Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i18.2887

Article

Identification of bean genotypes resistant to angular leaf spot by molecular markers

Janette Zavala-Páramo[1]
Jorge Alberto Acosta-Gallegos[1]
Ana Paulina Rodríguez-Vera[1]
Brenda Zulema Guerrero-Aguilar[1]
José Luis Anaya-López[1]
Víctor Montero-Tavera[1,§]

1 Campo Experimental Bajío-INIFAP. Carretera Celaya-San Miguel de Allende km 6.5, Celaya, Guanajuato, México. CP. 38110. (ibq.janettezavala@yahoo.com.mx; acosta.jorge@inifap.gob.mx; paulinarodriguez.verapaulinarodriguez.vr@gmail.com; guerrero.brenda@inifap.gob.mx; anaya.jose@inifap.gob.mx).

Autor para correspondencia: montero.victor@inifap.gob.mx.

Abstract

Angular leaf spot caused by *Pseudocercospora griseola* (Sacc.) Crous & Braun is one of the most common diseases of beans, reducing up to 80% of the harvest if weather conditions are favorable. Genetic improvement for resistance to this disease is one of the most effective strategies. One of the most efficient ways to control this disease is the introduction of genetic resistance mediated by molecular marker-assisted selection. Therefore, the present work aimed to identify genotypes of beans (*Phaseolus vulgaris*) with resistance to angular leaf spot in Mexican varieties and lines of black, bay, and red beans. To this end, eight specific molecular markers of the SCAR type were used and the correlation between the presence of the markers and the resistance of each genotype measured by inoculation with two strains of the fungus from different physiographic regions under greenhouse conditions was determined. The varieties Negro Comapa, Negro Cotaxtla, Negro Papaloapan, Ouro Negro and the advanced lines of black beans SEN 56, SEN 26, and SCN 7 were resistant to angular leaf spot; in contrast, Bayo Azteca, Bayo INIFAP, the red bean lines SCR 13 and SER 83, as well as the black bean lines SEN 44 and SEN 70 were susceptible. In general, the genotypes with the highest resistance presented the markers CV542014, SAA19, SM02, and SN02. Although the genetic materials were heterogeneous, these molecular markers, considered to be associated with genes with additive effects, could support the selection of *P. griseola* resistant genotypes and the actions of breeding programs to obtain new resistant varieties.

Keywords:

Phaseolus vulgaris, Pseudocercospora griseola, black bean, resistance, SCAR.

DOI: https://doi.org/10.29312/remexca.v15i18.2887

Introduction

In Mexico, beans are one of the most important foods due to their high contribution of protein, calcium, and iron. Their cultivation ranks second in terms of planted area and therefore represents the second most important agricultural activity due to the number of producers engaged in cultivation. The main producing states are Zacatecas, Sinaloa, Chihuahua, Durango, Nayarit, Chiapas, Guanajuato, San Luis Potosí, Puebla and Hidalgo (SIAP, 2019).

Angular leaf spot is an endemic bean disease caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun. It is easily and widely spread and is mainly found in the tropics and subtropics (León-Saavedra, 2009). Infected leaves have spots that originate on the underside and are delimited by the veins, evolve into grayish lesions, later turn brown and lack coloration at the edges (de Jesús *et al*., 2001). The losses caused range between 50% and 80% of normal production (de Jesús *et al*., 2001)

To date, five resistance genes have been identified, *Phg-1* to *Phg-5*, located on chromosomes Pv01, Pv08, Pv04 and Pv10; *Phg-1* to *Phg-3* were identified in the Mesoamerican collection and *Phg-4* and *Phg-5* in the Andean collection. There are also some gene candidates that have been shown to confer resistance to angular leaf spots in some genotypes but still require validation studies in others, such as *Phg-ON*, QTL ALS5.1UC, QTL ALS5.2UC and others (Nay *et al*., 2019b).

Sequence-characterized amplified region (SCAR) molecular markers are based on the identification of highly repeated RAPD fragments, which are associated with the resistance response; they are sequenced and specific primers are designed from their sequence. These types of markers have been widely used for their specificity in disease characterization, identification of resistant genotypes, and molecular marker-assisted selection (MAS) for the development of new resistant progenitors (Beebe *et al*., 2000, 2001; Rodrigues *et al*., 2016; Miller *et al*., 2018; Gil *et al*., 2019; Okii *et al*., 2019; Nay *et al*., 2019a).

The objective of this research was to identify SCAR molecular markers associated with resistance to angular leaf spot in bean varieties generated by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), for its acronym in Spanish. Additionally, a preliminary estimate of their predictive capacity was made by studying them in non-inoculated materials.

Materials and methods

Germplasm and molecular markers

Contrasting bean genotypes were used for their response to angular leaf spot, including resistant black beans (N. Papaloapan and Ouro Negro), black beans of unknown reaction (N. Comapa and N. Cotaxtla), susceptible bay varieties (B. Azteca and B. INIFAP), five black bean lines (SCN 7, SEN 26, SEN 44, SEN 56, SEN 70), two red lines (SCR 13 and SER 83) with unknown reaction to the angular leaf spot, and three segregating lines derived from the SEN 26/N. Papaloapan cross (110, 111, and 122). All genetic materials were provided by the Bean Improvement Program of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) based in the Bajío Experimental Field (CEBAJ), for its acronym in Spanish.

The SCAR markers CV542014 and TGA 1.1 (Gonçalves-Vidigal *et al*., 2011), SH13, SAA19, SBA16 and SM02 (de Queiroz *et al*., 2004), SN02 (Nietsche *et al*., 2000), E-ACA/MCCT (Mahuku *et al*., 2004), specific to the resistance genes *Phg1*, *Phg2* and *ON* were used. All the primers were synthesized by the SIGMA® company. Two strains of *P. griseola* from isolates from Cotaxtla, Veracruz (strain 2) and Valle de Santiago, Guanajuato (strain 4) were inoculated, either individually or forming an equivalent mixture (strain 2+4), to inoculate various bean genotypes.

DOI: https://doi.org/10.29312/remexca.v15i18.2887

Seeding and collection of leaf tissue samples

Twenty seeds of the black and bay bean varieties were germinated in a wet chamber at 23 °C in order to purify seedling DNA and amplify SCAR markers. The same genetic materials were sown in the greenhouse, in which five plants of each genotype were inoculated with each of the *P. griseola* strains or with a mixture of both. In the field, the five lines of black beans and two of red beans were shown, of which 80 plants of each were selected to verify the presence of resistance gene markers.

DNA extraction

Genomic DNA was isolated using Doyle (1990) method from a leaflet of each plant in the V5 developmental stage. The samples were ground in the FastPrep® homogenizer with 600 µl of CTAB extraction buffer, then the procedure recommended by the author of the technique was followed. The DNA concentration was standardized to 10 ng μl^{-1}. DNA quality was verified by 1% agarose gel electrophoresis and its amplifiability was determined by amplification of the *26S* ribosomal gene according to Montero-Tavera *et al*. (2017).

Amplification of molecular markers

DNA amplification of all genotype materials with the eight molecular markers was performed by polymerase chain reaction (PCR) using the following conditions.

For the CV542014 and TGA1.1 markers, the following was programed: an initial denaturation at 95 °C for 3 min, followed by 35 compound cycles of denaturation at 92 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min; finally, an extension cycle at 72 °C for 5 min was added. For the SH13, SAA19, SBA16, SM02, and E-ACA/MCCT330 markers, it was modified using denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1.5 min. For the SN02 marker, it was modified using annealing at 65 °C for 1 min. PCR reactions included 10X buffer (2 µl), 50X $MgCl_2$ (1 µl), 2.5Mm dNTPs (2 µl), 5 µm primer (4 µl), 1 U Taq polymerase (0.2 µl), 10 ng μl^{-1} DNA (3 µl) and deionized water (7.8 µl).

The amplified fragments were separated by 1% agarose gel electrophoresis with 1X TBE buffer at 120 V and photographically documented with the Gel Logic 112 equipment (Carestream, WoodBridge, CT, USA). The identification of each fragment was made based on its estimated size in base pairs.

Inoculation of selected varieties of black and bay beans

The two strains were grown at 27 °C in V8 medium according to what was published by Schwartz *et al*. (1982). Subsequently, each strain was suspended in sterile distilled water at a concentration of 2×10^4 conidia ml^{-1}; in addition, an equivalent mixture was prepared with the two strains, thus leaving three, so there were three sources of inoculum.

The resistant varieties N. Papaloapan and Ouro Negro, the as-yet-undetermined reaction varieties N. Comapa and N. Cotaxtla and the susceptible varieties B. Azteca and B. INIFAP were inoculated. Inoculation was carried out by spraying on the upper side and underside of the trefoils of 10 plants of each line or genotype variety five weeks after sowing. Subsequently, four readings of the progress of the disease were made using the scale the Librelon *et al*. (2015) spaced every two weeks, with which the area under the disease progression curve (AUDPC) was calculated as a measure of the severity of damage (Fihlo *et al*., 1997).

Results and discussion

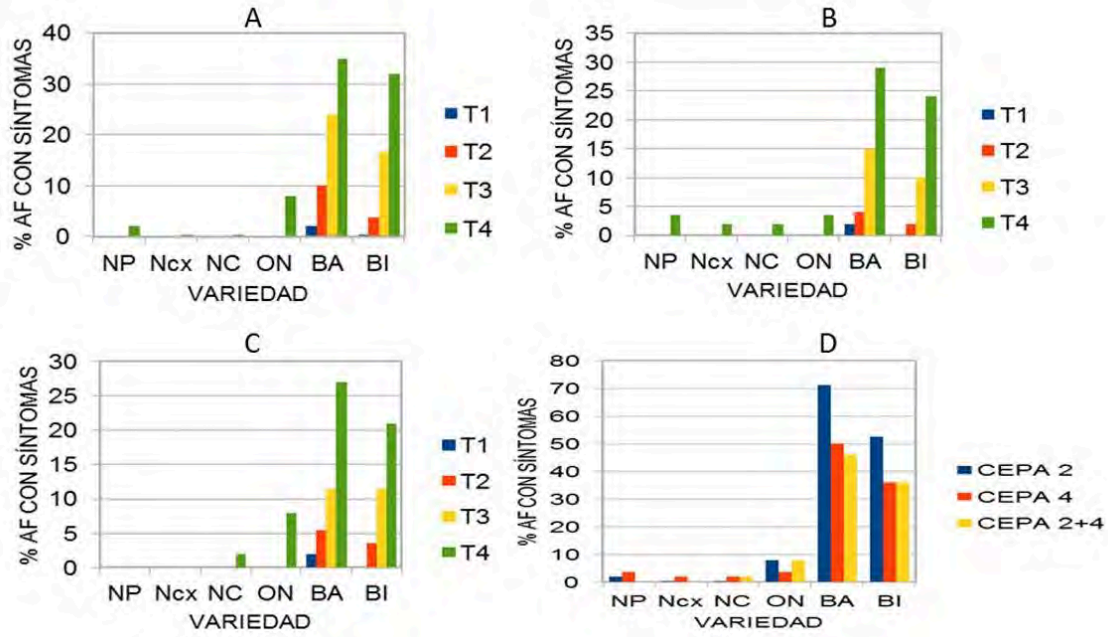
Artificial inoculation in the greenhouse

Artificial inoculation with the two strains 2, 4 and 2+4 confirmed that the varieties N. Comapa, N. Cotaxtla and N. Papaloapan are resistant since the severity of the angular leaf spot measured

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v15i18.2887

as AUDPC had a value of less than 5%, while Ouro Negro with strains 2 and 2+4 presented 8% severity. In contrast, the varieties B. Azteca and B. INIFAP had AUDPC values of 35% and 30%, respectively, with strains 2 and 4 (Figure 1).

Figure 1
Area under the disease progression curve. a) strain 2; b) strain 4; c) strains 2+4 and d) severity as a percentage of leaf area with symptoms.



In general, Ouro Negro, N. Papaloapan, N. Comapa and N. Cotaxtla presented mild symptoms of angular leaf spot, and in some cases, they were visible until the stage prior to natural defoliation by senescence (end of stage R9). N. Comapa and N. Cotaxtla had a higher level of resistance than the Ouro Negro variety, which is used as an international reference for resistance to angular leaf spot.

The susceptible varieties, B. Azteca and B. INIFAP, presented symptoms from the first reading, which worsened over the days, increasing the AUDPC (Figure 1). In general, strains 2 and 4 induced greater severity of damage than the mixture of strains; on the other hand, strain 4 induced greater damage to black bean varieties than strain 2.

Panel D of Figure 1 shows the final percentage of leaf area with damage caused by *P. griseola*. It was observed that it did not reach 10% in the black variety, but it reached up to 70% in the bay varieties. Bayo Azteca was the most susceptible variety and strain 2 the most severe. The mixture of strains was not synergistic since its AUDPC values were similar to strain 4.

Identification of molecular markers in bean genotypes

The markers amplified the expected fragments in all genotypes (Table 1). The 450 bp resistance allele of the CV542014 marker was identified in recombinant inbred lines (RILs) of the AND 277/Ouro Negro cross and verified in RILs of AND 277/Rudá. In the Mesoamerican genotypes, Ouro Negro and Rudá from the Mesoamerican collection are susceptible to the strains of *P. griseola* 31.17, 31.39, 61.31, 63.19, 63.23, 63.31, 63.35, and 61.41 (Gonçalves-Vidigal *et al*., 2011); nevertheless, O. Negro presented the resistance allele and not the susceptibility allele of 350 bp, as reported by the authors. The resistance allele was identified in the four varieties of black beans, the two of the bay type, and two advanced lines of black beans; however, in N. Comapa, its frequency was less than 50%.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i18.2887

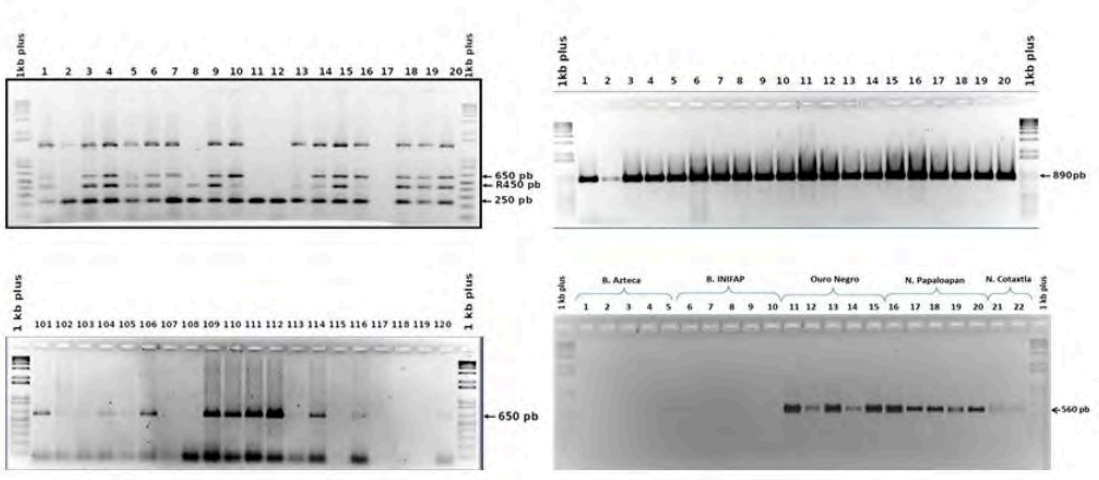
Table 1. Presence of resistance markers and susceptibility to angular leaf spot in bean varieties and lines.

Marker	Origin	Gene	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CV542014	AND277	*Phg-1*	450R 350S 250	100 - 100	96 - 100	27 - 100	54 - 100	70 - 97	89 - 100	58 - 100	79 - 100	- - 100	- 100 -	- 100 -	- 100 -	- 100 -	nd	nd	nd
TGA1.1	AND277	*Phg-1*	570R	-	-	-	-	-	-	-	-	-	-	-	-	-	81.2	83.7	22.5
SH13	O. Negro	*Phg-1*	650 520R	95 -	7 75	10 67	50 6.6	20 10	34 24	65 -	75 -	65 -	- 35	85 -	45 -	25 -	- -	- -	- -
SN02	Cornell 49242	*Phg-2*	890R	-	-	-	-	-	-	95	100	95	100	-	-	-	nd	nd	nd
E-ACA/ MCCT330	G10474	-	305R 280S	100 -	100 -	100 -	84 -	70 -	95 -	95 -	90 -	100 -	95 -	95 -	100 -	100 -	62.5 -	- -	60 -
SAA19	O. Negro	-	650R	40	-	-	3.3	43	-	80	80	55	88	-	10	55	-	-	-
SBA16	O. Negro	-	560R 500	100 -	93 24	74 11	59 22	37 -	36 100	95 -	70 30	100 -	55 -	85 -	45 10	90 5	78.7 -	45 -	85 -
SM02	O. Negro	-	460R	100	93	100	100	97	93	100	100	100	100	100	100	95	nd	nd	nd
Res. Mark*				4	5	4	4	3	3	6	6	5	5	3	2	4	3	1	2

1= Ouro Negro; 2= N. Papaloapan; 3= N. Comapa; 4= N. Cotaxtla; 5= B. Azteca; 6= B. INIFAP; 7= SCN7; 8= SEN56; 9= SEN26; 10= SEN70; 11= SCR13; 12= SER83; 13= SEN44; 14= 110; 15= 111; 16= 122; nd= not determined; R and S= alleles of resistance and susceptibility; the fragments without letters represent the unknown additional bands. Res Mark= resistance markers; *= those with a frequency equal to or greater than 50%, not including additional bands.

The 350 bp fragment associated with susceptibility was present in the SEN 70, SCR 13, and SER 83 lines. SEN 26 did not have any of these alleles, but it did have an additional 250 bp fragment, which was present in all materials, except in those with the susceptibility allele. The sequence of this fragment is not known; nonetheless, it seems to be associated with resistance to angular leaf spot and complementary studies are suggested to define its role. Figure 2 shows representative photographs of agarose gels with amplified fragments.

Figure 2. Example of amplified fragments. a) CV542014 marker in SEN56; b) SN02 marker in SEN56; c) SAA19 marker in B. Azteca and d) SBA16 marker in B. Azteca, B. INIFAP, Ouro Negro, N. Papaloapan and N. Cotaxtla.



The TGA1.1 marker also comes from RILs of the AND 277/Ouro Negro cross and produces a resistance allele of 570 bp (Gonçalves-Vidigal *et al*., 2011). This marker is considered to be present

DOI: https://doi.org/10.29312/remexca.v15i18.2887

exclusively in genotypes of the Andean collection; nevertheless, it was identified in the three segregating lines (Mesoamerican collection). The SH13 marker was identified in RILs of the Ouro Negro/Rudá cross, both from the Mesoamerican collection. Unlike the markers described above, here the Ouro Negro variety was used as a parent resistant to *P. griseola* races 31.23, 31.55, 63.31, and 63.19 (Faleiro *et al*., 2003; de Queiroz *et al*., 2004).

The resistance allele (520 bp) was found in a frequency greater than 50% only in the Papaloapan variety (75%); however, it was also present in five other genotypes at a maximum frequency of 35%(Table 1). This marker also amplified an additional 650 bp fragment present at varying frequencies (7% to 95%) in most of the genotypes studied and it was not identified only in the three segregating lines in SEN 70; nonetheless, it does not seem to be associated with the angular leaf spot resistance response.

The SN02 marker is associated with the *Phg-2* gene and was obtained from RILs of the Cornell 49242/Rudá cross. Cornell 49242 is resistant to the race 31-17 of *P. griseola* and the source of resistance of this gene is Mexico 54 (Nietsche *et al*., 2000). It was identified in the SCN7, SEN56, SEN26, and SEN70 lines, which accumulated the highest number of markers associated with resistance genes. Therefore, it seems to be related to resistance in these genotypes; nevertheless, the source of *Phg-2* in these materials is unknown.

The E-ACA/MCCT330 marker is linked to the resistance gene of the Mesoamerican genotype G 1047, which is resistant to the 63.63 race of *P. griseola*, one of the most virulent known. It was obtained from RILs of the G 10474/Sprite cross, 'universal' susceptible. It amplifies a 305 bp fragment associated with resistance and a 280 bp fragment associated with susceptibility (Mahuku *et al*., 2004). The resistance allele was present with frequencies greater than 50% in all genotypes, except for segregating line 111. Therefore, it is inferred that this marker does not discriminate resistant genotypes from susceptible ones.

The susceptibility allele was not found in any material. The SAA19 marker was identified from RILs of the Ouro Negro/US Pinto 111 cross and was verified in RILs of the TO/Ouro Negro cross (de Queiroz *et al*., 2004). This marker was found in three black varieties with frequencies below 50%, five advanced lines of black beans and one advanced line of red beans. De Queiroz *et al*. (2004) reported that when this marker is used as a selection criterion together with the SM02 marker, an effectiveness of 97% was obtained. SCN7, SEN56, SEN26, and SEN70 presented both markers at frequencies greater than 50%; in addition, the first three also have the SN02 and CV242014 markers or their additional band; this combination seems to be very important in inducing resistance.

The SBA16 marker, like SH13, was identified in RILs of the Ouro Negro/Rudá cross and was verified in RILs of TO/Ouro Negro (Faleiro *et al*., 2003; de Queiroz *et al*., 2004). In Ouro Negro, it amplifies a 560 bp fragment associated with resistance. It was found in all genotypes although at frequencies less than 50% in bay beans and in SER83.

It generates an additional band of 500 bp, which is found in four varieties, including Bayo INIFAP, with a frequency of 100%, but due to its distribution and frequency, it does not seem to be related to resistance. The SM02 marker was obtained from RILs of Ouro Negro/US Pinto 111 and validated in RILs of the TO/Ouro Negro cross. This marker was identified with high frequency in all genotypes, so it does not discriminate resistant genotypes from susceptible ones.

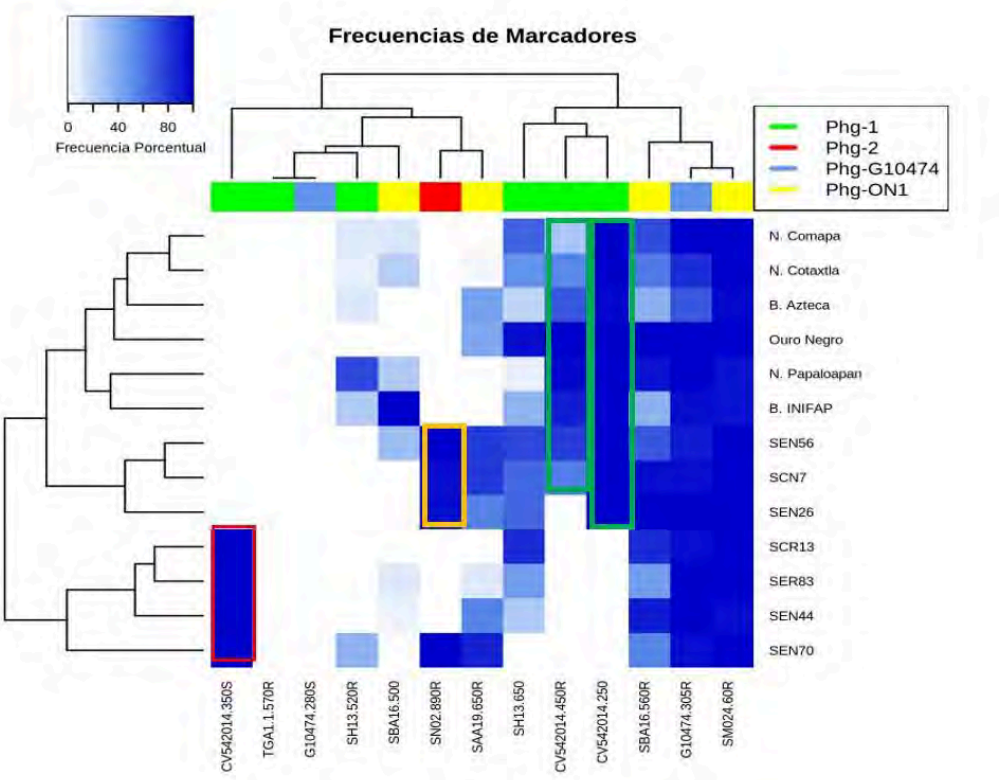
Molecular marker-based clustering analysis

The frequencies of fragments amplified by the eight markers were used to perform a clustering analysis, which was represented by means of a heatmap (Figure 3).

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i18.2887

Figure 3. Frequency-based heatmap. The row dendrogram shows the similarity rela onships between genotypes and the column dendrogram between markers. The box on the right indicates the genes linked to each marker. The name of each marker included a le er at the end that indicates its rela onship with resistance or suscep bility. The red box shows the suscep bility allele of the CV542014 marker, the green boxes show its resistance allele and an additional band that could be related to resistance. The yellow box shows the resistance allele of the SN02 marker.



The dendrogram based on genotypes (left side) shows three groups; in group one is N. Papaloapan and Ouro Negro, resistant to angular leaf spot, N. Comapa, N. Cotaxtla, B. Azteca, and B. INIFAP. The above could suggest that all of them are potentially resistant; however, N. Papaloapan presents five resistance markers with frequencies greater than 50%; Ouro Negro, N. Comapa, and N. Cotaxtla have four, while B. Azteca and B. INIFAP have only three; the markers that could make the difference so that these two genotypes do not have a resistance reaction by inoculation are SBA16 (low frequencies) and SN02 (absence in these genotypes) (Figure 3).

Group two was made up of the three genotypes with the highest number of markers with a frequency greater than or equal to 50%: SEN56, SCN7 and SEN26 with 6, 6 and 5 markers; respectively, which made it possible to predict that they would be resistant. Group three included four genotypes that share the characteristic of being the only ones that have the susceptibility allele of the CV242014 marker; in addition, two of them were the ones that had the lowest cumulative number of markers with a frequency greater than 50%, they were SCR13 and SER83.

It is notorious that SEN 70, although it accumulated five markers, also lacks the resistance allele of CV542014. The results allow us to predict that these materials will be susceptible. Resistance-related markers are those that have higher frequencies when resistant and susceptible genotypes are compared. This is the case of CV542014, absent only in the susceptible genotypes of group 3, SN02, present in the three genotypes of group 2, and SAA19, present in the black bean lines in complement with the SM02 marker. SCR 13, SER 83, SEN 44, and SEN 70 have this marker, but they also have the susceptibility allele of the CV542014 marker.

DOI: https://doi.org/10.29312/remexca.v15i18.2887

The efficient use of molecular markers requires a validation phase, which must include inoculation as a confirmatory means; this confirmatory phase is followed by the predictive phase, where the single use of the markers must be sufficient to perform assisted selection at larger scales. Validation should be performed with uniform genetic materials in order to avoid uncontrolled variations in marker frequencies.

The results suggest that the genes represented by the markers have additive effects, so the greater the number of markers, the greater the resistance. Thus, the genotypes with the highest potential resistance to angular leaf spots are those with the highest number of markers with high frequencies (SCN7 and SEN56 with six markers and N. Papaloapan and SEN26 with five), while the genotypes with the lowest number of markers are potentially susceptible (SER83, SCR13, B. Azteca, and B. INIFAP).

Conclusions

The *P. griseola* strains presented differences in severity of the damage caused, with strain 2, from Valle de Santiago, being more pathogenic. Molecular markers vary widely in their association with resistance to angular leaf spots. In the bean genotypes studied and with the two strains considered, the markers linked to resistance were CV542014, SAA19, SM02 and SN02, which allowed us to demonstrate, together with the artificial inoculation of *P. griseola*, that the varieties N. Comapa and N. Cotaxtla are resistant to the disease, those of bay beans and the advanced lines of red beans are susceptible; nevertheless, the three advanced lines, SEN 56, SEN 7, and SEN 26, have greater resistance potential due to their cumulative number of molecular markers. Markers can be considered as linked to genes with additive effects and they are a useful strategy for the selection of resistant genotypes.

Bibliography

1 Beebe, S.; Rengifo, J.; Gaitan, E.; Duque, M. C. and Tohme, J. 2001. Diversity and origin of Andean landraces of common bean. Crop Sci. 41(3):854-862. https://doi.org/10.2135/cropsci2001.413854x.

2 Beebe, S.; Skroch, P. W.; Tohme, J.; Duque, M. C.; Pedraza, F. and Nienhuis, J. 2000. Structure of genetic diversity among common bean landraces of Mesoamerican origin based on correspondence analysis of RAPD. Crop Sci. 40(1):264-273. https://doi.org/10.2135/cropsci2000.401264x.

3 de Jesús, W. C. J.; Vale, F. X. R.; Coelho, R. R.; Hau, B.; Zambolim, L.; Costa, L. C. and Filho, A. B. 2001. Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathol. 91(11):1045-1049. https://doi.org/10.1094/PHYTO.2001.91.11.1045.

4 de Queiroz, V. T.; Sousa, C. S.; Costa, M. R.; Sanglad, D. A.; Arruda, K. M. A.; Souza, T. L. P. O.; Ragagnin, V. A.; Barros, E. G. and Moreira, M. A. 2004. Development of SCAR markers linked to common bean angular leaf spot resistance genes. Ann. Rep. Bean Improv. Coop. 47:237-238.

5 Doyle, J. J. 1990. Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

6 Faleiro, F. G.; Ragagnin, V. A.; Schuster, I.; Corrêa, R. X.; Good-God, P. I.; Brommonshenkel, S. H.; Moreira, M. A. and Barros, E. G. 2003. Mapeamento de genes de resistência do feijoeiro à ferrugem, antracnose e mancha-angular usando Marcadores RAPD. Fitopat. Brasil. 28:2-5. http://dx.doi.org/10.1590/S0100-41582003000100009.

7 Fihlo, A. B.; Carneiro, S. G.; Godoy, C. V.; Amorim, L.; Berger, R. D. and Hau, B. 1997. Angular leaf spot of *Phaseolus* beans: relationships between disease, healthy leaf area, and yield. Phytopathol. 87(5):506-509. https://doi.org/10.1094/PHYTO.1997.87.5.506.

8 Gil, J.; Solarte, D.; Lobaton, J. D.; Mayor, V.; Barrera, S.; Jara, C.; Beebe, S. and Raatz, B. 2019. Fine-mapping of angular leaf spot resistance gene *Phg-2* in common bean and

DOI: https://doi.org/10.29312/remexca.v15i18.2887

development of molecular breeding tools. Theor and Applied Genet. 132(7):2003-2016. https://doi.org/10.1007/s00122-019-03334-z.

9 Gonçalves-Vidigal, M. C.; Cruz, A. S.; Garcia, A.; Kami, J.; Vidigal, F.; Pedro, S.; Sousa, L. L.; McClean, P. P. G. and Pastor-Corrales, M. A. 2011. Linkage mapping of the *Phg-1* and *Co-14* genes for resistance to angular leaf spot and anthracnose in the common bean cultivar AND 277. Theor and Applied Genet. 122:893-903. Doi: 10.1007/s00122-010-1496-1.

10 León-Saavedra, I. 2009. La antracnosis y la mancha angular del frijol común (*Phaseolus vulgaris* L.). Tem. Cienc. Tecnol. 13(39):23-25.

11 Librelon, S. S.; Souza, E. A. D.; Pereira, R.; Pozza, E. A. and Abreu, A. D. F. B. 2015. Diagrammatic scale to evaluate angular leaf spot severity in primary leaves of common bean. Australas. Plant Pathol. 44(4):385-395. https://doi.org/10.1007/s13313-015-0360-9.

12 Mahuku, G.; Montoya, C.; Henríquez, M. A.; Jara, C.; Teran, H. and Beebe, S. 2004. Inheritance and characterization of angular leaf spot resistance gene present in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Sci. 44(5):1817-1824. https://doi.org/10.2135/cropsci2004.1817.

13 Miller, T.; Gepts, P.; Kimno, S.; Arunga, E.; Chilagane, L. A.; Nchimbi-Msolla, S. Namusoke, A.; Namayanja, A. and Tedla, Y. R. 2018. Alternative markers linked to the *Phg-2* angular leaf spot resistance locus in common bean using the *Phaseolus* genes marker database. Afr. J. of Biotechnol. 17(26):818-828. https://doi.org/10.5897/AJB2018.16493.

14 Montero-Tavera, V.; Escobedo-Landín, M. A.; Acosta-Gallegos, J. A.; Anaya-López, J. L. and Ruiz-Nieto, J. E. 2017. 26S: novel reference genes from leaves and roots of common bean for biotic stress expression studies based on PCR. Legum. Res. 40(3):429-433. Doi: 10.18805/lr.v0iOF.7859.

15 Nay, M. M.; Mukankusi, C. M.; Studer, B. and Raatz, B. 2019a. Haplotypes at the *Phg-2* locus are determining pathotype-specificity of angular leaf spot resistance in common bean. Front. Plant Sci. 10:1126. https://doi.org/10.3389/fpls.2019.01126.

16 Nay, M. M.; Souza, T. L.; Raatz, B.; Mukankusi, C. M.; Gonçalves#Vidigal, M. C.; Abreu, A. F. B; Melo, C. L. and Pastor#Corrales, M. A. 2019b. A review of angular leaf spot resistance in common bean. Crop Sci. 59(4):1376-1391. https://doi.org/10.2135/cropsci2018.09.0596.

17 Okii, D.; Badji, A.; Odong, T.; Talwana, H.; Tukamuhabwa, P.; Male, A. Mukankusi, C. and Gepts, P. 2019. Recombination fraction and genetic linkage among key disease resistance genes (*Co-4* ^{2}/*Phg-2* and *Co-5*/*P. ult*) in common bean. Afr. J. Biotechnol. 18(29):819-830. https://doi.org/10.5897/AJB2019.16776.

18 Rodrigues, L. A.; Coelho, G. R.; Valdisser, P. A. M.; Vianello, R. P. and Souza, T. L. P. 2016. Marker-assisted selection integrated to the embrapa common bean breeding program. Embrapa Arroz e Feijão-Artigo em periódico indexado (ALICE). 199-200 pp.

19 Sietsche, S.; Borém, A.; Carvalho, G. A.; Rocha, R. C.; Paula, T. J.; Barros, E. G. and Moreira, M. A. 2000. RAPD and SCAR markers linked to a gene coffering resistance to angular leaf spot in common bean. J. Phytopathol. 148(2):117-121. Doi: 10.1046/j.1439-0434.2000.00479.x.

20 SIAP. 2019. Sistema de Información Agroalimentaria y Pesquera. Panorama agroalimentario. Secretaría de Agricultura y Desarrollo Rural. Ciudad de México. 1ra . Ed. 218 p.

21 Schwartz, H. F.; Correa, F. y Pastor-Corrales, M. 1982. La mancha angular del frijol y su control; guía de estudio. CIAT. Cali, Colombia. 24 p.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i18.2887

Identification of bean genotypes resistant to angular leaf spot by molecular markers

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 July 2024
Date accepted: 01 October 2024
Publication date: 14 December 2024
Publication date: Nov-Dec 2024
Volume: 15
Issue: 8
Electronic Location Identifier: e2887
DOI: 10.29312/remexca.v15i18.2887

Categories

Subject: Articles

Keywords:

Keywords:

Phaseolus vulgaris
Pseudocercospora griseola
black bean
resistance
SCAR

Counts

Figures: 3
Tables: 1
Equations: 0
References: 21
Pages: 0