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Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, 'G 2333'

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Abstract Two independently assorting dominant genes conditioning resistance to bean anthracnose were identified in an F_2 population derived from the highly resistant bean differential cultivar, 'G 2333'. One gene was allelic to the *Co-4* gene in the differential cultivar 'TO' and was named *Co-4²*, whereas the second gene was assigned the temporary name *Co-7* until a complete characterization with other known resistance genes can be conducted. Two RAPD markers linked to the *Co-4²* allele were identified. One RAPD, $OAS13_{950}$, co-segregated with no recombinants in two segregating populations of 143 F_2 individuals, whereas the second RAPD, $OAL9_{740}$, mapped at 3.9 cM from the *Co-4²* allele. Two 24-mer SCAR primers (SAS13), developed from the $OAS13_{950}$ RAPD marker, were dominant and polymorphic, similar to the original RAPD, and supported the tight linkage between the marker(s) and the *Co-4²* allele. The markers were present in germplasm with known resistance alleles at the *Co-4* locus. The presence of the markers in two other differential cultivars not previously characterized and in four navy bean cultivars suggests the existence of a gene family for anthracnose resistance at or near the *Co-4* locus. Since the *Co-7* gene was present only in germplasm which also possessed the *Co-4²* and *Co-5* genes, the SAS13 markers were used in combination with standard inoculation techniques to identify F_3 lines in which the *Co-7* gene was homozygous and the *Co-4²* allele was absent. A similar strategy of marker-assisted dissection is proposed to identify resistant lines in which the *Co-5* gene is absent and the *Co-7* gene is present by selecting against the $OAB3_{450}$ marker, which has been shown previously to be linked to the *Co-5* gene. These genes cannot be distinguished using traditional screening methods since all current races of the pathogen virulent to the *Co-5* gene are avirulent to the *Co-4²* and *Co-7* genes. We describe the use of molecular markers tightly linked to resistance genes to facilitate the identification of an uncharacterized resistance gene for which no discriminating race of the pathogen is known.

Key words RAPD · SCAR · Allelism · *Colletotrichum lindemuthianum* · *Phaseolus vulgaris*

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Introduction

One of the most severe, widespread diseases of common bean (*Phaseolus vulgaris* L.) is anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lambs.-Scrib. (Pastor-Corrales et al. 1995; Schwartz 1991; Tu 1992). Genetic variability of *C. lindemuthianum* has been observed in different parts of the world where beans are grown (Beebe and Pastor-Corrales 1991; Pastor-Corrales and Tu 1989), and many races of the pathogen have been characterized (Balardin et al. 1997; Rava et al. 1993; Garrido-Ramirez and Romero-Cova 1989; Pastor-Corrales and Tu 1989; Menezes and Dianese 1988; Yerkes and Teliz-Ortiz 1956). Due to the highly variable nature of the pathogen and the continual emergence of new races, genetic resistance in the host is not durable. Currently, races of *C. lindemuthianum* are classified by inoculating isolates onto a universal set of 12 differential cultivars. Races are assigned a cumulative numerical value for each

susceptible differential cultivar based on an established binary system (CIAT 1988; Pastor-Corrales 1991). Only 6 of the 12 differential cultivars have been genetically characterized for anthracnose resistance: 'Michigan Dark Red Kidney', *Co-1* gene (Burkholder 1918; Young and Kelly 1997); 'Cornell 49242', *Co-2* gene (Mastenbroek 1960); 'Mexico 222', *Co-3* gene; 'TO', *Co-4* gene; 'TU', *Co-5* gene (Fouilloux 1979); and 'AB 136', *Co-6* gene (Young and Kelly 1996a). There is limited evidence that some of the remaining 6 differentials, such as 'Perry Marrow' and 'Kaboon', may carry more than one resistance factor (Cardenas et al. 1964; Muhalet et al. 1981). For instance, using race 521, Pastor-Corrales et al. (1994) demonstrated that anthracnose resistance in differential cultivar 'G 2333', was controlled by two unknown resistance genes. In addition to these two unknown genes, Young and Kelly (1996a) showed that 'G 2333' also carried the *Co-5* gene, which was not detected by Pastor-Corrales et al. (1994) since race 521 is virulent to the *Co-5* gene. The presence of more than one gene in 'G 2333' may account for its broad resistance to all known races of *C. lindemuthianum* (Balardin et al. 1997; Pastor-Corrales et al. 1994; Schwartz et al. 1982). If resistance in 'G 2333' is conditioned by three resistance genes (Young and Kelly 1996a), one of which has been previously described (*Co-5*), the two other genes need to be characterized. Since no discriminating race(s) of the pathogen is available to distinguish effectively between these resistance genes, it is impossible to know which gene or gene combination is being transmitted to the progeny. When combined in a single genotype, those unknown resistance genes, that cannot be identified by traditional pathological techniques, can be distinguished from each other using a marker tightly linked to one of the resistance genes. The use of markers to dissect the genetic basis of complex traits in other crops has been described (marker-assisted dissection, Allard 1996).

In this paper we discuss the use of tightly linked random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) markers to assist in the identification of independently inherited resistance genes for which no discriminating races of the pathogen are available. To demonstrate the application of marker-assisted dissection, we chose to study the anthracnose resistance genes present in the bean cultivar, 'G 2333'. We have found a new resistance gene for bean anthracnose, identified RAPD and SCAR markers linked to that gene, and used linked markers to identify and isolate the third resistance gene present in 'G 2333'.

Materials and methods

Plant material

The study of resistance to anthracnose in 'G 2333' was investigated using two breeding lines, 'SEL 1360' and 'SEL 1308', derived through backcrossing with 'G 2333'. The cultivar 'G 2333' is highly photoperiod sensitive, which makes it difficult to work with in temperate regions. Both lines were resistant to a number of races of *C. lindemuthianum*, and each line was known to carry different resistance gene(s) since they displayed different phenotypic reactions after inoculation with a wide array of races of the pathogen (Young and Kelly 1996a). 'SEL 1360' possesses a single dominant resistance gene characterized as *Co-5*. Resistance in 'SEL 1308', however, was concluded to be similar to that of the 'G 2333' parent, since no race was found to discriminate between the resistance genes present (Young and Kelly 1996a). Crosses between 'SEL 1308' and cultivars 'Catrachita' [*Co-6* gene (Beebe and Pastor-Corrales 1991)] and 'Black Magic' [susceptible (Kelly et al. 1987)] were made, and these two segregating populations were used to characterize the genetic resistance in 'SEL 1308'. The 'Catrachita/SEL 1308' and 'Black Magic/SEL 1308' populations were composed of 72 and 99 F_2 individual plants, respectively, and the $F_{2:3}$ progeny of each individual F_2 plant were tested to discriminate homozygous from heterozygous resistant genotypes. In order to test for allelism we crossed SEL 1308 to 6 cultivars, each carrying a single independent and characterized resistance gene; 'Michigan Dark Red Kidney' (*Co-1*), 'Blackhawk' (*Co-2*, Ghaderi et al. 1990), 'Mexico 222' (*Co-3*), 'TO' (*Co-5*), and 'Catrachita' (*Co-6*, Young and Kelly 1996a). Since the number of genes in 'SEL 1308' was unknown, 'SEL 1360' (*Co-5* gene) was crossed with the original parent, 'G 2333', to generate a population where the unknown genes would be segregating and the *Co-5* gene would be fixed.

Preparation of races of *C. lindemuthianum*

Each race of *C. lindemuthianum* used for the characterization of the genetic resistance in 'SEL 1308' was first tested against the anthracnose differential set to confirm its identity. Monosporic cultures grown and maintained in stocks of fungus-colonized filter paper were the source of inoculum used for each race. Races 1545 (source CIAT) and 73 (ATCC 96512), pathogenic on 'Catrachita' and 'Black Magic', respectively, were chosen to determine the inheritance of resistance in 'SEL 1308' (Table 1). For the allelism tests, race 73 was

Table 1 Phenotypic characterization and observed ratio of F_2 progeny and $F_{2:3}$ families for their reaction to *Colletotrichum lindemuthianum*

Population[a]	Race	Anthracnose reaction						
		Number of F_2 plants			Number of $F_{2:3}$ plants			
		R-	rr	Probability[b]	RR	Rr	rr	Probability[c]
Catrachita × SEL 1308	1545	52	20	0.68	16	30	19	0.72
Black Magic × SEL 1308	73	78	21	0.45	15	45	24	0.31

[a] Susceptible × resistant crosses in all cases
[b] Value for a 3:1 (resistant, R-; susceptible, rr) expected ratio
[c] Value for a 1:2:1 (resistant, RR; resistant, Rr; susceptible, rr) expected ratio

Table 2 Allelism test for genetic characterization of the resistance to *Colletotrichum lindemuthianum* in 'SEL 1308'

Population[a]	Loci tested[b]	Race[c]	Anthracnose reaction			
			Number of F_2 plants			
			R-	rr	$\chi^{2\,d}$	P^e
MDRK × SEL 1308	*Co-1*/SEL 1308	73	76	2	1.23	0.27
Blackhawk × SEL 1308	*Co-2*/SEL 1308	64	96	3	1.24	0.26
Mex 222 × SEL 1308	*Co-3*/SEL 1308	521	86	8	0.48	0.49
TO × SEL 1308	*Co-4*/SEL 1308	73	97	0	5.44	0.02
TU × SEL 1308	*Co-5*/SEL 1308	73	90	9	0.92	0.34
Catrachita × SEL 1308	*Co-6*/SEL 1308	73	65	4	0.00	1.00
SEL 1360 × G 2333	*Co-7*[f]/SEL 1308	521	86	6	0.00	1.00
SEL 1360 × G 2333	*Co-7*/SEL 1308	1545	80	3	0.59	0.44

[a] Resistant × resistant crosses in all cases
[b] Characterized resistance gene × SEL 1308 resistance gene
[c] Race of *Colletotrichum lindemuthianum* used in each case
[d] 15 : 1 (resistant, R-; susceptible, rr) expected ratio for two independently assorting genes
[e] P = probability of estimated value
[f] *Co-7*, third independent gene in G 2333

used in the populations derived from 'Michigan Dark Red Kidney', 'TO', 'TU', and 'Catrachita' whereas race 64 (ATCC 18987) was used in the cross 'Blackhawk/SEL 1308' and race 521 (source CIAT) was used in the 'Mexico 222/SEL 1308' and 'SEL 1360/G 2333' populations (Table 2). In addition to using race 521, we confirmed the disease reaction of the 'SEL 1360/G 2333' population with race 1545, which is pathogenic on the *Co-5* gene but avirulent to the other two resistance genes in 'G 2333'. Inoculum preparation, inoculation methods and disease characterization using different races was conducted as described previously (Young and Kelly 1996a).

RAPD and SCAR analyses

Individual F_2 plants from the 'Catrachita/SEL 1308' and 'Black Magic/SEL 1308' populations were used as two independent mapping populations to test for putative linkages between RAPD markers and the resistance gene in 'SEL 1308'. Bulked segregant analysis (Michelmore et al. 1991) was used to form two independent and contrasting DNA bulks composed of equal volumes of fluorometrically standardized DNA from 6 F_2 homozygous resistant and 6 F_2 homozygous susceptible plants derived from both mapping populations. The polymerase chain reaction (PCR) procedure was similar to that described by Young and Kelly (1996b) using random primers (Operon Technologies, Alameda, Calif.) and Stoffel DNA polymerase (Perkin Elmer). The thermal profile used consisted of 3 cycles of 1 min at 94°C/1 min at 35°C/2 min at 72°C; 34 cycles of 10 s at 94°C/20 s at 40°C/2 min at 72°C, followed by 1 cycle of 5 min at 72°C. The RAPD amplification products linked to the *Co-4*[2] gene were purified using the QIAquick gel extraction kit (Qiagen, Chatsworth, Calif.) and cloned by means of the TA cloning system (Invitrogen Corp, San Diego, Calif.). Cloned amplification products were sequenced following the procedure described by Melotto et al. (1996). SCAR markers for the $OAS13_{950}$ and $OAL9_{740}$ RAPD markers were generated and tested in both mapping populations. Amplification of the DNA fragment by the SCAR primers was carried out as described by Melotto et al. (1996). The PCR procedure consisted of 34 cycles 10 s at 94°C/2.4 min at 72°C, followed by 1 cycle of 5 min at 72°C. The presence or absence of DNA amplification in the parental genotypes and in selected samples from the two populations was confirmed for the $OAS13_{950}$ RAPD and its derived SCAR marker SAS13 using *Taq* DNA polymerase (Gibco, BRL). In addition, the $OAS13_{950}$ and SAS13 markers were used for purposes of marker-assisted selection to identify F_2 individuals which did not possess the marker and to screen other differential and commercial cultivars.

Linkage analysis

Simple inheritance of the disease phenotype and the putative linked RAPD and SCAR markers was confirmed using Chi-square tests. Linkage analyses were performed using the program LINKAGE-*1* (Suiter et al. 1983). Linkage estimates between loci are expressed in centimorgans (cM), as calculated using Kosambi's function by the LINKAGE-*1* computer program.

Results

The observed phenotypic ratios of F_2 progeny and $F_{2:3}$ families based on their reaction to races 1545 and 73 in the 'Catrachita/SEL 1308' and 'Black Magic/SEL 1308' populations, respectively, suggest that a single dominant gene is responsible for the anthracnose resistance in 'SEL 1308'. The Chi-square test performed on the two F_2 population supported a fit to the 3 : 1 expected ratio of resistant to susceptible plants (R- : rr), and confirmed a 1 : 2 : 1 (RR : Rr : rr) ratio on the $F_{2:3}$ families derived from each F_2 population (Table 1). Allelism tests for independence of the single dominant resistance gene in SEL 1308 from the six other reported genes are shown in Table 2. In five F_2 populations, the observed segregation fit a 15 : 1 (R- : rr) ratio, indicating that the single dominant gene conditioning resistance to anthracnose in 'SEL 1308' segregated independently from the *Co-1*, *Co-2*, *Co-3*, *Co-5*, and *Co-6* dominant genes. However, no segregation was observed in the F_2 population derived from the cross 'TO/SEL 1308', suggesting that the dominant gene in 'SEL 1308' is located at the same locus as the *Co-4* gene in the cultivar 'TO'. A previous study showed that race 2047 was virulent to cv 'TO' but avirulent to the 'SEL 1308' genotype (Young and Kelly 1996a), suggesting that the resistance gene in 'SEL 1308' is a different allele at the *Co-4* locus. Therefore, it is proposed that this single dominant allele in

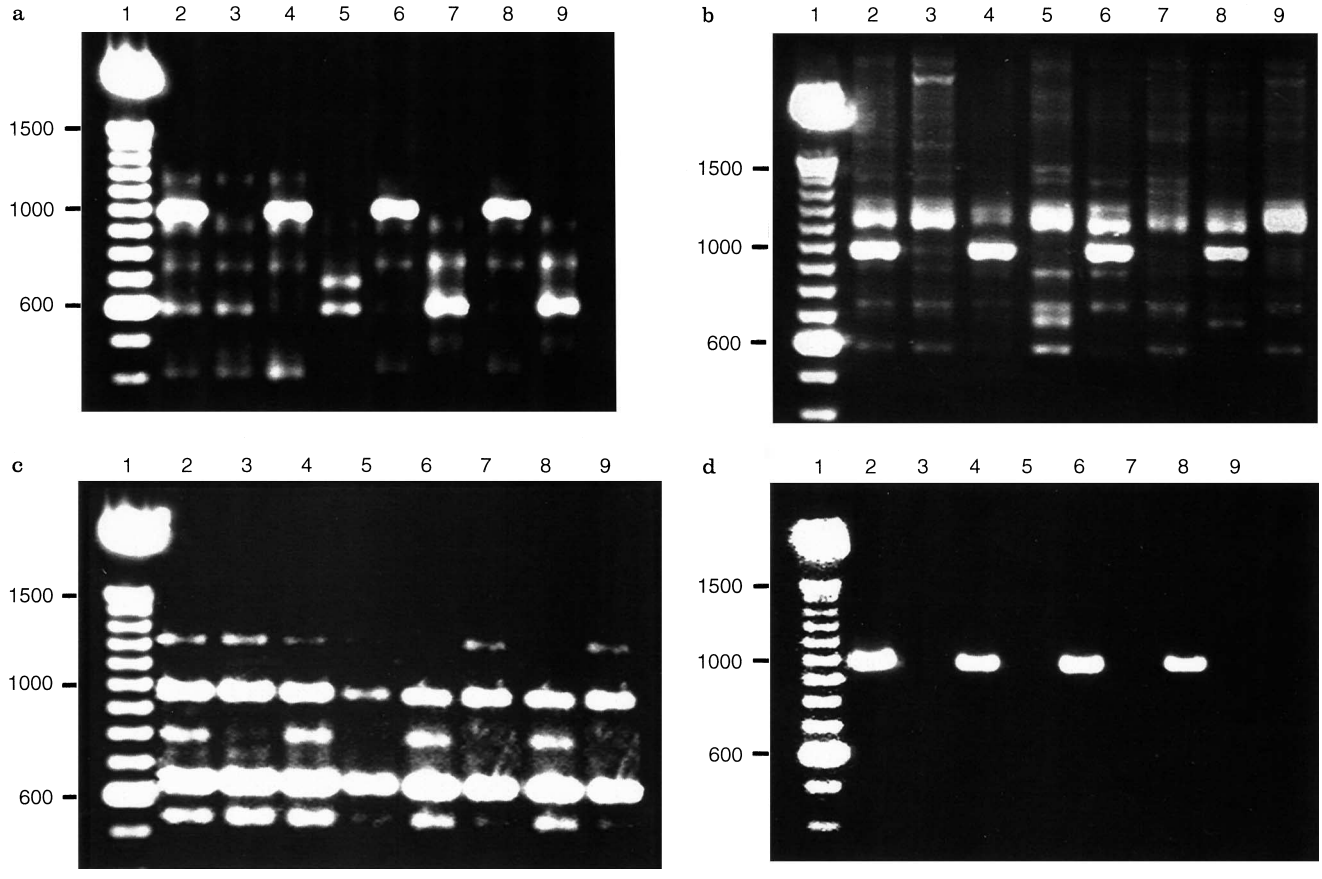

Fig. 1a–d DNA amplification of eight bean cultivars using **a** the $OAS13_{950}$ RAPD marker with Stoffel DNA polymerase, **b** the $OAS13_{950}$ RAPD marker with *Taq* DNA polymerase, **c** the $OAL9_{740}$ RAPD marker with Stoffel DNA polymerase, **d** the SAS13 SCAR marker. *Lane 1* 100-bp DNA ladder, *2* resistant DNA bulk (*Co-4²*/*Co-4²*), *3* susceptible DNA bulk (*co-4²*/*co-4²*), *4* 'SEL 1308' (*Co-4²*/*Co-4²*), *5* 'SEL 1360' (*co-4²*/*co-4²*, *Co-5*/*Co-5*), *6* 'G 2333' (*Co-4²*/*Co-4²*, *Co-5*/*Co-5*), *7* 'Catrachita (*Co-6*/*Co-6*), *8* 'TO' (*Co-4*/*Co-4*), *9* 'Black Magic' (*co-4²*/*co-4²*)

'SEL 1308' be named *Co-4²*. The independence of *Co-4²* from a third resistance gene in 'G 2333' was tested by inoculating the F_2 population of 'SEL 1360/G 2333' with races 521 and 1545, both of which are pathogenic on the *Co-5* gene present in both parents (Young and Kelly 1996a). The observed segregation fit a 15:1 (R-:rr) ratio, indicating that the third resistance gene in 'G 2333' is a single dominant gene which segregated independently from *Co-4²* in 'G 2333' or in its offspring, 'SEL 1308' (Table 2). It is proposed that this gene be tentatively named *Co-7* (Bassett 1996) until allelism tests are conducted with the other characterized resistance genes (*Co-1*, *Co-2*, *Co-3*, and *Co-6*).

A total of 260 decamer primers were used to screen for RAPD markers linked to the *Co-4²* gene, and two linked RAPD markers were identified. One marker, designated $OAS13_{950}$ (generated by a 5′-CAC-GGACCGA-3′ decamer; Fig. 1a), co-segregated with the resistance gene in 143 individuals in both populations, and no recombinants were observed (Table 3). This marker was present in 'SEL 1308', 'G 2333', and in the differential cultivars 'Widusa', 'PI 207262', and 'TO' which carries the *Co-4* gene. The marker was absent from all other differential cultivars, including 'SEL 1360' and those with characterized resistance genes. In a survey of 24 commercial bean cultivars representing the two gene pools of *P. vulgaris*, the $OAS13_{950}$ RAPD marker was present in 4 related navy bean cultivars, 'Monroe', 'Sanilac', 'Seafarer', and 'Seaforth'. Similar results were observed using *Taq* DNA polymerase (Fig. 1b).

A second marker, designated $OAL9_{740}$ (generated by a 5′-TGGCGCACAC-3′ decamer; Fig. 1c), segregated in a 3:1 ratio as a dominant factor in both mapping populations. Combining data from all 143 F_2 individuals, the two-point chi-square analysis showed that $OAL9_{740}$ RAPD mapped 3.9 ± 2 cM from the *Co-4²* allele in the two populations (Table 3). The $OAL9_{740}$ RAPD did not produce as clear a polymorphic band as the $OAS13_{950}$ marker and, since it was not linked as tightly with the *Co-4²* allele, the marker was not utilized further.

The two specific 24-mer SCAR primers synthesized on the basis of the DNA fragment sequence amplified by the $OAS13_{950}$ RAPD marker are shown in Table 4. The SCAR marker SAS13 amplified a single DNA

Table 3 Chi-square analysis for $OAL9_{740}$ and $OAS13_{950}$ RAPD marker loci segregating in two F_2 populations and two-point chi-square analysis and linkage estimates for marker loci and the *Co-4²* resistant allele (*n.d.* not defined)

Population	Locus tested	Expected ratio	Observed frequency	χ^2	P[a]	cM[b] ($r \pm$ SE)
Catrachita × SEL 1308	$OAL9_{740}$	3:1	47:19	0.32	0.57	
Black Magic × SEL 1308	$OAL9_{740}$	3:1	71:24	0.0	1.0	
Catrachita × SEL 1308	$OAS13_{950}$	3:1	47:19	0.32	0.57	
Black Magic × SEL 1308	$OAS13_{950}$	3:1	70:25	0.03	0.86	
Catrachita × SEL 1308	$OAL9_{740}$/*Co-4²*	3:6:3:1:2:1	15:29:0:1:0:17	57.5	0.0	3.1 ± 2
Black Magic × SEL 1308	$OAL9_{740}$/*Co-4²*	3:6:3:1:2:1	12:44:3:0:1:21	62.8	0.0	4.5 ± 2
Across populations	$OAL9_{740}$/*Co-4²*	3:6:3:1:2:1	27:73:3:1:1:38	119.5	0.0	3.9 ± 2
Catrachita × SEL 1308	$OAS13_{950}$/*Co-4²*	3:6:3:1:2:1	16:29:0:0:0:17	62.0	0.0	0.0 ± n.d.
Black Magic × SEL 1308	$OAS13_{950}$/*Co-4²*	3:6:3:1:2:1	12:45:0:0:0:24	81.0	0.0	0.0 ± n.d.
Across populations	$OAS13_{950}$/*Co-4²*	3:6:3:1:2:1	28:74:0:0:0:41	143.0	0.0	0.0 ± n.d

[a] P = probability estimated value
[b] Linkage analysis based on 1:2:1 genotypic segregation ratio for the *Co-4²* locus and 3:1 ratio for the $OAL9_{740}$ and/or $OAS13_{950}$ RAPD marker

Table 4 SCAR primer sequences derived from the RAPD marker $OAS13_{950}$ linked to the *Co-4²* allele in common bean

SCAR marker	Primer	Sequence[a]
SAS13	SAS13.24XP	5′-CACGGACCGAATAAGCCACCAACA-3′
	SAS13.24RP	5′-CACGGACCGAGGATACAGTGAAAG-3′

[a] Underlined sequences are the original $OAS13_{950}$ RAPD marker

fragment of the expected size (950 bp) and appeared as a single polymorphic band in agarose gels (Fig. 1d). In addition, SAS13 followed the same co-segregation with the *Co-4²* allele as the $OAS13_{950}$ RAPD marker in both F_2 populations, supporting the tight linkage between the marker and the resistance gene.

SCAR sequences of different lengths (15-mer, 18-mer, 23-mer) generated from the $OAL9_{740}$ RAPD produced a single monomorphic band in both susceptible and resistant lines, regardless of the combinations selected, the PCR file chosen, or after digestion with different endonucleases.

The $OAS13_{950}$ and SAS13 markers were used to identify F_2 individuals which did not possess the *Co-4²* allele in the SEL 1360/'G 2333' population. A progeny test of F_3 families (derived from F_2 individuals without the $OAS13_{950}$ marker) was performed using race 521 to discriminate homozygous resistant *co-4²co-4²*/*Co-7Co-7* genotypes from the heterozygous *co-4² co-4²*/*Co-7co-7* individuals. Six F_2 individuals in the 'SEL 1360/G 2333' population were first identified as susceptible to race 521; these are thus recessive for both genes (Table 3). Only 55 individuals from the remaining 86 F_2 individuals were harvested due to problems with photoperiod sensitivity in the cross. Among these, 14 individuals lacking the $OAS13_{950}$ marker were identified. The F_3 progeny (consisting mainly of 15 individuals) of these 14 F_2 individuals were screened with race 521, and 3 homozygous resistant F_3 families (*co-4²co-4²*/*Co-7Co-7*) consisting of 15 individuals were identified.

Discussion

The characterization of the genetic resistance in 'G 2333' to *C. lindemuthianum* is of major importance to bean breeders because of the broad-based resistance exhibited by this cultivar (Pastor-Corrales et al. 1994). The combination of resistance genes present in 'G 2333' would be of great utility for long-term resistance if bred into commercial bean cultivars. In this study, we confirm that 'G 2333' carries three independently inherited resistance genes, one of which is a second resistance allele, *Co-4²*, at the *Co-4* locus. The finding of different alleles at the same locus conferring differential resistance to races of *C. lindemuthianum* is not unique (Fouilloux 1979). The second allele at the *Co-4* locus offers improved potential in breeding for anthracnose resistance since it conditions resistance to a broader array of pathogenic races, being thereby similar to the second allele at the *bc-2* locus which conditions resistance to bean common mosaic virus (Drijfhout 1991).

The current study supports the importance of Mexican bean germplasm as a valuable source of resistance to anthracnose. Of the 12 differential cultivars 5 either come directly from Mexico or were derived through crossing with Mexican germplasm. Two Mexican landrace cultivars, 'PI 207262', named 'Tlalnepantla 64', and 'G 2333', named 'Colorado de Teopisca' (Pastor-Corrales et al. 1994), are used directly as differentials. Fouilloux (1979) identified the three *Mexique* genes (*Co-3*, *Co-4*, *Co-5*) in Mexican germplasm and transferred

them into the differential cultivars, 'Mexico 222', 'TO', and 'TU', respectively. Although the *Co-5* gene in 'G 2333' is the same as the *Mexique III* gene in 'TU', the second gene in 'G 2333' is unique as it is an allele of the *Co-4* (*Mexique II*) gene with a broader resistance pattern. The third gene, *Co-7*, appears to offer equal potential in breeding for resistance since, in combination with the *Co-4*^{2} allele, it has not been overcome by any existing race of the pathogen (Balardin et al. 1997; Pastor-Corrales et al. 1994). Bean breeders interested in controlling anthracnose may wish to utilize Mexican germplasm as a source of new and useful Middle American genes for resistance to anthracnose. This is analogous to the research on breeding for rust resistance in common bean where germplasm from Guatemala appears to offer the best potential as a source of resistance to that variable pathogen (Stavely 1990).

RAPD and SCAR markers linked to anthracnose resistance genes in common bean have been reported previously (Adam-Blondon et al. 1994; Young and Kelly 1996b, 1997). However, the finding of the $OAS13_{950}$ and $OAL9_{740}$ markers in the three differential cultivars, 'Widusa', 'PI 207262', and 'TO' was unexpected. We anticipated finding the markers only in 'G 2333' so their presence in the differential cultivar 'TO' (*Co-4* gene) raises some question about the linkage with the *Co-4*^{2} allele. Since no recombinants were observed between the $OAS13_{950}$ marker and the *Co-4*^{2} allele in two populations, a tight linkage is assumed. Similar tight linkages have been observed in common bean for the rust resistance *Ur-4* gene and the $OA14_{1100}$ marker (Miklas et al. 1993). One major difference between these studies is that the $OAS13_{950}$ marker was detected in two populations (143 individuals) with similar genetic backgrounds, i.e., Middle American gene in a Middle American background, whereas Miklas et al. (1993) found no recombinants (70 individuals) between the $OA14_{1100}$ marker and the Andean *Ur-4* gene. Recombination was restricted in the latter population because the Andean *Ur-4* gene had been backcrossed into genetically distant Middle American germplasm. Young and Kelly (1996b) observed lower recombination values between RAPD markers and the Middle American *Co-2* gene expressed in an Andean background than in a Middle American background. The presence of the $OAS13_{950}$ marker in two cultivars, 'TO' and 'SEL 1308', which carry alleles at the *Co-4* locus, is not a major limitation in breeding for resistance. The markers can be used to indirectly select either allele at the *Co-4* locus, depending on the germplasm available to the breeder. The presence of the markers in 'Widusa' and 'PI 207262' suggests that they possess a different allele at the *Co-4* locus in addition to other resistance gene(s). Since neither 'Widusa' nor 'PI 207262' possess greater levels of resistance than 'TO', they cannot carry either characterized *Co-4* allele. Allelism tests among 'Widusa', 'PI 207262', 'TO' and 'SEL 1308' are planned since the resistance gene(s) in 'Widusa' and 'PI 207262' have not been characterized fully.

The authors have no explanation for the presence of the markers in navy bean cultivars 'Monroe', 'Sanilac', 'Seafarer', and 'Seaforth'. Other related navy bean cultivars such as 'Michelite', 'Mayflower', 'C-20', 'Bunsi', 'Kentwood', 'Fleetwood', 'Harofleet', 'Harokent', and 'OAC-Rico' were screened but the marker was absent. The marker was absent from all other market classes of dry bean and snap bean surveyed. Since all bean genotypes with the $OAS13_{950}$ RAPD and SCAR markers possess different genes for resistance to anthracnose, a cluster of anthracnose resistance genes possibly, exists at or near the *Co-4* locus. The existence of clusters of resistance genes have been demonstrated in *Lactuca* and *Lycopersicon* spp (Maisonneuve et al. 1994; Salmeron et al. 1996). Although the *Co-1* gene in 'Sanilac' and 'Seafarer' and the *Co-2* gene in 'Seaforth' are independent of the *Co-4* locus, all these cultivars carry additional resistance to anthracnose races *beta* and *gamma* (Cardenas et al. 1964). The resistance in 'Monroe' is unknown, but it may carry the same resistance to races *beta* and *gamma* as its parent, 'Michelite'. In common bean, Adam-Blondon et al. (1994) mapped nine genes of known function, the majority of which represent gene families such as the three to ten genes for the phaseolin storage protein, six genes for chalcone synthase, and four for phenylalanine ammonia lyase. In all cases only one member of the gene family was mapped (Adam-Blondon et al. 1994). The presence of clustered genes for phaseolin supports the theory that specific genes for anthracnose resistance may exhibit a similar clustering pattern based on the presence of the marker in genotypes with different resistance genes. Additional evidence for the presence of complex loci of linked resistance genes in common bean was demonstrated for genes controlling resistance to bean rust at the *Ur-5* locus (Stavely 1984).

Although the broad-based anthracnose resistance of 'G 2333' has been widely tested and confirmed by different research groups (Balardin et al. 1997; Pastor-Corrales et al. 1994; Schwartz et al. 1982), the identification of individual genes is needed to evaluate their potential in breeding for resistance. In the absence of a race of the pathogen virulent on 'G 2333', the use of tightly linked markers, as suggested by Maisonneuve et al. (1994), to identify and combine different genes was evaluated. In the F_2 population ('SEL 1360/G 2333') where the *Co-5* gene is fixed and the *Co-4*^{2} and *Co-7* genes are segregating independently, six of the nine possible genotypic combinations will carry at least one dominant *Co-4*^{2} allele. The other three genotypes would be homozygous for the recessive *Co-4*^{2} allele, one of which is the double recessive *co-4*^{2}*co-4*^{2}/*co-7 co-7*, identified by its susceptible reaction to race 521, which is also virulent to the *Co-5* gene. All three genotypes were identified by selecting F_2 individuals which

do not possess the $OAS13_{950}$ RAPD and SAS13 SCAR markers linked in coupling with the *Co-4²* allele. By eliminating the double recessive individuals based on inoculation we were able to identify the other two genotypes. These genotypes possess the recessive *co-4²* allele and differ at the *Co-7* locus for homo- or heterozygosity. The homozygous individuals were identified as pure breeding resistant F_3 families after inoculation with race 521 and will be used as parents in allelism tests with other known resistance genes to fully characterize the *Co-7* gene.

In addition, the $OAS13_{950}$ RAPD and SCAR markers were used to construct the resistant and susceptible DNA bulks for screening to detect markers linked to the *Co-7* gene. Individuals included in the susceptible bulk were detected in the 'SEL 1360/G 2333' population after inoculation with race 521 of the pathogen. We confirmed that all members of the susceptible bulk did not have the $OAS13_{950}$ marker as expected. Since no race is available to distinguish the *Co-4²* and *Co-7* genes, only those resistant individuals which do not possess the $OAS13_{950}$ markers linked to the *Co-4²* gene will be included as members of the resistant bulk. This ensures that the resistance to race 521 can only be derived from the *Co-7* gene. Screening of the bulks with random decamer primers will be conducted to find a coupling RAPD marker linked to the new resistance gene, since the resistant F_2 heterozygotes were not distinguished from the F_2 homozygotes when the resistant bulk was formed. Once detected, the marker can be used to ensure that the *Co-7* resistance gene is incorporated into different genetic backgrounds, independent of other anthracnose resistance genes.

Finally, the use of this information in developing a better differential series for the classification of new races of anthracnose is proposed. Despite its broad resistance, 'G 2333' is not the ideal differential cultivar since it carries three different resistance genes. One suggestion would be to replace 'G 2333' with 'SEL 1308', which has been shown to carry only the single *Co-4²* allele, and consider including upon identification a genotype known to carry only the *Co-7* gene. The development of lines in which the *Co-7* gene is present alone becomes a priority. The *Co-7* gene represents a unique and important source of resistance to anthracnose and should be invaluable, when fixed alone in a host genotype, for better classifying potential new races of the anthracnose pathogen and for use in breeding durable anthracnose resistant germplasm.

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