B4. The possibility of the locus of linkage group B7 being located in the cluster that includes the resistance specificities showing monogenic segregation can be considered. Table 7 shows the joint segregations for resistances to races, 449, 3, 6, 7, 38, and 39, compared to the expected frequencies under the assumptions that the locus conferring resistance to race 449 proceeding from TU is located in this cluster and no intra-cluster recombination is present. Differences between the observed and expected frequencies are not significant ($\chi^2 = 6.19$; $P > 0.05$). In this case, no evidence of intra-cluster recombination was observed and the relative location within the B7 cluster of the locus conferring resistance to 449 cannot be inferred.

With respect to race 1545, the results shown in Table 1 indicate that two independent dominant loci conferring resistance to race 1545 are present in the parental cultivar MDRK. The contingency χ^2 tests corresponding to the joint segregations of the resistance to race 1545 and each one of the different markers analyzed in the F_2 TU × MDRK population are shown in Table 5. These results indicate that one of the two loci conferring resistance to race 1545 is located in linkage group B1, and the other is located in linkage group B4. In order to confirm these possibilities, resistance to race 1545, and molecular markers, $OF10_{530}$ (located in linkage group B1), $OF10_{1000}$, SW12, and PV-ctt001 (located in linkage group B4), were analyzed in the two F_3 families, F_3-29 and F_3-74, and in the progenies (F_4 families) of such F_3 families, respectively. The F_3-29 family was homozygous for the alleles corresponding to the parental TU markers $OF10_{1000}$, SW12, and PV-ctt001, and showed monogenic segregations for the resistance to race 1545 (5 R:13 R/S:6 S; $\chi^2 = 0.25$, $P = 0.88$) and for the molecular marker $OF10_{530}$ (22 T/–:3M/M; $\chi^2 = 2.25$, $P = 0.13$). The locus for resistance to race 1545 present in this family was linked to marker $OF10_{530}$ at 8.9 cM (LOD = 2.15). The F_3-74 family was homozygous for the allele corresponding to parental TU marker $OF10_{530}$, and showed monogenic segregations for the resistance to race

Table 3 Joint segregation for resistance to races, 3, 6, 7, 38, and 39, of *Colletotrichum lindemuthianum* in the F_3 TU × MDRK families

Resistance spectrum of F_3 families[a]					Frequency
Race 3	Race 6	Race 7	Race 38	Race 39	
R	R	R	R	R	22
S	S	S	S	S	18
R/S	R/S	R/S	R/S	R/S	32
R/S	R	R/S	R/S	R	1[b]
S	S	S	R/S	S	2[c]

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

[b] Family F_3-83, showing evidence of recombination between resistance specificities. The segregations of resistant (R) and susceptible (S) plants within this family were: 6 R/3 S (race 3), 25 R/0 S (race 6), 14 R/2 S (race 7), 8 R/2 S (race 38), 31 R/0 S (race 39)

[c] Families F_3-11 and F_3-86, showing evidence of recombination between resistance specificities. The segregations of resistant (R) and susceptible (S) plants within these families were: family F_3-11, 0 R/16 S (race 3), 0 R/17 S (race 6), 0 R/28 S (race 7), 10 R/7 S (race 38), 0 R/37 S (race 39); family F_3-86, 0 R/33 S (race 3), 0 R/17 S (race 6), 0 R/16 S (race 7), 34 R/16 S (race 38), 0 R/16 S (race 39)

1545 (10 R:10 H:7 S; $\chi^2 = 2.48$, $P = 0.29$), and for the molecular markers $OF10_{1000}$ (19 T/–:6M/M; $\chi^2 = 0.01$, $P = 0.91$), SW12 (19 T/–:8M/M; $\chi^2 = 0.31$, $P = 0.58$), and PV-ctt001 (7 T/T:13 T/M:7M/M; $\chi^2 = 0.04$, $P = 0.98$). The locus for resistance to race 1545 present in this family was loosely linked to the markers $OF10_{1000}$ (20.5 cM; LOD = 1.66) and SW12 (26.2 cM; LOD = 1.34), and tightly linked to the marker PV-ctt001 (5.7 cM; LOD = 8.23) on B4.

Resistance to race 102

The results shown in Table 1 indicate that three independent dominant loci conferring resistance to race 102 are present in the parental cultivar TU. Table 5 shows the contingency χ^2 tests corresponding to the joint segregations of the resistance to race 102 and each one of the different markers analyzed in the F_2 TU × MDRK population. These results indicate that one of the three loci conferring resistance to race 102 is located in linkage group B7 (most probably in the B7 cluster including the loci for specific

Table 4 Linkage analysis between the cluster conferring resistance to races 3, 6, 7, 38, and 39, and each one of the DNA or protein markers, $OF10_{530}$, $OF10_{1000}$, SW12, PV-ctt001, BM183, SAS8, SpI, Pha, SCARAZ20, SAB3, BM210, SpB, SBB14, SCAreoli, and $OAK20_{890}$

Markers		F_3 TU × MDRK families															RF	LOD
		R					R/S					S						
Name	LG	T/T	T/–	T/M	M/–	M/M	T/T	T/–	T/M	M/–	M/M	T/T	T/–	T/M	M/–	M/M		
$OF10_{530}$	B1	–	16	–	–	5	–	25	–	–	10	–	15	–	–	4	0.50	0.00
$OF10_{1000}$	B4	–	12	–	–	8	–	27	–	–	7	–	8	–	–	11	0.43	0.19
SW12	B4	–	14	–	–	7	–	27	–	–	7	–	11	–	–	8	0.47	0.03
PV-ctt001	B4	4	–	8	–	8	11	–	18	–	6	0	–	11	–	8	0.45	0.17
BM183	B7	–	16	–	–	6	–	25	–	–	8	–	11	–	–	8	0.45	0.13
SAS8	B7	–	16	–	–	3	–	28	–	–	7	–	8	–	–	11	0.32	1.61
SpI	B7	–	20	–	–	2	–	25	–	–	7	–	3	–	–	13	0.22	4.19
Pha	B7	18	–	3	–	1	3	–	24	–	5	1	–	2	–	13	0.15	11.21
SCARAZ20	B7	19	–	3	–	0	0	–	32	–	0	1	–	0	–	18	0.05	23.83
SAB3	B7	–	22	–	–	0	–	31	–	–	4	–	3	–	–	15	0.12	8.88
BM210	B7	16	–	5	–	0	2	–	28	–	5	1	–	2	–	16	0.12	15.10
SpB	B7	13	–	–	9	–	8	–	–	24	–	1	–	–	15	–	0.25	3.35
SBB14	B8	6	–	9	–	6	11	–	14	–	10	6	–	9	–	4	0.50	0.00
SCAreoli	B11	–	13	–	–	9	–	19	–	–	16	–	12	–	–	7	0.50	0.00
$OAK20_{890}$	?	4	–	–	13	–	7	–	–	21	–	2	–	–	14	–	0.43	0.17

Linkage values are expressed as RF (recombination fraction) and LOD

LG Linkage group in which the marker is located

R F_3 families having all individuals resistant to races 3, 6, 7, 38 and 39, *R/S* F_3 families having individuals resistant and susceptible, *S* F_3 families having all individuals susceptible. The three F_3 families showing evidence of intracluster recombination have not been considered

T/T Homozygous for the TU alleles of the corresponding marker, *T/–* homozygous for the TU alleles or heterozygous, *T/M* heterozygous, *M/–* homozygous for the MDRK alleles or heterozygous, *M/M* homozygous for the MDRK alleles

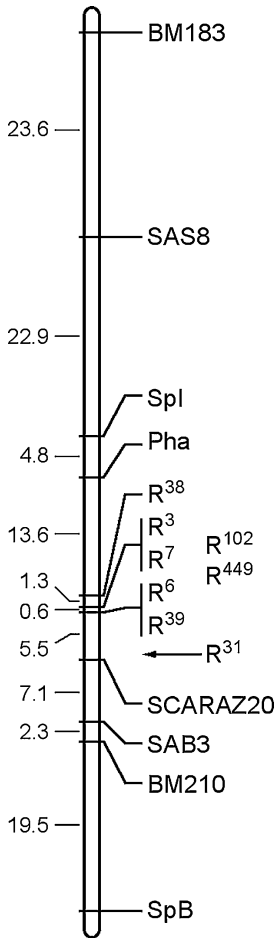

Fig. 1 Relative positions in the bean linkage group B7 of the molecular markers BM183, SAS8, SCARAZ20, SAB3, and BM210; the seed protein loci Pha (phaseolin), SpI, and SpB; and the *Co*-5 cluster including genes conferring specific resistance to anthracnose races, 3 (R^3), 6 (R^6), 7 (R^7), 31 (R^{31}), 38 (R^{38}), 39 (R^{39}), 102 (R^{102}), and 449 (R^{449}), in the differential cultivar TU. The relative positions within the cluster of the genes R^{102} and R^{449} are undetermined. Map distances, on the left, are expressed in centiMorgans, estimated using the Kosambi mapping function

Table 5 Contingency χ^2 tests corresponding to the joint segregations for each one of the resistances to races 31 (R^{31}), 449 (R^{449}), 1545 (R^{1545}), and 102 (R^{102}) and each one of the different markers

Name	LG	R^{31}		R^{449}		R^{1545}		R^{102}	
		Cont. χ^2	*P*	Cont. χ^2	*P*	Cont. χ^2	*P*	Cont. χ^2	*P*
$OF10_{530}$	B1	0.82	n.s.	1.04	n.s.	18.80	**[b]	1.02	n.s.
$OF10_{1000}$	B4	4.50	n.s.	8.96	*[b]	10.19	**[b]	2.73	n.s.
SW12	B4	3.63	n.s.	9.85	**[b]	11.95	**[b]	4.02	n.s.
PV-ctt001	B4	5.77	n.s.	10.44	*[b]	23.13	**[b]	5.32	n.s.
BM183	B7	0.08	n.s.	1.09	n.s.	1.36	n.s.	1.47	n.s.
SAS8	B7	7.28	*[a]	0.77	n.s.	0.78	n.s.	9.76	**[a]
SpI	B7	10.13	**[a]	6.91	*[a]	0.69	n.s.	4.91	n.s.
Pha	B7	23.99	**[a]	16.34	**[a]	3.37	n.s.	10.27	*[a]
SCARAZ20	B7	46.65	**[a]	14.15	**[a]	3.26	n.s.	17.54	**[a]
SAB3	B7	18.19	**[a]	1.48	n.s.	1.10	n.s.	4.67	n.s.
BM210	B7	32.30	**[a]	10.12	*[a]	2.17	n.s.	9.20	n.s.
SpB	B7	7.05	*[a]	1.69	n.s.	1.00	n.s.	1.85	n.s.
SBB14	B8	0.73	n.s.	2.20	n.s.	2.16	n.s.	6.95	n.s.
SCAreoli	B11	1.78	n.s.	0.08	n.s.	3.16	n.s.	1.63	n.s.
$OAK20_{890}$	?	3.71	n.s.	0.67	n.s.	0.75	n.s.	1.19	n.s.

LG Linkage group in which the marker is located, *n.s.* not significant

[a] Significance due to an excess of resistant F_3 families showing the allele of the marker present in TU

[b] Significance due to an excess of resistant F_3 families showing the allele of the marker present in MDRK

* $0.05 > P > 0.01$, ** $0.01 > P$

resistance to races, 3, 6, 7, 31, 38, 39, and 449), the locations of the other two loci remain unknown.

Discussion

Concerning the differential cultivar TU, the results obtained indicate the presence of at least three independent loci conferring resistance to anthracnose. One locus is formed by a cluster of different genes conferring specific resistance to races, 3, 6, 7, 31, 38, 39, 102, and 449. According to the preliminary results of Campa et al. (2005), this cluster is located in linkage group B7 (Fig. 1) and would correspond to the *Co*-5 gene, originally known as *Mexique 3*, and first described in the genotype TU (Fouilloux 1976). The linkage found between the resistance cluster and SAB3 (Table 4) agrees with the linkage previously found between *Co*-5 and the RAPD $OAB3_{450}$ (Young and Kelly 1997), from which the SCAR SAB3 was developed (Vallejo and Kelly 2001).

This resistance cluster in TU corresponding to *Co*-5 also was closely linked to the marker SCARAZ20 (Table 4; Fig. 1), which seems contradictory at first glance because SCARAZ20 has been described as linked to a resistance gene present in the anthracnose differential cultivar AB136, and assumed to be the *Co*-6 gene (Queiroz et al. 2004; Campa et al. 2007). The *Co*-6 gene was first described in the cultivar Catrachita and shown to be independent (allelism tests) from the resistance gene *Co*-5, present in the cultivar TU (Young and Kelly 1996). A close linkage between the *Co*-6 gene and the $OAK20_{890}$ marker (7.3 cM) was later established using three different mapping populations involving Catrachita as the resistant parent and the three susceptible cultivars Black Magic, Blackhawk, and Raven (Young and Kelly 1997). In agreement with these results, the independent segregation found between the resistance cluster present in TU and the $OAK20_{890}$ marker (Table 4), confirms that *Co*-5 is independent from the resistance gene present in Catrachita (*Co*-6).

Since Catrachita was derived from the cross BAT1225 × AB136, it was assumed that the anthracnose resistance present in AB136 was due to the *Co*-6 gene (Young and Kelly 1996). However, no allelism tests involving AB136 and TU were carried out. Recently, Campa et al. (2007) reported the presence of at least two independent anthracnose resistance loci in AB136. One of

Table 6 Joint segregation of F_3 TU × MDRK families for resistance to *C. lindemuthianum* races, 3, 6, 7, 38, 39, and 31, compared to the expected frequencies under the assumption of two dominant genes proceeding from TU being responsible for the resistance to race 31; one of them located in the B7 cluster, including resistance to races, 3, 6, 7, 38, and 39, and the other one not linked to this cluster. In this assumption, intra-cluster recombination has not been considered

Resistance spectrum of F_3 families[a]						Observed frequency	Expected frequency
Race 3	Race 6	Race 7	Race 38	Race 39	Race 31		
R	R	R	R	R	R	20	16.50 (4/16)
R	R	R	R	R	R/S	1[b]	0.00
R	R	R	R	R	S	0	0.00
S	S	S	S	S	R	0	4.13 (1/16)
S	S	S	S	S	R/S	9	8.25 (2/16)
S	S	S	S	S	S	7	4.13 (1/16)
R/S	R/S	R/S	R/S	R/S	R	8	8.25 (2/16)
R/S	R/S	R/S	R/S	R/S	R/S	21	24.75 (6/16)
R/S	R/S	R/S	R/S	R/S	S	0	0.00

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

[b] Family showing evidence of recombination between resistance to race 31 and the remaining resistance specificities

Table 7 Joint segregation of F_3 TU × MDRK families for resistance to *C. lindemuthianum* races 3, 6, 7, 38, 39, and 449, compared to the expected frequencies under the assumptions that one of the two loci conferring resistance to race 449 is located in the B7 cluster (including resistance specificities to races, 3, 6, 7, 38, and 39) and that no intra-cluster recombination is present

Resistance spectrum of F_3 families[a]						Observed frequency	Expected frequency
Race 3	Race 6	Race 7	Race 38	Race 39	Race 449		
R	R	R	R	R	R	20	14.50 (4/16)
R	R	R	R	R	R/S	0	0.00
R	R	R	R	R	S	0	0.00
S	S	S	S	S	R	3	3.63 (1/16)
S	S	S	S	S	R/S	7	7.25 (2/16)
S	S	S	S	S	S	1	3.63 (1/16)
R/S	R/S	R/S	R/S	R/S	R	10	7.25 (2/16)
R/S	R/S	R/S	R/S	R/S	R/S	17	21.75 (6/16)
R/S	R/S	R/S	R/S	R/S	S	0	0.00
$\chi^2 = 6.19$ ($P = 0.29$)							

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

these loci, formed by a cluster including genes conferring specific resistance to races 81 and 449, was located in linkage group B7, closely linked to markers SCARAZ20 and SZ04 (derived from the RAPD $OZ04_{560}$), in a relative position similar to the one found in this work for the *Co*-5 gene. This suggests that the anthracnose resistance locus present in AB136, previously linked to markers SCARAZ20 (Queiroz et al. 2004) and $OZ04_{560}$ (Alzate-Marin et al. 1999; Gonçalves-Vidigal et al. 2001), and located in linkage group B7, flanked by the *P* (basic color gene) and *Phs* (phaseolin) genes (Freyre et al. 1998; Kelly et al. 2003; Rodríguez-Suárez et al. 2007), corresponds to the *Co*-5 gene.

With respect to the remaining two loci present in TU, one confers resistance to races 31 and 102, and the other conditions resistance to race 102. The location of these loci remains unknown. The results shown in Table 4 indicates that these resistance loci segregate independently from the molecular markers, $OF10_{530}$, linked to the *Co*-1 gene (Young and Kelly 1997); SCAreoli, linked to *Co*-2 (Geffroy et al. 1998); SW12 and PV-ctt001, linked to *Co*-3/*Co*-9 (Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2008); SBB14, linked to *Co*-4 (Awale and Kelly 2001); $OAK20_{890}$, linked to *Co*-6 (Young and Kelly 1997); and $OF10_{1000}$, linked to *Co*-10 (Alzate-Marin et al. 2003).

Concerning MDRK, from the results of the contingency tests (Table 5) and the segregation analyses of the two F_3 families, F_3-29 and F_3-74, there are two independent genes present in MDRK which confer resistance to race 1545. One gene (segregating in family, F_3-29) is linked to marker $OF10_{530}$, (8.9 cM; LOD = 2.15). This RAPD marker was previously linked to the anthracnose resistance locus *Co*-1 using a $F_{2:3}$ mapping population derived from the cross between the near-isogenic lines N85006 S and N85006 R (Young and Kelly 1997). Later, it was located in linkage group B1 (Méndez de Vigo 2001; Rodríguez-Suárez et al. 2007; Geffroy et al. 2008; Vallejo and Kelly 2008). The second resistance gene present in MDRK (segregating in family, F_3-74) is loosely linked to the markers $OF10_{1000}$ (20.5 cM; LOD = 1.66) and SW12 (26.2 cM; LOD = 1.34), and tightly linked to the marker PV-ctt001 (5.7 cM; LOD = 8.23). The marker $OF10_{1000}$ is linked to the anthracnose resistance locus *Co*-10 (Alzate-Marin et al. 2003), whereas the markers SW12 and PV-ctt001 are linked to the anthracnose resistance locus *Co*-3/*Co*-9 (Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2008). The two loci *Co*-3/*Co*-9 and *Co*-10 reside on B4 (Kelly et al. 2003), in positions sufficiently separate to be independent (Alzate-Marin et al. 2003). The results obtained here can be explained if it is assumed that $OF10_{1000}$ is located between *Co*-3/*Co*-9 and *Co*-10, with the tighter linkage found between PV-ctt001 and the gene conferring resistance to race 1545, which taken together suggest a plausible location of this gene within the *Co*-3/*Co*-9 cluster. Concerning resistance to race 449, the results of the contingency tests (Table 5) indicate that the gene present in MDRK conferring resistance to this race is located in linkage group B4, the *Co*-3/*Co*-9 cluster in MDRK conferring resistance to both races, 1545 and 449, being the most reasonable explanation. This would agree with the presence of anthracnose resistance specificities in the *Co*-3/*Co*-9 cluster found in other Andean materials such as JaloEEP558, and would support the ancestral nature of this resistance gene cluster (Geffroy et al. 1999).

In allelism tests conducted to date, it was assumed that *Co*-1 was the only anthracnose resistance gene present in MDRK, and *Co*-5 the only gene present in TU (Kelly and Vallejo 2004). The results obtained herein confirm the presence of the *Co*-1 and the *Co*-5 loci in MDRK and TU, respectively, but also clearly indicate that additional genes conferring resistance to anthracnose are present in these differential cultivars. Presence of additional genes is supported further by interpretation of the results obtained from the previous allelism tests. For instance, the absence of segregation for resistance in the F_2 from the cross between MDRK and Widusa was taken as an evidence for the resistance in Widusa being due to *Co*-1 (Gonçalves-Vidigal and Kelly, 2006). With the presence in MDRK of anthracnose resistance genes at both the *Co-1* and the *Co*-3/*Co*-9 loci, the absence of segregation for resistance in the MDRK × Widusa F_2 should be reinterpreted as evidence for the resistance in Widusa being due either to *Co*-1 or to *Co*-3/*Co*-9. This reinterpretation would agree with the results of Rodríguez-Suárez et al. (2008) that, using direct mapping, demonstrated the presence of anthracnose resistance genes in the *Co*-3/*Co*-9 cluster in Widusa.

Acknowledgments This work was supported by grants AGL2004-08145-C02 and AGL2007-66563-C02 from the Ministerio de Educación y Ciencia, Spain. Ana Campa was recipient of a salary fellowship from the Instituto de Investigación y Tecnología Agraria y Alimentaria (INIA), Spain. Anthracnose races 7, 31, 39, 449, and 1545 were kindly provided by James D. Kelly (Michigan State University).

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