Phytopathology "First Look" paper • http://dx.doi.org/10.1094/PHYTO-01-17-0012-R • posted 07/25/2017
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1 **Identification of clusters that condition resistance to**
2 **anthracnose in the common bean differential cultivars AB136**
3 **and MDRK**

4

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1 **Abstract** The correct identification of the anthracnose resistance systems
2 present in the common bean cultivars AB136 and MDRK is important because
3 both are included in the set of 12 differential cultivars proposed for use in
4 classifying the races of the anthracnose causal agent, *Colletrotrichum*
5 *lindemuthianum*. In this work, the responses against seven *C. lindemuthianum*
6 races were analyzed in a recombinant inbred line population derived from the
7 cross AB136 x MDRK. A genetic linkage map of 100 molecular markers
8 distributed across the 11 bean chromosomes was developed in this population
9 to locate the gene or genes conferring resistance against each race, based on
10 linkage analyses and chi-square tests of independence. The identified
11 anthracnose resistance genes were organized in clusters. Two clusters were
12 found in AB136: one located on linkage group Pv07, which corresponds to the
13 anthracnose resistance cluster Co-5, and the other located at the end of linkage
14 group Pv11, which corresponds to the Co-2 cluster. The presence of resistance
15 genes at the Co-5 cluster in AB136 was validated through an allelism test
16 conducted in the F_2 population TU x AB136. The presence of resistance genes
17 at the Co-2 cluster in AB136 was validated through genetic dissection using the
18 $F_{2:3}$ population ABM3 × MDRK, in which it was directly mapped to a genomic
19 position between 46.01 and 47.77 Mb of chromosome Pv11. In MDRK, two
20 independent clusters were identified: one located on linkage group Pv01,
21 corresponding to the Co-1 cluster, and the second located on LG Pv04
22 corresponding to the Co-3 cluster. This report enhances the understanding of
23 the race-specific *P. vulgaris*-*C. lindemuthianum* interactions and will be useful in
24 breeding programs.
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1 **Introduction**

2 Common bean (*Phaseolus vulgaris* L.) is an important grain legume for
3 human consumption (http://faostat3.fao.org/home/E). This species is organized
4 into two differentiated gene pools, Mesoamerican and Andean, with
5 distinguishable morphologic and molecular characteristics (Gepts and Bliss
6 1985; Kwak and Gepts 2009; Bitocchi et al. 2013).

7 Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. &
8 Magnus) Lams.-Scrib, is a serious and widespread fungal disease that affects
9 common bean (Schwartz et al. 2005). The fungus has a high pathogenic
10 variability that is classified into physiological races based on the response
11 profile of a standardized set of 12 differential common bean cultivars. A binary
12 value is assigned to each cultivar and the race is named as the sum of the
13 values of all susceptible cultivars (Pastor-Corrales 1991). More than 100
14 different races have been reported worldwide using this system (Sicard et al.
15 1997; Sharma et al. 1999; Mahuku and Riascos 2004; Mota et al. 2016). Some
16 races are widely dispersed, such as race 73, while other races are specific to
17 certain countries or regions (Balardin et al. 1997). In northern Spain races 3, 6,
18 19, 38 and 102 were identified, among which race 38 was the most prevalent
19 (Ferreira et al. 2008).

20 The *P.vulgaris* - *C. lindemuthianum* interaction is very specific and can
21 be considered as a reference model for studying race-specific resistance in
22 plants. The first anthracnose resistance genes were described based on the
23 interpretations of allelism tests (Barrus 1911; Fouilloux 1976). At present, more
24 than 20 resistances genes (named as Co-; *Co-1* to *Co-17*, and *Co-u* to *Co-z*)
25 have been described in common bean. Most of these genes show complete

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1 dominance and have been located in the genetic map of common bean
2 organized in clusters of closely linked genes. Seven main anthracnose
3 resistance clusters have been identified in linkage groups (LGs) Pv01, Pv02,
4 Pv03, Pv04, Pv07, Pv08 and Pv11 (Ferreira et al. 2013; Trabanco et al. 2015).

5 The anthracnose differential cultivar set has been a valuable source of
6 resistance genes for breeding purposes. However, there is still a lack of
7 information and several contradictions in the literature regarding the resistance
8 genes present in some of these cultivars. The differential cultivar AB136 was
9 first described by Schwartz et al. (1982) as a potential anthracnose resistance
10 source included in the Mesoamerican gene pool. Despite the importance of this
11 cultivar, its anthracnose resistance system has not been clearly established. A
12 dominant resistance gene identified as *Co-6* (Young and Kelly 1996), and a
13 recessive gene identified as *co-8* (Alzate-Marín et al. 1997) have been reported
14 in AB136, although the existence of a recessive gene has never been
15 confirmed. The differential cultivar MDRK (Michigan Dark Red Kidney) belongs
16 to the Andean gene pool and was first described by Yerkes and Ortiz (1956). So
17 far, the only anthracnose resistance gene described in MDRK was *Co-1*
18 (Melotto and Kelly 2000; Kelly and Vallejo 2004); therefore, this cultivar has
19 been widely used in allelism tests to confirm the presence of this gene in other
20 cultivars. However, some evidence suggests that anthracnose resistance in
21 MDRK could be controlled by other loci apart from *Co-1* (Campa et al. 2009).

22 This work further analyzed the genetic control of anthracnose resistance
23 in the two important common bean differential cultivars AB136 and MDRK. The
24 resistance to seven *C. lindemuthianum* races was analyzed in a recombinant
25 inbreed line population (RIL) population derived from the cross AB136 x MDRK

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1 (ABM RIL population), for which a linkage map was developed. An allelism test
2 and a genetic dissection were additionally conducted using F_2 and $F_{2:3}$
3 populations to validate the results obtained in the ABM RIL population.
4

5 Materials and Methods

6 **Plant material.** The following three different populations were developed in this
7 work: (i) A mapping population formed by 110 F_7 RILs was obtained from the
8 cross AB136 × MDRK (ABM RIL population) using the single seed descent
9 method from individual F_2 plants (Fehr 1987). (ii) A population of 122 F_2 seeds
10 was obtained from the cross TU x AB136. TU is one of the twelve anthracnose
11 differential cultivars in which the *Co-5* gene was first described (Fouilloux 1976)
12 and mapped on LG Pv07 (Campa et al. 2009). (iii) A population of 97 $F_{2:3}$
13 families was obtained from the cross between RIL ABM3 and MDRK. In this
14 case, F_2 seeds were self-pollinated and individually harvested to generate the
15 corresponding $F_{2:3}$ families. F_3 seedlings were used to characterize the race-
16 specific anthracnose reaction of the respective F_2 plant. In addition to AB136,
17 MDRK and TU, the 9 remaining differential cultivars were used to confirm the
18 identity of the *C. lindemuthianum* races.
19 **Inoculation procedure and disease scoring.** Seven isolates classified in
20 different races were used: races 73, 449 and 1545 from the collection of the
21 Crop and Soil Sciences Department (Michigan State University, USA) and races
22 3, 6, 19 and 38 from the SERIDA (The Regional Agrifood Research and
23 Development Service) collection. These races were chosen for this study based
24 on the reaction of the parental lines: AB136 was resistant to races 3, 6, 19, 38,
25 73 and 449 and MDRK was resistant to races 73, 449, and 1545. Inoculation

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1 procedure was conducted as described by Rodríguez-Suárez et al (2007).
2 Seedlings were evaluated after 7-9 days using a 1-9 scale where 1 is no visible
3 symptoms and 9 very severely diseased or dead (Van Schoonhoven and
4 Pastor-Corrales 1987). Seedlings with no visible symptoms (value 1) or showing
5 small lesions on leaves and stems (values 2 or 3) were considered resistant
6 (R), while seedlings with large sporulation lesions (values 4 to 8) or that died
7 (value 9) were considered susceptible (S). Between 8 to 10 seedlings per RIL
8 and 16 to 20 F_3 seedlings per $F_{2:3}$ family were evaluated for each race. RILs
9 were classified as resistant or susceptible, and $F_{2:3}$ families were classified as
10 homozygous resistant or susceptible (all F_3 seedlings showed the same
11 parental phenotype) or heterozygous (the two parental phenotypes were
12 detected). The response to each race was evaluated in two independent tests in
13 the RIL and $F_{2:3}$ populations. A third evaluation in the same way as described
14 above was conducted for RILs or $F_{2:3}$ families that showed unclear
15 classifications. RILs, F_2 seedlings and $F_{2:3}$ families were individually randomized
16 over the whole climate room. In each test, the parental lines and the
17 anthracnose differential cultivars were included as controls.

18 Resistance genes were tentatively named according to Ferreira et al.
19 (2013) considering the relative position of the anthracnose resistance clusters
20 (Co- cluster), the name of the race (in superscript) followed by the bean
21 genotype in which the resistance gene was identified.

22 **Marker analyses.** Genomic DNA was extracted from young trifoliate leaves of
23 non-inoculated plants (21- to 30-day-old plants) using the FastDNA kit (MP
24 Biomedicals, Illkirch, France) following the supplier's instructions. Molecular
25 markers based on PCR analyses were used to build a genetic linkage map of

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1 the RIL and the $F_{2:3}$ populations. A set of SSR (Simple Sequence Repeat), InDel
2 (insertion-delection polymorphism; Moghaddam et al. 2013) and SCAR
3 (Sequence Characterized Amplified Region) markers were selected based on
4 their physical positions on the *P. vulgaris* genome v1 (GenBank assembly
5 GCF_000499855.1). Among them, the following markers labeling the main
6 anthracnose resistance clusters identified in common bean were included:
7 markers CV5420314 and TGA1.1 labeling the Co-1 cluster on LG Pv01
8 (Gonçalves-Vidigal et al. 2011); Pvctt001 and 254-G15F labeling the Co-3
9 cluster on LG Pv04 (David et al. 2008; Rodríguez-Suárez et al. 2008); Phs and
10 SCARZ20 labeling the Co-5 cluster on LG Pv07 (Campa et al. 2009); 78L17a
11 labeling the Co-4 cluster on LG Pv08 (Trabanco et al. 2015) and SH13b
12 labeling the Co-2 on LG Pv11 (Campa et al. 2014). Cluster Co-17, which was
13 recently identified on LG Pv02 (Trabanco et al. 2015), was tagged with marker
14 BM156. PCR amplifications were performed in a Verity Thermal Cycler (Applied
15 Biosystems, Life Technologies, CA, USA) in a final volume of 20 µL solution
16 containing 25 ng of genomic DNA, 100 mM Tris–HCl, 100 mM KCl (pH 8.3), 4
17 mM MgCl2, 0.2 mM each dNTP (Bioline, London, UK), 0.2 µM each primer, and
18 1.25 U of Biotaq DNA polymerase (Bioline). Amplification products were
19 resolved on 8% polyacrylamide gels with 1x TBE buffer (89 mM TRIS, 89 mM
20 boric acid, 2 mM EDTA), stained with SYBR safe (Invitrogen, Life Technologies,
21 CA, USA) and visualized under UV light.

22 The morphological trait of growth habit, controlled by the *Fin,fin* gene,
23 was also included in the ABM genetic linkage map, recorded as indeterminate
24 (*FinFin*) versus determinate (*finfin*) growth habit.

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1 **Genetic analyses.** Goodness-of-fit of observed to expected ratios was tested
2 by using chi-square. MAPMAKER Macintosh version 2.0 software (Lander et al.
3 1987) was used for the map construction with a log of the likelihood ratio (LOD)
4 threshold of 3.0 and a recombination fraction of 0.25. The order of the markers
5 was estimated based on multipoint compare, order and ripple analyses.
6 Distances between ordered loci (in centimorgans) were calculated using the
7 Kosambi mapping function. LGs were named according to Pedrosa-Harand et
8 al. (2008). When the observed segregation suggested the presence of one
9 resistance gene, it was directly included in the genetic map. When the
10 segregation suggested the presence of more than one gene, chi-square tests of
11 independence were used to test the association between the two categorical
12 variables "segregation of a resistance" and "segregation of a molecular marker".
13 A significant association suggested that the chromosomal region tagged with
14 the molecular marker was involved in the genetic control of the resistance
15 response. Significance thresholds were determined using Bonferroni correction
16 from the α-level of 0.05 (Bonferroni 1936).
17

18 ## Results

19 **Genetic linkage map.** A total of 100 of the 175 molecular markers tested were
20 polymorphic between AB136 and MDRK and were analyzed in the RIL
21 population: 70 SSR markers, 23 SCAR, 6 InDel and the morphological trait
22 growth habit (Supplementary Table S1). The resulting map included a total of
23 11 LGs with an average of 9 markers per LG (a minimum of 5 markers on LG
24 Pv09, and a maximum of 12 markers on LG Pv04). The ratio of missing values
25 was less than 10% for all loci. A total of 29 markers showed a deviated

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segregation from that expected for one locus. Distorted markers were concentrated in five chromosomal regions on LGs Pv04, Pv05, Pv06, Pv10 and Pv11. In most cases distortions were due to an excess of the AB136 allele, except on LG Pv06 in which it was due to an excess of the MDRK allele. The resulting map covers 1,000.6 cM, with an average marker distance of 12.7 cM (minimum 0.7, maximum 44.4). The relative marker order obtained on each LG is indicated in Fig. 1 (Supplementary Table S1), and it is in agreement with the physical positions of the markers on the bean genome.

Resistance segregation in the ABM population. Seven *C. lindemuthianum* races were analyzed in the ABM RIL population (Table 1). Resistance segregation to races 3, 38, 449, and 1545 conformed to the 3 R: 1 S ratio expected for two independent genes in a RIL population. Resistance segregation to races 6, 19, and 73 fit the 7 R: 1 S ratio expected for three independent genes.

Co-segregation was observed between races 3 and 38 in 95 RILs (80 RILs were resistant against the two races, R^3-R^{38}, and 15 RILs were susceptible, S^3-S^{38}) and evidence of recombination was found in 9 lines (4 RILs showing the haplotype, R^3-S^{38}, and 5 showing the haplotype S^3-R^{38}). Thus, the genes conferring resistance against races 3 and 38 may be linked. Co-segregation was also observed between races 6 and 19 in 92 RILs (82 RILs were resistant against the two races, R^6-R^{19}, and 10 were susceptible, S^6-S^{19}), and evidence of recombination was found in 8 cases (3 RILs showing the haplotype, R^6-S^{19}, and 5 showing the haplotype S^6-R^{19}). This result suggested a linkage between the genes conferring resistance against races 6 and 19.

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Chi-square tests of independence. Fig. 1 provides histograms representing the probabilities obtained for each chi-square test of independence performed between each race and each molecular marker. There were significant associations with markers located on LGs Pv01, Pv04, Pv07, Pv10 and Pv11.

Races 3 and 38. Considering the response to race 3, chi-square values showed significant associations with the markers located on LG Pv07 that labeled the Co-5 cluster, and with the markers located on LG Pv11 that labeled the Co-2 cluster (Fig. 1). Therefore, the two independent genes from AB136 that appeared to be involved in the control of resistance against race 3 (Table 1) could be located, one at the Co-5 cluster position and the other at the Co-2 cluster position (these genes are tentatively named $Co\text{-}5^{3\text{-}AB136}$ and $Co\text{-}2^{3\text{-}AB136}$; Fig. 2A). According to Fig. 1 and based on the co-segregation observed between races 3 and 38 the same situation was determined for the response to race 38 (genes $Co\text{-}5^{38\text{-}AB136}$ and $Co\text{-}2^{38\text{-}AB136}$; Fig. 2A).

Races 6 and 19. For race 6, the chi-square values showed significant associations with the markers labeling the Co-5 cluster on LG Pv07 (Fig. 1), which suggested the presence of one resistance gene against race 6 at this cluster ($Co\text{-}5^{6\text{-}AB136}$; Fig. 2A). A significant association was also observed with the markers of LG Pv10, although this result should be carefully considered because most of the markers forming the LG PV10 did not fit the expected segregation ratio for one locus. The third resistance gene suspected to be involved based on the segregation ratio (Table 1) was not clearly identified by the chi-square analysis, although low probability values ($0.05 < p > 0.001$) were observed when compared with markers that labeled the Co-2 cluster on LG

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1 Pv11. For race 19, chi-square tests showed significant associations when
2 compared with the markers that labeled the Co-5 cluster on LG Pv07 and the
3 Co-2 cluster on LG Pv11 (Fig. 1), suggesting the presence of two independent
4 resistance genes (*Co-5*$^{19\text{-}AB136}$ and *Co-2*$^{19\text{-}AB136}$; Fig. 2A). The third resistance
5 gene suspected to be involved based on the segregation ratio (Table 1) was not
6 clearly identified by the chi-square analysis, although low probability values
7 ($0.05 < p > 0.001$) were also observed when compared with those of the
8 markers on LG Pv10.
9 *Race 1545.* In this case, chi-square tests showed significant associations with
10 the markers labeling the Co-1 and Co-3 clusters, respectively (Fig. 1). In
11 agreement with the results shown in Table 1, the presence of two independent
12 genes against race 1545 in MDRK were deduced: one located at the Co-1
13 cluster on LG Pv01 and the second at the Co-3 cluster on LG Pv04 (genes *Co-*
14 *1*$^{1545\text{-}MDRK}$ and *Co-3*$^{1545\text{-}MDRK}$; Fig. 2A).
15 *Race 73.* For race 73, significant associations were observed with the markers
16 that labeled the Co-5 cluster on LG Pv07 and the Co-1 cluster on LG Pv01 (Fig.
17 1). The third resistance gene suspected to be involved based on the
18 segregation ratio (Table 1) was not clearly identified by the chi-square analysis,
19 although low probability values ($0.05 < p > 0.001$) were observed when
20 compared with markers labeling the Co-3 cluster. Thus, the presence of one
21 resistance gene against race 73 at the Co-5 cluster on LG Pv07, probably from
22 AB136, and a second gene at the Co-1 cluster on LG Pv01, probably from
23 MDRK, was concluded (genes *Co-5*$^{73\text{-}AB136}$ and *Co-1*$^{73\text{-}MDRK}$; Fig. 2A).
24 *Race 449.* Results of the chi-square tests of independence showed significant
25 associations with the markers that labeled the Co-5 and Co-3 clusters,

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1 respectively (Fig. 1). It was concluded that one resistance gene was located at
2 the Co-5 resistance cluster on LG Pv07, probably from AB136, and the second
3 gene was located at the Co-3 cluster on LG Pv04, probably from MDRK (genes
4 *Co-5*$^{449\text{-}}$AB136 and Co-*3*$^{449\text{-}MDRK}$; Fig. 2A).

5 **Allelism test**. To verify the presence of a resistance gene in AB136 located at
6 the Co-5 cluster, an allelism test was conducted using the F_2 population TU x
7 AB136. This allelism test was conducted using race 38 because in the
8 differential cultivar TU a gene conferring resistance to race 38 was mapped on
9 LG Pv07 at the Co-5 cluster (Campa et al. 2009). A total of 122 F_2 plants
10 derived from the cross TU x AB136 (R x R cross) were inoculated with race 38
11 and all of them showed a resistance response.

12 **Genetic dissection**. To validate the presence of resistance genes at the Co-2
13 cluster in AB136, a genetic dissection was performed using the $F_{2:3}$ population
14 ABM3 x MDRK. The ABM3 RIL from the ABM population was selected
15 according to its specific genetic combination, which was based on the
16 resistance profile and genotype for specific markers. Line ABM3 was
17 susceptible to race 449 and showed the MDRK genotype for markers that
18 labelled the Co-5 cluster (markers Phs, Pvatcc003, IND7_80043, IND7_85201,
19 SZ04 and SCARAZ20). Thus, this line was expected to lack resistance genes
20 from AB136 at Co-5. In addition, this line was resistant to races 3, 6, 38 and 19
21 indicating that other resistance genes from AB136 were present in ABM3. Table
22 2 shows the segregation ratios for resistance to five *C. lindemuthianum* races in
23 the $F_{2:3}$ population ABM3 x MDRK. Line ABM3 was resistant to four of the five
24 races tested, while line MDRK was resistant to only one race. Resistance
25 segregation to races 3, 38, and 19 fit the 1R: 2R/S: 1S ratio expected for one

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1 locus. For race 6, a slight deviation from the 1R: 2R/S: 1S ratio was observed,
2 due to a lack of homozygous resistant $F_{2:3}$ families. However, this is the best fit
3 for race 6 and the linkage map obtained support the possibility that resistance to
4 race 6 in this population may be determined by one gene. Resistance
5 segregation to race 73 (ABM3 susceptible x MDRK resistant) fit a 7R: 8R/S: 1S
6 ratio indicating that two independent genes conferred resistance against race
7 73 in MDRK.

8 To determine more accurate localizations of these resistance genes, 16
9 molecular markers were analyzed in the ABM3 x MDRK population
10 (Supplementary Table S2). The genes conferring resistance against races 3, 6,
11 19 and 38 were directly mapped on LG Pv11 (Fig. 2B), forming a cluster of
12 closely linked genes (genes *Co-2*$^{3\text{-}AB136}$, *Co-2*$^{6\text{-}AB136}$, *Co-2*$^{19\text{-}AB136}$ and *Co-2*$^{38\text{-}AB136}$
13). The physical position of this cluster, between 46.01 Mb (marker
14 IND11_460165) and 47.77 Mb (marker IND11_477711) of chromosome Pv11
15 corresponded to that of cluster Co-2. For race 73, its segregation revealed the
16 presence of more than one gene (Table 2). Thus, the localization of these
17 resistance genes was investigated using chi-square tests of independence.
18 Results revealed a significant association of the resistance with both markers
19 CV542014 and SF10 that labeled the Co-1 and Co-3 clusters, respectively
20 (CV542014-race 73: χ^2 = 16.38; p = 0.00; SF10-race 73: χ^2 = 9.95; p = 0.01).
21 According to these results, two independent genes conferred resistance against
22 race 73 in MDRK and they were probably located at clusters Co-1 and Co-3.

23

24 ## Discussion

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1 Results obtained in this work indicated that anthracnose race-specific
2 resistance genes in AB136 are located in, at least, two independent
3 chromosomal regions on LGs Pv07 and Pv11. On LG Pv07, the genes
4 conferring specific resistance against races 3, 6, 19, 38, 73 and 449 were
5 located between markers Phs and SCARAZ20. The presence of anthracnose
6 resistance genes in AB136 on LG Pv07 is well documented in the literature. In
7 analyzing different F_2 populations monogenic segregations against races 23
8 (delta), 31 (kappa), 64, 69, 73, 81 and 89 were detected in AB136 (Gonçalves-
9 Vidigal et al. 1997, 2001; Alzate-Marín et al. 1997, 1999, 2000; Poletine et al.
10 2000). RAPD marker $OPZ04_{560}$ was linked to resistance genes against races
11 64, 73, 81, and 89 (Alzate-Marín et al. 1999). In other mapping populations this
12 RAPD marker, and the corresponding SZ04 SCAR marker (Queiroz et al.
13 2004), were located on LG Pv07 (Freyre et al. 1998; Rodríguez-Suárez et al.
14 2007). The gene conferring specific resistance against race 89 in AB136 was
15 also linked to RAPD marker $OPAZ20_{940}$ in the $F_{2:3}$ population Rudá x AB136
16 (Alzate-Marín et al. 2000), and the corresponding SCAR marker SCARZ20
17 (Queiroz et al. 2004) was mapped on LG Pv07 (Campa et al. 2009).
18 Subsequently, specific resistance genes against races 81 and 449 were
19 mapped on LG Pv07 linked to markers SCARAZ20 and SZ04 using the $F_{2:3}$
20 population AB136 × Michelite (Campa et al. 2007). This locus present in AB136
21 on LG Pv07 was identified as the *Co-6* gene (Young and Kelly 1996) although
22 the genetic analyses were conducted using the line Catrachita derived from
23 AB136 (BAT1225 × AB136). Results of an allelism test indicated that the gene
24 present in Catrachita, which was thought to be *Co-6*, was independent from the
25 *Co-5* gene described in TU (Fouilloux 1976). *Co-5* in TU was identified as a

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1 cluster of genes protecting against races 3, 6, 7, 38, 39, 102 and 449, located
2 on LG Pv07 between markers Phs and SCARAZ20 (Campa et al. 2009). The
3 cluster of AB136 identified in this work on LG Pv07 has the same relative
4 position to that of the Co-5 cluster described in TU. On the other hand, no
5 segregation was found in the allelism test conducted with race 38 in the F_2
6 population TU x AB136. According to these evidences the cluster identified in
7 this work on LG Pv07 in AB136 corresponds to Co-5.

8 On LG Pv11, four resistant genes against races 3, 6, 19, and 38 derived
9 from AB136 were mapped through genetic dissection. These genes were
10 closely linked forming a cluster between physical positions 46.01 and 47.77 Mb.
11 This position on LG Pv11 corresponds to *Co-2*, a gene widely deployed in
12 common bean breeding programs (Kelly and Vallejo 2004; Ferreira et al. 2012,
13 2017). This chromosomal region has been identified as an important and
14 complex cluster of resistance genes, which includes genes that protect against
15 diseases other than anthracnose (Schmutz et al. 2014; Meziadi et al. 2016;
16 Hurtado-Gonzales et al. 2017). This is the first report that identifies Co-2 as a
17 cluster involved in the genetic control of anthracnose resistance in the AB136
18 differential cultivar.

19 In addition to Co-5 and Co-2, segregation data obtained for races 6 and
20 19, suggested the presence of a third independent anthracnose resistance
21 locus in AB136. Results of chi-square tests of independence revealed a
22 significant association between race 6 and markers GATS11B and BM157,
23 which were closely linked on LG Pv10. The GATS11B marker labeled a
24 chromosomal region identified as a resistant gene analog region (RGA) related
25 to a minor gene contributing to partial resistance to anthracnose isolate 5DOM

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1 (López et al. 2003). Recently, data obtained from GWAS analyses in a diversity
2 panel of 230 Andean beans, identified a region in chromosome Pv10 that was
3 involved in moderate levels of resistance to anthracnose race 7 (Zuiderveen et
4 al. 2016). On the other hand, the recent sequencing and assembled of the
5 common bean genome (Schmutz et al. 2014) revealed the existence of a
6 cluster of resistant-related genes at the end of chromosome Pv10. Thus, the
7 localization of anthracnose resistance genes on chromosome Pv10 would not
8 be unexpected. However, the data obtained in this work were not conclusive
9 and should be carefully interpreted because most of the molecular markers
10 analyzed for LG Pv10 showed deviations from the segregation ratio expected
11 for one locus, with an excess of the AB136 allele. The preferential transmission
12 of Mesoamerican alleles was described in common bean in crosses between
13 the two gene pools (Paredes and Gepts 1995). Higher frequencies of distorted
14 markers are expected in RIL populations derived through single seed descent,
15 in which distorted segregation represents the cumulative effect of both genetic
16 and environmental factors on multiple generations (Paredes and Gepts 1995).
17 For the differential cultivar MDRK, two chromosomal regions containing
18 race-specific resistance genes were located on LGs Pv01 and Pv04. On LG
19 Pv01, genes conferring specific resistance against races 73 and 1545 were
20 located in a position corresponding to cluster Co-1. On LG Pv04, genes
21 conferring specific resistance against races 73, 449 and 1545 were located in a
22 position corresponding to cluster Co-3. Results obtained in this work for races
23 1545 and 449 are in agreement with those obtained for these races in the $F_{2:3}$
24 population TU x MDRK (Campa et al. 2009). Resistance to race 73 was
25 analyzed in the $F_{2:3}$ population ABM3 × MDRK (S x R cross) and the

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1 segregation observed fit the expected ratio for two independent genes from
2 MDRK located at the Co-1 and Co-3 clusters. As far as we know, this is the first
3 S x R cross conducted to study the resistance against race 73 in MDRK,
4 although it is a more appropriate type of cross for inferring the number and
5 mode of action of genes that confer resistance. To date, resistance to race 73 in
6 MDRK has been assumed to be conditioned only by the Co-1 cluster, and it has
7 been widely used in allelism tests in R × R crosses. For instance, using the F_2
8 population MDRK x Perry Marrow (R × R cross) a lack of segregation (all
9 seedlings resistant) was observed for race 73, which was interpreted as the *Co-*
10 *1* gene conditioning resistance to race 73 in Perry Marrow (Melotto and Kelly
11 2000). However, based on the results of the present work the resistance to race
12 73 in Perry Marrow could be conditioned by Co-1, Co-3 or by both. In the F_2
13 population MDRK × SEL 1308 (R × R cross) the segregation ratio obtained for
14 race 73 was interpreted as a 15 R: 1 S segregation ratio (73 R: 2 S; χ^2 = 1.81, *p*
15 = 0.18) corresponding to the presence of two independent genes, one from
16 MDRK and one from SEL1308 (Young et al. 1998). Nevertheless, this ratio also
17 fit a 63 R: 1 S segregation ratio corresponding to three independent genes (χ^2=
18 0.59; *p*= 0.44) and could be reinterpreted based on the results obtained here.
19 The genetic base for resistance against race 73 was recently analyzed by
20 GWAS and significant associations with SNP markers ss715645251 on
21 chromosome Pv01 and ss715649432 on chromosome Pv04 were identify
22 (Zuiderveen et al. 2016), which is in accordance with the results obtained in this
23 work. Resistance to race 73 in the differential cultivar MDRK could not be the
24 most appropriate for an allelism test because it can be conditioned by two
25 independent genes. However, when an allelism test is conducted the genetic

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1 background should be considered, because anthracnose resistance genes
2 showing a complementary mode of action have been described in common
3 bean (Cardenas et al. 1964; Muhalet et al. 1981; Campa et al. 2011; Campa et
4 al. 2014), more common in Andean genotypes such as MDRK, so different
5 number of resistance genes could be identified in a genotype against the same
6 race, depending on the type of cross (Ferreira et al. 2013).

7 Although RIL populations are more informative for studying complex
8 segregation patterns than other types of populations, the size of the RIL
9 population used in this work was not very large and the number of molecular
10 markers used in the genetic map was limited. For these reason, part of the
11 results interpreted from the RIL population were validated using complementary
12 crosses and genetic dissection. These validations support the results obtained
13 and endorse the use of chi-square tests of independence for the localization of
14 major genes involved in complex segregations. Results obtained in this work
15 can be relevant to the interpretation of future allelism tests and in the
16 implementation of breeding programs focused on protecting bean crops against
17 anthracnose.

18

19 **Acknowledgements** This work was partially financed by grant RTA2011-0076-
20 CO2-01 from Instituto de Investigación y Tecnología Agraria y Alimentaria
21 (INIA)- Ministerio de Economía y Competitividad, Spanish Government and co-
22 financed with the European Regional Development's funds (FEDER). A Campa
23 is recipient of a salary from the INIA-CCAA (INIA-DR13-0222), Spain. We thank
24 J. D. Kelly of Michigan State University for providing some of the isolates of *C.*

Phytopathology "First Look" paper • http://dx.doi.org/10.1094/PHYTO-01-17-0012-R • posted 07/25/2017
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2 technical assistance.

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1 **Table 1.** Segregations observed for resistance to seven *C. lindemuthianum* races in
2 the ABM RIL population. Segregation ratio tested in each case, and chi-square
3 goodness-of-fit test are indicated. R, resistant; S, susceptible
4

	Parental phenotype		ABM RIL population						
			Observed		Expected				
Race	AB136	MDRK	R	S	R	S	Ratio[a]	χ^2	*p*
3	R	S	86	20	79.5	26.5	3:1	2.13	0.14
6	R	S	92	18	96.3	13.7	7:1	1.50	0.22
19	R	S	89	15	91.0	13.0	7:1	0.35	0.55
38	R	S	87	21	81.0	27.0	3:1	0.78	0.18
73	R	R	97	9	92.7	13.3	7:1	1.56	0.21
449	R	R	74	33	80.3	26.7	3:1	1.95	0.16
1545	S	R	82	23	78.7	26.3	3:1	0.54	0.46

5 [a] Ratio 3 resistant: 1 susceptible, expected for two independent genes in a RIL population
6 Ratio 7 resistant: 1 susceptible, expected for three independent genes in a RIL population
7
8

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1 **Table 2.** Segregations observed for resistance to six *C. lindemuthianum* races in the
2 $F_{2:3}$ population ABM3 × MDRK. The segregation ratio considered in each case and the
3 chi-square goodness-of-fit test is indicated. R, resistant; S, susceptible
4

	Parental phenotypes		$F_{2:3}$ families ABM3xMDRK [a]							
			Observed			Expected				
Race	ABM3	MDRK	R	R/S	S	R	R/S	S	$\chi^{2\ b}$	*p*
3	R	S	15	35	21	17.75	35.50	17.75	1.03	0.60
6	R	S	11	47	26	21.00	42.00	21.00	6.55	0.04
19	R	S	18	28	18	16.00	32.00	16.00	1.00	0.61
38	R	S	20	35	19	18.50	37.00	18.50	0.24	0.89
73	S	R	20	27	6	13.25	26.50	13.25	2.63	0.27

5 [a] R, F_3 families having all individuals resistant; R/S, F_3 families having resistant and susceptible
6 individuals; S, F_3 families having all individuals susceptible
7 [b] For races 3, 6, 19 and 38 the ratio was 1 resistant: 2 heterozygous: 1 susceptible expected for
8 one locus. For race 73 the ratio was 7 resistant: 8 heterozygous: 1 susceptible expected for two
9 independent genes
10
11
12

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1 **Figure legends**

2 **Fig. 1.** Histograms showing the probability of the chi-square test obtained
3 between the response to a specific race and each loci included in the genetic
4 map. In each LG, markers are represented according to the order obtained in
5 the linkage map (left-hand corresponds to 0 Mb of the chromosome and right-
6 hand corresponds to final position). Asterisks represent significant associations
7 after Bonferroni correction ($p < 0.001$). Red line represents a significance level
8 of $p = 0.05$.

9

10 **Fig. 2. A)** LGs Pv01, Pv04, Pv07 and Pv11 in which anthracnose resistance
11 genes were indirectly located using the ABM RIL population. Resistance genes
12 are named by using its location in the genetic map (*Co*-anthracnose resistance
13 cluster), name of the isolate or race (in superscript), followed by the bean
14 genotype in which the resistance gene was identified (A, AB136; M, MDRK).
15 Map distances are expressed in centiMorgans, estimated using the Kosambi
16 mapping function. *Fin*, locus controlling indeterminate versus determinate
17 growth habit. **B)** LG Pv11 in which four anthracnose resistance genes were
18 directly mapped using the $F_{2:3}$ population ABM3 × MDRK. Map distances are
19 expressed in centiMorgans, estimated using the Kosambi mapping function.

20

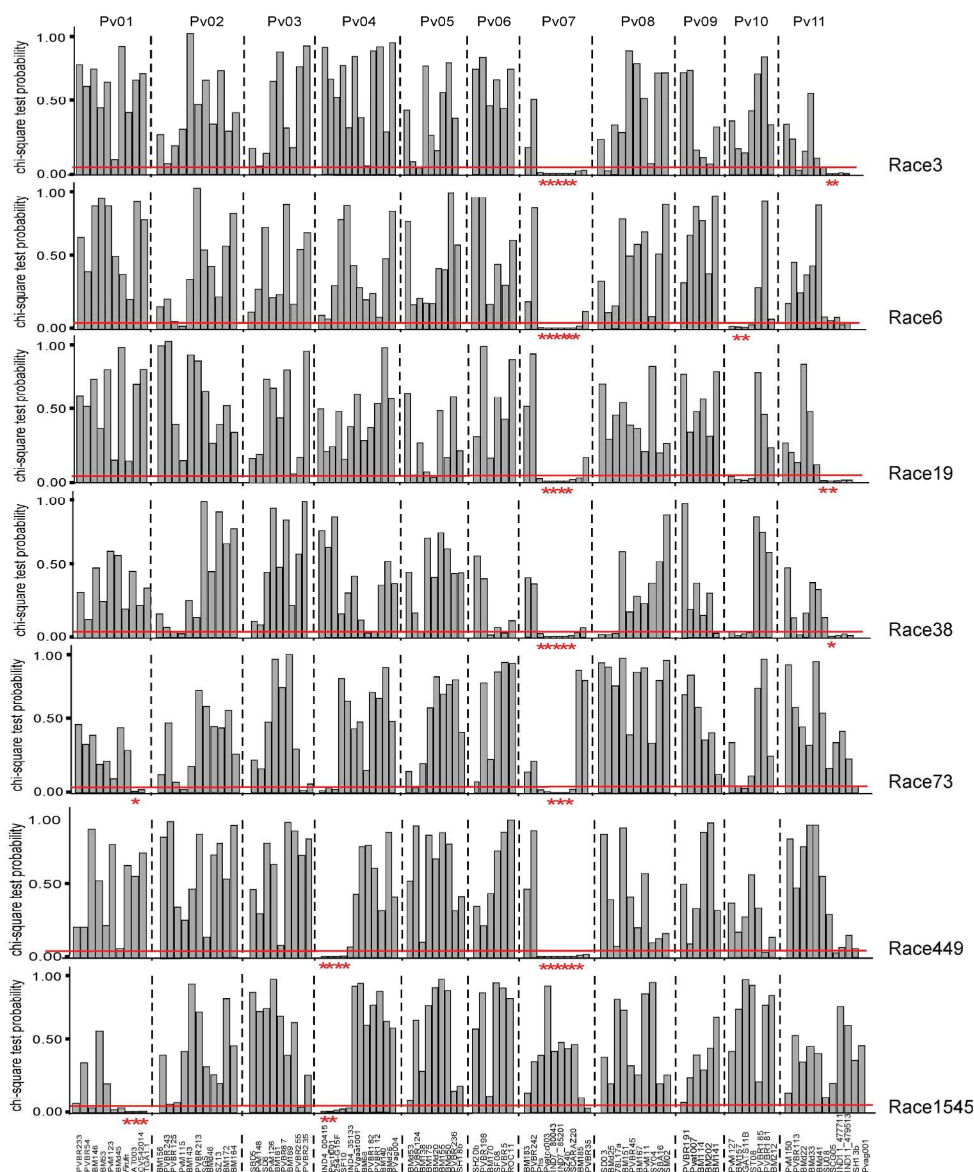

Fig. 1. Histograms showing the probability of the chi-square test obtained between the response to a specific race and each loci included in the genetic map. In each LG, markers are represented according to the order obtained in the linkage map (left-hand corresponds to 0 Mb of the chromosome and right-hand corresponds to final position). Asterisks represent significant associations after Bonferroni correction (p <0.001). Red line represents a significance level of p= 0.05.

246x299mm (300 x 300 DPI)

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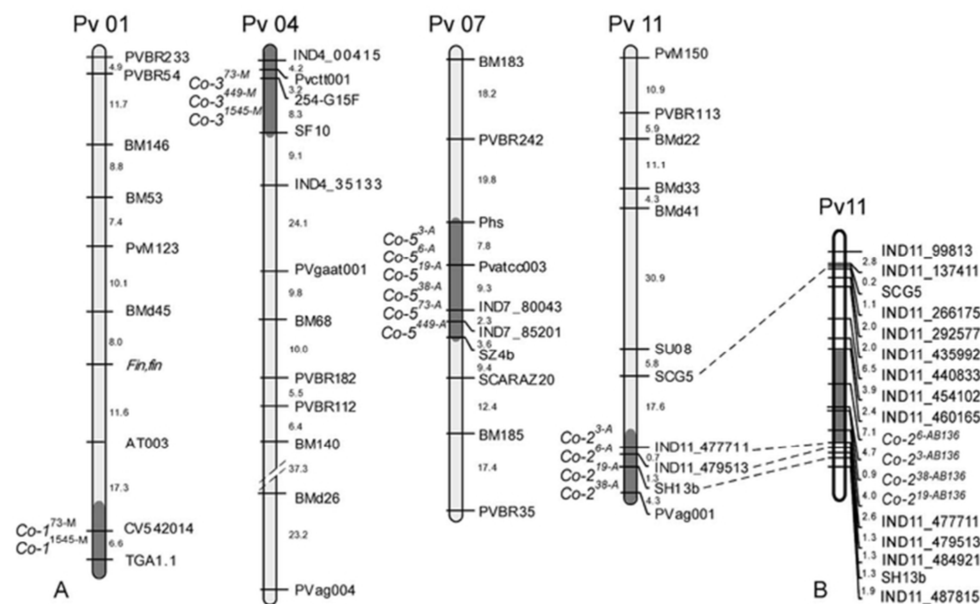

Fig. 2. A) LGs Pv01, Pv04, Pv07 and Pv11 in which anthracnose resistance genes were indirectly located using the ABM RIL population. Resistance genes are named by using its location in the genetic map (Co- anthracnose resistance cluster), name of the isolate or race (in superscript), followed by the bean genotype in which the resistance gene was identified (A, AB136; M, MDRK). Map distances are expressed in centiMorgans, estimated using the Kosambi mapping function. Fin, locus controlling indeterminate versus determinate growth habit. B) LG Pv11 in which four anthracnose resistance genes were directly mapped using the F2:3 population ABM3 × MDRK. Map distances are expressed in centiMorgans, estimated using the Kosambi mapping function.

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Table S1. Genetic linkage map obtained in the RIL population AB136 x MDRK. Accumulate distances are expressed in centimorgans. Molecular markers tagging anthracnose resistance clusters (*Co-number*) are indicated. Chi-square goodness-of-fit to one locus segregation ratio is indicated. ns, not significant; *, $0.05 < p > 0.01$; **, $p < 0.01$.

Marker	LG	Dist[a]	χ^2	*p*
PVBR233	Pv01	0.0	0.42	ns
PVBR54	Pv01	4.9	0.62	ns
BM146	Pv01	16.6	0.01	ns
BM53	Pv01	25.4	2.98	ns
PvM123	Pv01	32.8	2.71	ns
BMd45	Pv01	42.9	4.65	*
Fin.fin	Pv01	50.9	0.11	ns
AT003	Pv01	62.5	0.42	ns
CV542014(*Co-1*)	Pv01	79.8	1.80	ns
TGA11(*Co-1*)	Pv01	86.4	5.07	*
BM156(*Co-17*)	Pv02	0.0	0.55	ns
PVBR243	Pv02	7.4	0.11	ns
PVBR125	Pv02	14.2	0.11	ns
PvM115	Pv02	24.8	2.28	ns
BM143	Pv02	33.4	0.05	ns
PVBR213	Pv02	39.0	0.05	ns
SG5	Pv02	52.3	5.38	*
BMd46	Pv02	75.8	0.18	ns
SCZ13	Pv02	88.4	0.00	ns
BM172	Pv02	130.4	0.05	ns
BM164	Pv02	133.6	2.84	ns
SBD5	Pv03	0.0	0.93	ns
PvM148	Pv03	9.4	0.20	ns
SD8	Pv03	17.5	0.00	ns
PvM126	Pv03	33.3	0.05	ns
BM181	Pv03	38.8	0.58	ns
PVBR87	Pv03	61.7	0.05	ns
BM189	Pv03	70.2	1.42	ns
PVBR255	Pv03	-	2.71	ns
PVBR235	Pv03	80.0	0.10	ns
IND4.00415	Pv04	0.0	1.11	ns
Pvctt001(*Co-3*)	Pv04	4.2	3.57	ns
254-G15F(*Co-3*)	Pv04	7.4	11.91	**
SF10	Pv04	15.7	6.40	*
IND4.35133	Pv04	24.8	9.33	**
PV-gaat001	Pv04	48.9	2.85	ns
BM68	Pv04	58.7	7.38	*
PVBR182	Pv04	68.7	5.44	*
PVBR112	Pv04	74.2	2.45	ns
BM140	Pv04	80.6	1.00	ns
BMd26	Pv04	117.9	0.64	ns
PV-ag004	Pv04	141.1	0.10	ns
BMd53	Pv05	0.0	7.18	*
PVBR124	Pv05	12.5	6.22	*
BM138	Pv05	24.9	4.55	*
BM175	Pv05	36.6	6.22	*
BMd20	Pv05	43.7	1.25	ns
BM155	Pv05	51.9	1.90	ns
BMd50	Pv05	58.3	2.65	ns
PVBR236	Pv05	68.5	0.71	ns
SH18b	Pv05	76.3	0.71	ns
SH20b	Pv06	0.0	0.73	ns
PVBR198	Pv06	31.7	1.36	ns
BM170	Pv06	51.2	2.04	ns
SF08	Pv06	63.8	12.84	**
PVBR5	Pv06	67.4	7.51	*
ROC11	Pv06	76.7	7.51	*
BM183	Pv07	0.0	5.63	*
PVBR242	Pv07	18.2	1.71	ns
Phs(*Co-5*)	Pv07	38.0	0.40	ns
Pv-atcc003	Pv07	45.8	3.60	ns
IND7.80043	Pv07	55.1	1.39	ns
IND7.85201	Pv07	57.4	1.39	ns
SZ4b	Pv07	61.0	3.60	ns
SCARAZ20(*Co-5*)	Pv07	70.4	1.64	ns
BM185	Pv07	82.8	1.49	ns
PVBR35	Pv07	100.2	1.71	ns
SD03	Pv08	0.0	0.04	ns
BMd25	Pv08	5.9	0.56	ns
78L17a(*Co-4*)	Pv08	16.6	2.84	ns
BM151	Pv08	61.0	0.01	ns
PVBR45	Pv08	66.0	0.11	ns
BM167	Pv08	69.2	1.00	ns
PvM11	Pv08	74.6	0.32	ns
SY4	Pv08	83.4	0.10	ns
SBA16	Pv08	119.0	0.78	ns
SM02	Pv08	127.9	10.00	**
PVBR191	Pv09	0.0	1.00	ns
Pv-at007	Pv09	15.2	2.59	ns
BM114	Pv09	-	0.05	ns
BM202	Pv09	43.6	0.05	ns
BM141	Pv09	60.0	5.07	*
PvM127	Pv10	0.0	33.05	**
BM157	Pv10	4.1	32.19	**
GATS11B	Pv10	3.4	33.91	**
ST08	Pv10	8.7	25.60	**
PVBR185	Pv10	35.0	19.32	**
PVBR181	Pv10	51.8	8.19	**
BM212	Pv10	72.1	0.59	ns
PvM150	Pv11	0.0	9.89	**
PVBR113	Pv11	10.9	24.82	**
BMd22	Pv11	16.8	16.11	**
BMd33	Pv11	27.9	9.56	**
BMd41	Pv11	32.2	8.24	**
SU08	Pv11	63.1	1.11	ns
SCG5	Pv11	68.9	0.12	ns
IND11.477711	Pv11	86.4	0.01	ns
IND11.479513	Pv11	87.1	0.11	ns
SH13b(*Co-2*)	Pv11	88.4	0.05	ns
Pv-ag001	Pv11	92.7	0.23	ns

[a]Accumulate distances in cM

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Table S2. Molecular marker segregations obtained in the F_2 population ABM3 × MDRK. Chi-square goodness-of-fit to one locus segregation ratio is indicated.

		Parental phenotypes		Observed phenotypes among F_2 ABM3 × MDRK plants[a]						
Marker	LG	ABM3	MDRK	A/A	A/-	A/M	-/M	M/M	χ^{2b}	*p*
IND11_99813	Pv11	-	200	23	-	-	74	-	0.09	0.77
IND11_137411	Pv11	190	150	28-		48	-	21	1.02	0.60
SCG5	Pv11	850	-	-	69	-	-	28	0.77	0.38
IND11_266175	Pv11	110	100	20	-	48	-	29	1.68	0.43
IND11_292577	Pv11	-	100	21	-	-	76	-	0.58	0.45
IND11_435992	Pv11	80	50	22	-	46	-	29	1.27	0.53
IND11_440833	Pv11	210	-	-	67	-	-	30	1.82	0.18
IND11_454102	Pv11	210	195	20	-	48	-	26	0.81	0.67
IND11_460165	Pv11	135	130	21	-	47	-	29	1.41	0.49
IND11_477711	Pv11	200	150	21	-	46	-	29	1.50	0.47
IND11_479513	Pv11	200	230	22	-	44	-	29	1.55	0.46
IND11_484921	Pv11	120	110	22	-	46	-	29	1.27	0.53
SH13b	Pv11	500	480	23	-	47	-	27	0.42	0.81
IND11_487815	Pv11	110	160	23	-	44	-	30	1.84	0.40
CV542014	Pv01	240	310	37	-	45	-	15	10.48	0.01
SF10	Pv04	1072	-	-	76	-	21	-	0.58	0.45

[a] A/A, homozygous for the ABM3 alleles; A/-, homozygous for the ABM3 allele or heterozygous; A/M; A/M, heterozygous; -/M, homozygous for the MDRK allele or heterozygous; M/M, homozygous for the MDRK allele

[b] For dominant markers the expected ratio was 3R: 1S or 1R :3S . For codominant markers the expected ratio was 1 R: 2H :1S