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ORIGINAL PAPER

Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.)

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Abstract Powdery mildew (PM) is a serious disease in many legume species, including the common bean (*Phaseolus vulgaris* L.). This study investigated the genetic control behind resistance reaction to PM in the bean genotype, Cornell 49242. The results revealed evidence supporting a qualitative mode of inheritance for resistance and the involvement of two independent genes in the resistance reaction. The location of these resistance genes was investigated in a linkage genetic map developed for the XC RIL population. Contingency tests revealed significant associations for 28 loci out of a total of 329 mapped loci. Fifteen were isolated or formed groups with less than two loci. The thirteen remaining loci were located at three regions in linkage groups Pv04, Pv09, and Pv11. The involvement of Pv09 was discarded due to the observed segregation in the subpopulation obtained from the Xana genotype for the loci located in this region. In contrast, the two subpopulations obtained from the Xana genotype for the BM161 locus, linked to the Co-3/9 anthracnose resistance gene (Pv04), and from the Xana genotype for the SCAReoli locus, linked to the Co-2 anthracnose resistance gene (Pv11), exhibited monogenic segregations, suggesting that both regions were involved in the genetic control of resistance. A genetic dissection was carried out to verify the involvement of both regions in the reaction to PM. Two resistant recombinant lines were selected, according to their genotypes, for the block of loci included in the Co-2 and Co-3/9 regions, and they were crossed with the susceptible parent, Xana. Linkage analysis in the respective F_2 populations supported the hypothesis that a dominant gene (*Pm1*) was located in the linkage group Pv11 and another gene (*Pm2*) was located in the linkage group Pv04. This is the first report showing the localization of resistance genes against powdery mildew in *Phaseolus vulgaris* and the results offer the opportunity to increase the efficiency of breeding programs by means of marker-assisted selection.

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Introduction

Powdery mildew (PM) can result in a devastating disease in many legume crops, including the common bean (*Phaseolus vulgaris* L.). In Northern Spain, PM is caused by the fungus, *Erysiphe diffusa* (Cooke and Peck) U. Braun and S. Takam (Trabanco et al. 2012) causing significant yield losses in bean crops. The fungus is an obligate biotrophic pathogen which produces small, round, greyish or whitish spots on leaves, stems and pods. In extreme cases, the fungus can cover all aerial parts of the plant resulting in premature defoliation, premature fall of flowers and pods and a reduction in plant development.

Control of the disease is difficult by conventional techniques, because the fungus produces spores that are easily spread by the wind. In addition, the fungus can be spread by infected seeds, plant debris or by some weeds. Use of resistant cultivars provides an effective approach for disease control, eliminates the use of fungicides, minimizes

crop losses and supports sustainable production management. The development of resistant cultivars requires information on the variation in the reaction against this fungus, potential resistance sources and the nature and inheritance of the resistance. In contrast with other legume species, such as mungbean, pea or soybean, only limited information is available to common bean breeders on the genetic control of PM.

With regard to resistance inheritance against this pathogen, several studies have reported a qualitative mode of inheritance controlled by either one dominant gene (Dundas 1936), one dominant and one recessive gene (Bett and Michaels 1995) or by two complementary genes (Ferreira et al. 1999). Recently, Trabanco et al. (2012) investigated the reaction of 245 accessions and five segregating populations using seedling tests under controlled conditions. They found that only 6 out of the 245 evaluated genotypes showed a complete resistance, without visible symptoms on the leaves: genotype Amanda, Belneb, Cornell 49242, Negro San Luis, Porrillo Sintetico, and a local accession, BGE003161. The observed reactions in the five segregating populations fitted Mendelian ratios with two different modes of inheritance. These were either dominant or complementary modes of action. The results also suggested that the resistant reaction in Cornell 49242 could be explained by two dominant and independent resistance genes, where one gene conferred complete resistance, and another gene moderated mycelial development on the leaves. Both genes showed a dominant epistatic relationship. However, the relative position of these resistance genes in the bean linkage map has not been analyzed.

Resistance genes are not distributed randomly in the bean genome. They appear to be clustered on particular chromosomes and specific regions. For example, anthracnose resistance genes are located in specific regions of the genome and they are organized in groups or clusters of loci in which individual gene(s) confer resistance to one isolate or race (Rodríguez-Suárez et al. 2007, 2008; Campa et al. 2009). To date, seven specific regions, which include these resistance specificities, have been identified in linkage groups (LGs) Pv01, Pv02, Pv03, Pv04, Pv07, Pv08 and Pv11 (Ferreira et al. 2012).

Genetic and molecular studies have revealed the presence of many plant disease resistance genes (R genes) in the genomes of monocotyledonous and dicotyledonous plants. R genes can have two functions in the plant–pathogen interaction. They can operate as sensors, directly or indirectly detecting pathogens, or they can activate downstream signaling that, in turn, leads to pathogen resistance (Chisholm et al. 2006; DeYoung and Innes 2006). R genes are often arranged in complex clusters or group of genes (Ameline-Torregrosa et al. 2008b; Meyers et al. 2003; Michelmore and Meyers 1998). Clusters of R genes have also been reported at the end of LGs Pv04 and Pv11 in the bean genome (Chen et al. 2010; David et al. 2009; Geffroy et al. 1998, 2009).

The objective of this study was to investigate the genetic control of PM resistance in the bean genotype Cornell 49242 and to identify the location in the genetic map of the genes conferring resistance against this pathogen. Mapping of these genes can supply markers suitable for marker-assisted selection in bean-breeding programs and provide the basis for the future genomic characterization of these resistance genes.

Materials and methods

Plant materials

A total of 109 F_7 recombinant inbred lines (RILs), derived from the cross Xana × Cornell 49242 by single seed descent (XC RIL population), were used to study the inheritance of the response to PM. Xana is a large white-seeded line bean variety, with determinate, type I growth habit, which belongs to the market-class fabada. It was developed at Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA, Villaviciosa, Spain) from a cross between two Andean genotypes, Andecha and V203. Cornell 49242 is a small-seeded black bean line showing indeterminate prostrate, type III growth habit. It belongs to the Mesoamerican gene pool and is included in the market class black turtle. Cornell 49242 is resistant to PM whereas Xana is susceptible (Trabanco et al. 2012).

Inoculation procedure and disease scoring

An isolate of PM obtained from a single spot in one infected bean plant at SERIDA-Villaviciosa was used in this work. The pathogen was grown and maintained on plants of susceptible bean cv. Xana in spore-proof chambers.

Resistance tests were carried out according Trabanco et al. (2012). Four seeds of each genotype were planted in a 15-cm plastic pot of 1.5 l containing 80 peat: 20 perlite. Plant response was recorded as resistant or susceptible considering the mycelial development on the areal part of the plants. The XC RIL population was evaluated in three separated tests. Two pots per recombinant line were arranged in each test in a randomized design. In each test, the susceptible line Xana and the resistant lines Cornell 49242 and Porrillo Sintético were included as reference of different reaction types.

Molecular marker development and analysis

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).

Two type of marker loci were added to the genetic map developed in XC RIL population (Pérez-Vega et al. 2010) to saturate specific regions or linkage groups. (1) Markers previously described in the literature with known position in the bean genetic map: the 254G15F SCAR marker developed from the BAT93 BAC clone 254G15 (David et al. 2008), the SQ4 SCAR marker (Awale et al. 2008) and the $OF10^{1100}$RAPD marker located on end of LG Pv04 (Rodríguez-Suárez et al. 2007). Several mapped microsatellite markers (Blair et al. 2003; Gaitán-Solis et al. 2002; Grisi et al. 2007; Hanai et al. 2010) were also analyzed. (2) Microsatellite markers designed from the accessions EU931620.1, FJ817289, FJ817291.1, and FJ817290 deposited in the Genebank database (David et al. 2009) and located in the Co-3/9 region (LG Pv04). WebSat platform (http://wsmartins.net/websat/; Martins et al. 2009) was used to identify microsatellite motifs and the corresponding forward (FW) and reverse (RV) primers. Sequences were screened for all possible dimeric and trimeric repeat motifs. Sequences containing a minimum of five di-nucleotides or four tri-nucleotides motif repeats were considered for primers design. The selected parameters in primers design were: between 18 and 24 bp (optimum 22 bp); annealing temperature between 50 and 68 °C (optimum 60 °C); GC content between 40 and 60 % and predicted size of the amplified fragment between 100 and 300 bp. Annealing temperature was selected using the OligoAnalyzer software (www.uku.fi/~kuulasma/OligoSoftware).

The polymerase chain reaction (PCR) amplifications were performed in 20 µl solution containing 25 ng of genomic DNA, 100 mM Tris–HCl, 100 mM KCl (pH 8.3), 4 mM MgCl2, 0.2 mM each dNTP (Bioline, London), 0.2 µM each primer, and 1.25 U of Biotaq DNA polymerase (Bioline, London). Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems, Life Technologies, Foster City, CA) programmed according to the corresponding author or following the recommendations supplied by the OligoAnalyzer software. 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 SYBR safe (Invitrogen, Life Technologies, CA, USA) and the amplification products were visualized under UV light. A 100-bp ladder (G.E. Healthcare Life Science, Fairfield, CT, USA) and the software GeneTools V4.01 (Syngene, Cambridge, UK) were used to measure the size of the fragments.

Linkage analyses

A genetic map developed for the XC RIL population was used to locate the genomic regions involved in the control of the response to PM. The linkage map consisted of 294 loci mapped by Pérez-Vega et al. (2010) and 35 added markers that were common to other bean maps (Gaitán-Solis et al. 2002; Grisi et al. 2007; Hanai et al. 2010). MAPMAKER Macintosh version 2.0 software (Lander et al. 1987) was used for map construction. 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 loci (cM) were calculated using the Kosambi mapping function. The obtained map had 11 linkage groups, which were aligned according to the common bean core linkage map using common molecular markers as anchor points. Linkage groups were named according to Pedrosa-Harand et al. (2008).

Contingency tests were used to investigate the association between the response to PM (classified as resistant or susceptible) and the loci included in the genetic map. The Chi-square test was used to test the goodness-of-fit of the observed-to-expected ratio in the qualitative traits and linkage analysis was performed using MAPMAKER Macintosh version 2.0. Loci showing significant deviations ($p < 0.01$) were not considered in the linkage analysis. Statistical analyses were performed using SPSS V12 software (SPSS Inc., Chicago, IL).

Genetic dissection

To verify the relative position of the resistance genes to PM in specific regions of the genetic map, a genetic dissection was undertaken. Recombinant lines showing resistant responses were selected according to their genotypes for specific loci located in the candidate regions where the resistance loci could be located. Selected recombinant lines were crossed using the susceptible cultivar, Xana, as the female. Crosses between the selected recombinant lines were also performed to study the mode of action of the different genes. F_1 plants were tested for their response to PM and resistant plants were self-crossed. The corresponding F_2 progenies were tested against a local isolate of PM and analyzed for the amplification of the specific markers used in the selection of recombinant lines.

Results

Response of the XC RIL population

Observed responses in the XC RIL population were classified into two groups: resistant and susceptible, like the parent, Xana. A total of 29 recombinant lines showed a susceptible response like the parent, Xana, while the 80 remaining lines were resistant. The observed segregation fitted the 3:1 expected ratio for two independent genes ($\chi^2_{3:1} = 0.15$, $p = 0.70$), which suggested that two independent genes control the resistant reaction in Cornell 49242. The mode of action of the genes (dominant or recessive) could not be deduced from this segregation.

Identification of candidate regions containing resistance genes

To identify the regions associated with PM resistance, contingency tests were undertaken among the loci included in the genetic map and the response to PM. Significant association was found for 28 loci out of a total of 329 mapped loci (Table 1 in ESM). Fifteen of them were isolated loci or formed groups with less than two loci in the genetic map. The thirteen remaining loci were located at three regions: three loci formed a large block in LG Pv04, including SCAR SW12, which is linked to the Co-3/9 anthracnose resistance gene; five loci were located on LG Pv11 and included the SCAR marker SCAreoli, linked to the Co-2 anthracnose resistance gene and; five loci were grouped in a wide region on LG Pv09, including the microsatellite marker BM141 and the AFLP marker McatEag^{131}. The marker locus BM161 (closely linked to SW12 on LG Pv04) and the SCAreoli locus on LG Pv11 showed greater deviations with respect to independent segregation with the response to PM. The deviations were due to an excess of resistant lines containing the Cornell 49242 genotype at these loci. In contrast, the deviation found for the five loci of LG Pv09 was due to an excess of resistant lines showing the Xana alleles (susceptible parent) for these markers, which suggested that this was a random association.

Marker enrichment of LGs Pv04 and Pv11

To precisely locate the genes conferring resistance to PM, twenty loci were added to LGs Pv04 and Pv11, with respect to the genetic map described by Pérez-Vega et al. (2010).

Linkage group Pv04

Fifteen new loci were incorporated into the LG Pv04. Microsatellite motifs were investigated in the accessions: EU931620.1, FJ817289, FJ817291.1 and FJ817290.1. A total of 77 microsatellites were designed: 38 with a dinucleotide repeat motif and 39 with a trinucleotide repeat motif. Twenty-seven microsatellites were tested in cvs. Xana and Cornell 49242 and 24 showed the amplification product (Table 2 in ESM). Seven microsatellites were shown to be polymorphic between both cultivars and they were analyzed in the XC RIL population. Table 1 shows the characteristics and the PCR primer pairs for the seven microsatellite markers that were designed. In all cases, the observed segregation fitted the expected ratio for one gene. Six microsatellites were mapped on LG Pv04, whereas the microsatellite, FZ-E9o, was located at the end of LG Pv11 (Fig. 1). The amplification product of SCAR 254G15F revealed three fragments in 2 % agarose gels, which were closely linked to the SW12 locus (Fig. 1). Twelve SSR markers, previously mapped on LG Pv04, were also analysed for polymorphism and five of them were added to the map: PV-ag004, BMd15, BMd9, BMarc22 and PVBR112. Finally, the RAPD fragment, OF10^{1100}, was added (Fig. 1). The added loci Contig-ah, 254G15E^{550}, 254G15E^{600}, 254G15E^{320}, FZ-E9 h, Contig IIIh, Contig IIIi, and OF10^{1100} were mapped on the Co-3/9 region and they were significantly associated with the response to PM also (Fig. 1; Table 1 in ESM).

Linkage group Pv11

Fourteen microsatellite markers mapped on Pv11 were investigated for polymorphism. Three of them were polymorphic and mapped on LG Pv11; PvBR113, BMd33 and PvM98. Finally, the SCAR marker, SQ4 was also analyzed and added to this LG (Fig. 1). The added loci SQ4 and FZ-E9o were mapped on the Co-2 region and they were significantly associated with the response to PM (Fig. 1; Table 1 in ESM).

Tentative mapping using subpopulations

To verify the involvement of the candidate regions in the response to PM, the resistant/susceptible segregations were investigated in different subpopulations of recombinant lines (Table 2). The subpopulation obtained from the Xana genotype for the McatEag^{131} locus (Pv09) exhibited a 36R:7S segregation for resistance to PM, which fitted the expected ratio for two genes ($\chi^2_{3:1} = 1.74$, $p = 0.19$). This finding suggested that this region on LG Pv09 was not involved in the genetic control of resistance to PM. A change in the segregation type with respect to the observed in the XC RIL population (non-segregation or monogenic segregation) would be expected if this tagged region was involved in the genetic control of the response to PM.

Table 1 Characteristics and PCR primer pairs amplifying seven microsatellite markers designed from the sequence FJ817291.1 and FJ817290.1 deposited in the Genebank data base

Marker name	Genebank entry	Motif		Primer sequence (5′ to 3′)	Tm (°C)	Predicted size
FZ-E9b	FJ817290.1	$(CA)_5$	Forward	ATTGGTAGAAACCGACTTTGGA	55	138
			Reverse	ATCCACAACAGCTTACAGGGTT		
FZ-E9m	FJ817290.1	$(AAG)_5$	Forward	CACTTAACAGGAGAAATCAGCTC	50	250
			Reverse	AGCTTCTGCACTACATCTTGTC		
FZ-E9n	FJ817290.1	$(TA)_{10}$	Forward	CGTTAGCCAAATTACAGAGCAA	50	160
			Reverse	AGACCCTTTCCTTCTCAATGC		
FZ-E9o	FJ817290.1	$(ATA)_4$	Forward	CAATCAAATCATGGAGAGGGTA	50	297
			Reverse	GAATCTGTGAATGGGACGAATA		
Contig-ah	FJ817291.1	$(ATA)_5$	Forward	CTGTTTCACAATCAACGGAT	58	186
			Reverse	TGCAAAAGGGACCTATCTAA		
Contig-III h	FJ817291.1	$(ATT)_4$	Forward	TGTAAGCTCTTTCCTCCCTCTG	58	289
			Reverse	TGACCATTGATTTCAGTAAGCC		
Contig-III i	FJ817291.1	$(AT)_6$	Forward	CACCATGTTCTGCTTCTTTT	50	249
			Reverse	CAGTTCAGAGGTGATTGTTTG		

In contrast, the subpopulation obtained from the Xana genotype for the BM161 locus or from the Xana genotype for the SCAReoli locus exhibited: 21R:24S and 21R:23S segregations, respectively (Table 2). In both cases, the observed segregation fitted the expected ratio for one gene ($\chi^2_{1:1} = 0.2$, $p = 0.35$; $\chi^2_{1:1} = 0.0\, p = 0.76$). These results suggested that one resistance gene to PM was linked to the BM161 locus (Co-3/9 region on LG Pv04) and the other was linked to the SCAReoli locus (Co-2 region on LG Pv11). This hypothesis is consistent with the segregation observed in the two subpopulations established from the Cornell genotypes for the loci BM161 and SCAReoli (Table 2). The segregations for subpopulations obtained from Cornell 49242 genotypes for these loci were: 45R:1S and 43R:1S. Both segregations did not fit the expected ratio for two independent genes or one gene. This finding suggested that a resistance gene was fixed in both subpopulations. The observed susceptible lines may be due to recombination events between the resistance locus and the respective marker loci. Finally, the involvement of both regions in the resistance control was consistent with the non-segregation observed (Table 2) in the subpopulation obtained from the Xana genotype for the BM161 and SCAReoli loci (all the lines were susceptible) or from the Cornell 49242 genotype for the BM161 and SCAReoli loci (all the lines were resistant).

The two subpopulations established from the Xana genotype for the BM161 and SCAReoli loci showed a monogenic segregation for resistance (Table 2) and so they were used in the linkage analysis. Subpopulation established from the Xana genotype for the SCAReoli locus (Pv11) revealed that the BM161 locus was tightly linked to the resistance gene (recombination fraction, RF = 0.02; LOD = 6.84). The SW12 and FZ-E9b loci, located in Co-3/9 region, also showed a close linkage to the resistance gene (LOD > 3.5). Subpopulations established from the Xana genotype for the BM161 locus (Pv04) revealed that the SH13b locus was tightly linked to the resistance gene (recombination fraction, RF = 0.01; LOD = 10.01). Loci SQ4, SCAreoli and PVag001, located in the Co-2 genomic region, also showed a close linkage to the resistance gene (LOD > 7.00). Figure 1 shows the relative position of the two resistance loci inferred from the linkage analysis.

Genetic dissection

A genetic dissection was carried out to confirm the hypothesis that two independent genes located at the end of LGs Pv04 and Pv11 conferred resistance to PM in Cornell 49242. Two resistant recombinant lines were selected according to their genotypes for the block of loci included in the Co-2 and Co-3/9 regions and, they were crossed with the susceptible parent, Xana. In all cases, the susceptible genotype, Xana, was used as the female parent. The resistant recombinant line 22 (XC22) carried Cornell 49242 genotypes for the Co-2 region markers (SCAreoli, SQ4, PVag001 and SH13b) and the Xana genotypes for markers of the Co-3/9 block (SI19, 254-G15F^{550}, 254-G15F^{600}, 254-G15F^{320}, FZ-E9b, SW12, BM161, FZ-E9b and OF10^{1100}; Fig. 1). Resistant recombinant line 217 (XC217) carried Xana genotype for the Co-2 region markers and the Cornell 49242 genotypes for markers of the Co-3/9 region. Seven F_1 plants, derived from the cross Xana × XC22, were tested and all of them were resistant. Eight F_1 plants, derived from

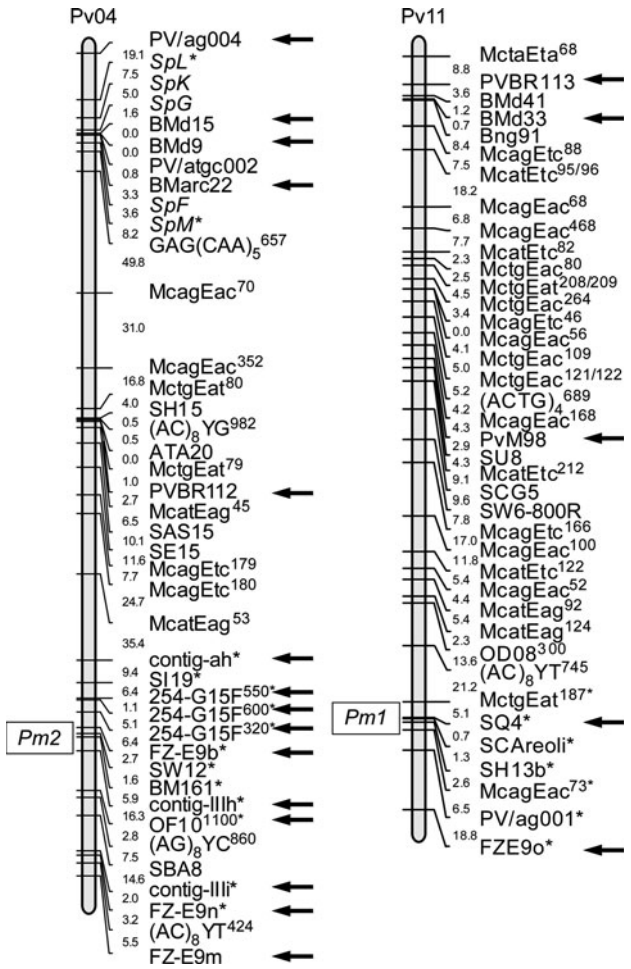

Fig. 1 Linkage map for the linkage groups Pv04 and Pv11 obtained in the RIL population developed from the cross Xana × Cornell 49242. Map distances, on the *left*, are expressed in centimorgans. *Asterisks* near the name of the loci indicate a significant association with the resistance to powdery mildew revealed by contingency tests ($p < 0.05$). *Arrows* indicate the new mapped loci in this study. Markers SCAreoli and SQ4 (Pv11) were described as linked to the genes *Co-2* and *Ur-11* conferring resistance to anthracnoses and rust, respectively. Marker SW12 (Pv04) was described as linked to the anthracnose resistance gene *Co-3* and marker $OF10^{1100}$ was described as linked to an anthracnose resistance gene tentatively named as *Co-10* (Ferreira et al. 2012)

the cross Xana × XC217, were tested and they all were also found to be resistant. Finally, the six F_1 plants obtained from the cross XC217 × XC22 (line XC217 was used as female parent) exhibited a resistant reaction like the resistant parent Cornell 49242.

F_1 plants derived from the crosses Xana × XC22 and Xana × XC217 were self-crossed and the F_2 populations derived from each cross were analysed for their response to PM and for markers located in the Co-2 or Co-3/9 regions. The F_2 population derived from the cross Xana × XC22 (86 plants) showed a 59R:27S segregation, which fitted the expected ratio for one dominant gene ($\chi^2_{3:1} = 1.88$; $p = 0.17$). The result suggested that resistance to PM was controlled by one dominant gene in the XC22 line. Four molecular markers, located in the Co-2 region, were analysed in most of the plants included in this F_2 population (Table 3). Linkage analysis corresponding to the joint segregation for resistance to PM and four molecular markers located in the Co-2 region (Table 3) confirmed that the resistance gene in XC22 line was located in LG Pv11. Resistance locus was closely linked to the SQ4 marker (RF = 3.6 %, LOD = 17.37).

Table 2 Observed segregation on different subpopulations established from Xana or Cornell 49242 genotype for the loci BM161 (Pv04) and/or SCAReoli (Pv11). Adjustment to an expected segregation controlled by two loci or one single locus is indicated

Genotype		Observed Segregation					
BM161 (Pv04)	SCAreoli (Pv11)	R	S	$\chi^2_{1:1}$	p	$\chi^2_{3:1}$	p
Cornell		45	1	32.1	0	12.8	0
	Cornell	43	1	40.1	0	12.1	0
Xana		21	24	0.2	0.35	19.3	0
	Xana	21	23	0	0.76	17.5	0
Xana	Xana	0	19	–	–	–	–
Cornell	Cornell	19	0	–	–	–	–
Xana	Cornell	20	1	17.9	0	5.19	0.02
Cornell	Xana	19	1	15.2	0	4.27	0.03

R, resistant; S, susceptible

The F_2 population derived from the cross Xana x XC217 (79 plants) showed a 53R:26S segregation. The observed segregation fitted the expected ratio for one dominant gene ($\chi^2_{3:1} = 2.64$; $p = 0.10$), which showed that resistance to PM in the XC217 line was controlled by a dominant gene. Eleven molecular markers, located in the Co-3/9 region, were also analysed in the majority of plants of this F_2 population (Table 4). Linkage analysis corresponding to the joint segregation of resistance to PM with molecular markers mapped in the Co-3/9 region (Table 4) confirmed that the resistance gene in the XC217 line was located in this region. This resistance locus was linked to the SBA8 marker (RF = 24.4 %, LOD = 3.36). In this segregation, seedlings classified as resistant (in contrast to Xana) showed moderate mycelial development on the leaves.

Discussion

This study investigated the inheritance of genetic resistance to PM in the bean genotype Cornell 49242, using a RIL population obtained from a cross between Xana and

Table 3 Linkage analysis between the gene conferring resistance to powdery mildew and four molecular markers located in LG Pv11 in a F_2 population derived from the cross Xana × XC22

Marker	Linked gene	Parental genotypes(bp)		F_2 plants										RF	LOD
				Resistant					Susceptible						
		Xana	XC22	XX	X_	XR	R_	RR	XX	X_	XR	R_	RR		
PVag001	*Co-2*	160	150	4		34		18	20		5		0	0.10	10.64
SH13b	*Co-2*	480	500	4		32		20	18		6		0	0.12	9.33
SQ4	*Co-2*	–	1,440	2			54		25			1		0.03	17.37
SCAReoli	*Co-2*	–	1,000	2			52		19			2		0.05	12.80

XX, homozygous for the Xana alleles of the corresponding marker; X_, homozygous for the Xana alleles or heterozygous; XR, heterozygous; R_, homozygous for the XC22 alleles or heterozygous; RR, homozygous for the XC22 alleles

Table 4 Linkage analysis between the gene conferring resistance to powdery mildew and nine molecular markers located in the Co-3/9 region (LG Pv04) in a F_2 population derived from the cross Xana × XC217

Marker	Linked gene	Parental genotypes (bp)		F_2 plants										RF	LOD
				Resistant					Susceptible						
		Xana	XC217	XX	X_	XR	R_	RR	XX	X_	XR	R_	RR		
FZ-E9b	*Co-3/9*	–	160	9			44		14			10		0.25	2.91
FZ-E9n	*Co-3/9*	175	170	4		29		19	8		14		3	0.36	0.94
BM161	*Co-3/9*	185	–		36			16		20			2	0.41	0.24
SBA8	*Co-3/9*	–	530	9			44		14			11		0.25	3.36
SI19	*Co-3/9*	460	–		32			21		19			6	0.41	0.21
SW12	*Co-3/9*	725	–		35			18		20			5	0.41	0.24
254-G15F	*Co-3/9*	–	320	5			46		11			13		0.27	2.23
	Co-3/9	550	–		34			17		20			4	0.37	0.39
	Co-3/9	–	600	3			48		10			14		0.26	2.21
Contig-IIIh	*Co-3/9*	300	–		35			17		20			5	0.41	0.24
Contig-IIIi	*Co-3/9*	250	–		32			18		21			3	0.31	0.93

XX, homozygous for the Xana alleles of the corresponding marker; X_, homozygous for the Xana alleles or heterozygous; XR, heterozygous; R_, homozygous for the XC217 alleles or heterozygous; RR, homozygous for the XC217 alleles

Cornell 49242. Previous studies that have investigated the response in F_2 populations suggested that two independent and dominant loci could be involved in the response against this pathogen (Trabanco et al. 2012). The results of this study revealed that there was a qualitative mode of inheritance for resistance and confirmed the involvement of two independent and dominant genes in the response to PM. The resistance genes were located in the genetic map using three complementary strategies: identifying potential regions associated with the resistance by contingency tests; mapping of resistance using two subpopulations established from the XC RIL population and, linkage analysis in the two F_2 populations obtained from a genetic dissection. All the results supported the hypothesis that a dominant gene (tentatively named as *Pm1*) is located at the end of the linkage group, Pv11 and another gene (tentatively named as *Pm2*) is located at the end of the linkage group, Pv04. Although different relative positions for the *Pm2* gene were estimated on Pv04 in the two linkage analyses (subpopulation of recombinant lines and the F_2 population), a significant linkage (LOD > 3) between the *Pm2* gene and markers for LG04 was found in both cases. Both genes seemed to control a different response to PM; gene *Pm1* conferred complete resistance to PM as the seedlings did not show any visible symptoms, while gene *Pm2* conferred a moderate resistance, in that there was limited mycelial development on the leaves, but there was no sporulation. In contrast, the resistant reaction (no visible symptoms) in the six F_1 plants derived from the cross between XC22 × XC217 suggested that both genes showed a

dominant epistatic relationship. This conclusion agreed with the genetic control reported by Trabanco et al. (2012) for their observed response in F_2 populations.

Resistance genes to PM were mapped at the end of the linkage groups Pv04 and Pv11. These relative positions are in agreement with genetic and genomic evidences. Clusters of resistance genes involved in the response to specific races of anthracnose, caused by the fungus *C. lindemuthianum*, were located in the same relative positions (Ferreira et al. 2012; Rodríguez-Suárez et al. 2007, 2008). Genes controlling resistance to bean rust were also mapped at the end of LG Pv11 and LG Pv04 (Awale et al. 2008; Miklas et al. 2002), as were genes controlling resistance to halo blight, caused by the bacteria, *Pseudomonas syringae* (Chen et al. 2010). Resistance gene analogs (RGAs) were mapped at the end of both linkage groups (López et al. 2003; Mutlu et al. 2006). The vast majority of R genes cloned so far encoded for R proteins containing nucleotide-binding sites (NBS) and the C-terminal leucine-rich repeat motif (LRR) (Dangl and Jones 2001; McDowell and Woffenden 2003). Clusters of sequences encoding NBS-LRR proteins were annotated in the same relative positions (Chen et al. 2010; David et al. 2009; Ferrier-Cana et al. 2003). Therefore, the location of the genes involved in the control of the response to PM in these regions was not unexpected. The involvement of genes encoding LRR proteins in the resistance to PM has been suggested in other legumes. In *Medicago truncatula*, Ameline-Torregrosa et al. (2008a) reported a cluster of genes encoding for LRR protein in the relative position where a QTL associated with the response to PM was mapped. In soybean, the candidate gene Glyma16g34090, encoding for a LRR protein (Jun et al. 2012) has been described at the same chromosome region including a resistance gene to PM. The *Mlo* genes constitute a family of transmembrane proteins, including the leucine rich domain, and many Mlo homologs have been identified in various plants (Singh et al. 2012). The Mlo gene was first identified in barley and its recessive allele leads to broad spectrum resistance against PM (Büschges et al. 1997).

Marker saturation of regions that affect the genetic control of specific traits represents one of the first steps in genomic characterization and can provide useful markers for indirect selection. To pinpoint the location of the gene conferring resistance to PM, a total of 20 new loci were incorporated on the LGs Pv04 and Pv11. The markers were selected according to their relative positions in previously reported genetic maps or were developed from BAC clone sequences obtained for the B4 resistance cluster (David et al. 2009). The resulting LG showed a higher level of saturation than the LGs described by Pérez-Vega et al. (2010). The previous linkage map included a gap in LG Pv04 and only three markers in the Co-3/9 region [SI19 and SW12, reported by Pérez-Vega et al. (2010), and BM161]. The map obtained in this study carried nine new loci in this region and the average genetic distances between loci was 6.1 cM for this region. All the markers were mapped on their expected relative positions, except the marker FZ-E9o, which was mapped on the end of LG Pv11. This microsatellite marker was developed from the FZ-E9 BAC clone sequence, which has been described to be at LG Pv04. A close relationship between the Co-3/9 region (end of LG Pv04) and the Co-2 region (end of LG Pv11) has been described previously (David et al. 2009) and it could explain the relative position of the FZ-E9o marker. The mapping of markers developed from BAC clone sequences confirmed the location of the sequences in chromosome 04.

This study supplies two markers closely linked to the *Pm1* gene (SQ4 and SCAReoli), which conferred complete resistance against the local isolate used in this work. Indirect selection using these markers is being successfully conducted to introgress genetic resistance to PM in common bean cv. Xana. With regard to the *Pm2* gene, the linkage analysis revealed different relative positions on the LG Pv04 in the two analyzed populations. The microsatellite marker BM161 showed the highest linkage to the resistance gene in the subpopulation established from the XC RIL population, whereas in the F_2 population, the highest linkage was with the SBA8 marker. These different relative positions could be explained by the presence of two linked resistance loci in the Co-3/9 genomic region. As previously mentioned, at least ten anthracnose resistance genes have been mapped at this position (Ferreira et al. 2012) and 26 candidate genes, coding for NBS-LRR proteins were annotated in this position with an interval of 30 cM (David et al. 2009). In consequence, the presence of two closely linked loci in the Co-3/9 region conferring to PM in the Cornell 49242 genotype could be possible.

To date, very little information has been available about the inheritance of the genetic reaction to PM in common bean. This has considerably limited the possibility of carrying out breeding programs focused on the introgression of resistance genes against this fungus in common bean genotypes. The results of this work confirmed that resistance to PM in cv. Cornell 49242 was controlled by two dominant and independent genes. One of the genes, located in LG Pv11 (*Pm1*), gave total resistance to this fungus and masked the action of the second gene (*Pm2*), located in LG Pv04. The *Pm1* gene was closely linked to the SQ4 marker, which offers the opportunity to increase the efficiency of breeding programs by means of marker-assisted selection. This is the first report that has investigated the localization of resistance genes against PM in common bean and it will be useful in the future identification of the genomic sequences involved in the genetic control of this resistance.

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