

Response of bean cultivars to inoculation and natural incidence of halo blight and usefulness of molecular markers for selection

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Abstract

In the Bajío, bean halo blight [*Pseudomonas syringae* pv. *phaseolicola* (*Psph*)] causes severe damage to varieties of the Azufrado and Cacahuete Bola types. To identify resistant genotypes, various bean materials were inoculated with two isolates, races 2 and 6. Likewise, readings of the reaction to natural infection by *Psph* in the field were recorded in two sets of Azufrado-type lines evaluated under rainfed conditions in Celaya, Guanajuato, in 2018, one with 10 and another with 16 lines established in June and July, respectively. In the marker laboratory, primers B10, W13, and T8, associated with genes of resistance against the bacterium in various bean genotypes, were used to determine the association between the presence of the markers and resistance to *Psph*. The Azufrado-type genotypes established in June were severely damaged (reaction from 5 to 8 on a scale of 1 to 9) by *Psph*, while those of July were damaged to a lesser degree. In the first trial, environmental conditions favored the attack of the bacterium, conditions that did not occur in the second trial. Most of the genotypes inoculated with race 6 in the greenhouse were susceptible. Genotypes positive for the presence of the three markers used were susceptible to races 2 and 6; that is, the marked resistance factors do not provide resistance to those races. All evaluated genotypes of the Azufrado-type of Nueva Granada race were susceptible to *Psph* races 2 and 6. Among the inoculation-resistant genotypes are Flor de Mayo M38, San Rafael, Pinto Laguna 80, and Pinto Saltillo.

Keywords:

Pseudomonas syringae pv. *phaseolicola* (Burkh), *Phaseolus vulgaris* L., resistance.



Introduction

In the Altiplano and Bajío of Central Mexico, the bean is limited by abiotic factors, such as intermittent drought under rainfed conditions, and in specific areas of the Bajío, by high temperatures that affect the yield and quality of the grain under irrigation, as well as by biotic factors (pests and diseases) that cause low yields (Anaya *et al.*, 2021). Recent studies showed an abundant presence of the bacterium that causes halo blight (*Psph*) in seeds of varieties of the Andean pool produced in Sinaloa (Félix-Gastelum *et al.*, 2016), varieties that, when sown in Guanajuato during the rainy season, succumb to the pathogen, as well as the landrace varieties of the Cacahuete Bola or Japanese type. Producers plant these types of varieties because of the high price at which they can sell their grain and because of the short cycle and ease of handling plants of determined habit under irrigation and rainfed conditions (Kelly, 2000).

The first symptoms of halo blight appear as small angular spots; as they grow, they turn brown, and a characteristic green-yellow halo appears around the spot. This halo is due to the action of a toxin produced by the bacterium (phaseolotoxin). Under favorable conditions for the development of the disease, the toxin moves systemically within the plant and also attacks the pods (Álvarez-Morales *et al.*, 2018), where a creamy white exudate was observed within the spots produced by the bacteria.

The infection penetrates the seeds during the process of pod maturation. At that stage, yellow and yellow-brown spots were observed on the seeds (Navarrete-Maya, 2013). At present, there are no varieties of the Azufrado and Cacahuete Bola types resistant to *Psph*, nor are the races present in the country known; in the Bajío and Sinaloa, race 6 has been detected to defeat all differential varieties (Félix-Gastelum *et al.*, 2016).

Recent studies on the collection and characterization of *Psph* isolates in Canada (Chatterton *et al.*, 2014) and states of the Midwest of the United States (Lamppa *et al.*, 2002) have indicated the presence of races 2, 6, and 8 (Gishing *et al.*, 2016); the particularity of race 6 is that it produces symptoms in all differential varieties of beans and to date only one resistant genotype has been described, PI 150414 from El Salvador (Taylor *et al.*, 1996).

Race 6 was described by Félix-Gastelum *et al.* (2016) as responsible for damaging Azufrado-type beans in Sinaloa. Collections and studies on the distribution and damage of bacteria attacking the crop in Africa have also been carried out (Chataika *et al.*, 2011). On the other hand, numerous improved varieties of beans have been developed in Mexico, and some have been described as resistant to natural infection by halo blight in the field; however, the resistance reaction described was not the product of controlled inoculation, indicating that it is not known to which races they are resistant (Anaya *et al.*, 2021).

In order to generate bean varieties resistant to halo blight, molecular markers have been developed for use in selection. These markers were associated with major resistance genes predicted in the bean genome by resistance analysis of progeny from directed crosses from a resistant genotype by a susceptible one (Fourie *et al.*, 2004; Miklas *et al.*, 2009; Miklas *et al.*, 2011). The objective of this research was to identify bean genotypes with resistance to halo blight for use in crosses with susceptible genotypes and verify the usefulness of six molecular markers described in the literature as associated with three resistance genes; this allowed us to implement assisted selection to transfer resistance to the main varieties used in the Bajío.

Materials and methods

Greenhouse. The seed of the following eight differential varieties used for the identification of *Psph* races was increased in a greenhouse: Canadian Wonder, Red Mexican UI 13, A 52 (ZAA 54), GUA 196B, Tendergreen, A 43 (ZAA 12), A 53 (ZAA 55) and Tepari 1072 (*Phaseolus acutifolius*).

In the phytopathology laboratory, two samples isolated in Guanajuato (considered as aggressive against different types of beans in previous inoculations) were processed following the procedure

described by Taylor *et al.* (1996), which, when inoculated on the set of differential varieties, turned out to be race 2 (overcomes six differentials), a strain isolated from a sample collected in Salvatierra, and race 6 (overcomes eight differentials) from a sample collected in Celaya, respectively (Miklas *et al.*, 2009).

Subsequently, 84 improved varieties described as resistant to natural *PspH* infection were inoculated, which included materials with different grain types. Due to space limitations in the greenhouse and considering the origin of the varieties, these were divided into two groups, 28 and 56, and sown in pots for inoculation with isolates of race 2 and race 6, respectively.

The seedlings were inoculated in the primary leaves following the standard methodology proposed by Mills and Silvernagel (1992) by using insulin syringes eight days after their emergence, the inoculum was at a concentration of 1×10^8 total colony forming units (cfu), a gauze soaked in the inoculum was placed on the underside of the leaves, which were injured with needles mounted on a tongue depressor so that they absorb it; from each inoculated material, a pot was left as control with seedlings inoculated with sterile water.

Each pot contained six seedlings, and each was considered an experimental unit. Three readings of the reaction to inoculation were made at 11, 16, and 21 days; to describe the reaction, the scale of Innes *et al.* (1984) was used; for statistical analysis, the readings taken at 21 days were used.

Field trials

In trials established in 2013, the reaction to natural infection by *PspH* was rated on a scale of 1 to 9 (Shoonhoven and Pastor-Corrales, 1987), where 1 to 3 is considered resistant, 4 to 6 intermediate reaction, and 7 to 9 susceptible. Field visits to assess the incidence of *PspH* were carried out in the reproductive stage. Crosses between susceptible and resistant varieties were made to obtain Fs1 (varieties of the Nueva Granada race as females and resistant plants of other races as a source of resistance). Parents and F1 plants were inoculated in the primary leaves as described above.

Identification of molecular markers associated with resistance

From the plants of the sown differential varieties, tissue from the youngest trifoliate leaf was collected in the vegetative stage for DNA extraction, following the CTAB procedure described by Doyle and Doyle (1987). For the quantification and determination of DNA purity, a NanoDrop 8000[®] spectrophotometer was used, placing 2 μ l of DNA on the pedestal and configuring the program for an optical density of 30 nanograms per microliter and absorbance at 260 nm.

Six sequence-tagged site (STS) markers associated with three genes of halo blight resistance described by Fourie *et al.* (2004); Miklas *et al.* (2009, 2011) were used. To determine the usefulness of these markers, 10 seeds of each differential variety were sown, and a mixture equivalent in weight of leaf tissue was made for the extraction of DNA from the seedlings of each one. DNA was used to amplify by PCR the six markers by following the indications of Fourie *et al.* (2004); Miklas *et al.* (2009, 2011). The amplification products were separated by 1.5% agarose gel electrophoresis with TBE buffer. The gels were left to run 2 h at 120 V, revealed with ethidium bromide, and documented by photograph on a UV transilluminator.

Results and discussion

Identification of resistant genotypes in the greenhouse

Inoculation with races 2 and 6 of *PspH* showed reaction heterogeneity in the symptoms of seedlings of some varieties; that is, in the same material, there were plants with susceptible and resistant reactions. There were also uniform resistant genotypes. Genotypes of intermediate reaction were also identified according to the scale used (0 to 1, resistant; 2 to 3, intermediate,

and 4, susceptible) (Innes *et al.*, 1984). Due to the diversity of resistant genotypes of different types, both races seem to be widely distributed in Mexico. The genotypes resistant to race 6 were: Pinto 168, Bayo 66, and Bayo 161 of the Jalisco race; Azufrado Tapatío, Lagunero 80, and Pinto Saltillo of the Durango race; and Bayo Azteca of the Mesoamericana race. In this group, Azufrado-type varieties were included, and all were susceptible.

Through inoculation with two isolates of *Psph* (Races 2 and 6), five varieties resistant to race 2 and eight to race 6 were identified, none of the Azufrado type (Table 1).

Table 1. Bean genotypes resistant to independent inoculation with *Psph* races 2 and 6 under controlled greenhouse conditions. Reaction of 0-1 on the scale of (Innes *et al.*, 1984).

Resistant to race 2	Resistant to race 6	
Pinto Anzalduas	Pinto 168	Pinto Saltillo
Flor de Mayo Anita	Bayo 66	Flor de Mayo M38
Pinto Durango	Azufrado Tapatío	Bayo Azteca
Flor de Mayo M38	Pinto Lagunero 80	Bayo 161
Negro Altiplano		

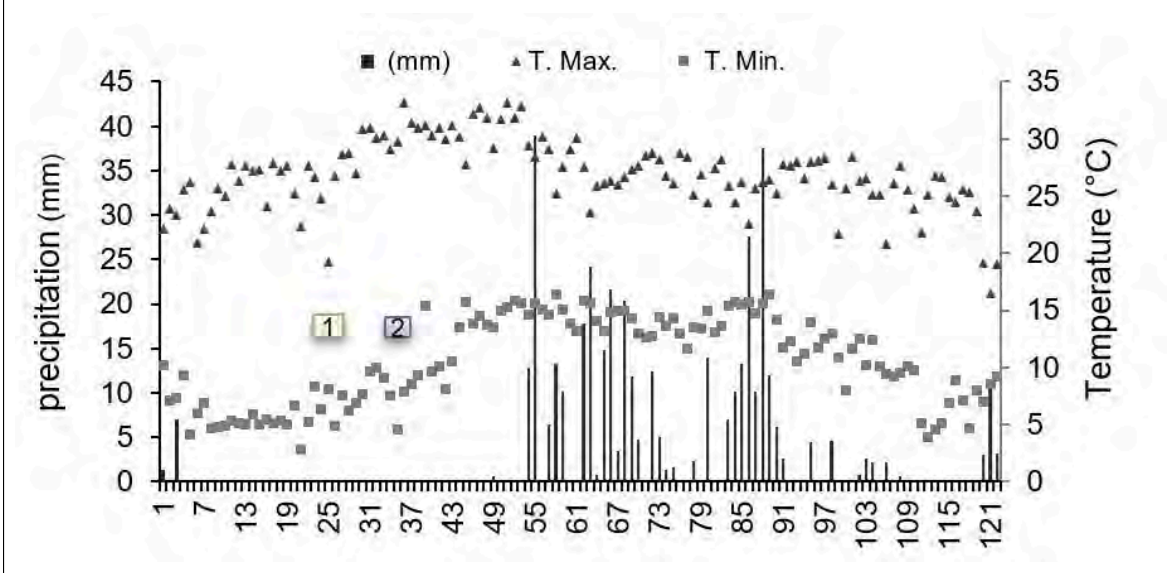
Guising *et al.* (2014, 2016) characterized the reaction of 283 bean accessions for their resistance to race 6 and identified five resistant ones, suggesting their use as sources of resistance, including a bean native to Puebla, Mexico. In Guanajuato, only the varieties Flor de Mayo M38 and Pinto Saltillo have been used in crosses; the rest of the resistant materials have not been used due to their undesirable agronomic characteristics, such as a type of grain without commercial value, long cycle, and climbing growth habit. Likewise, for selection in segregating populations, this should be done through inoculation with the races present in the region for which the varieties are being developed and not only based on field reaction, where escapes may occur (Ghising *et al.*, 2014).

Reaction of Azufrado genotypes established in the field

In the field, the 10 Azufrado-type materials established in June showed a severe attack by halo blight (data not shown). In this trial, precipitation was high in the vegetative phase during July, and temperatures were favorable for the development of halo blight (Figure 1). Due to the splashing of rain from infected plants onto healthy plants, *Psph* infection was uniform. It is also likely that *Psph* was present in the soil from previous bean sowings at the site.



Figure 1. Maximum and minimum temperature and decennial precipitation from January to November 2018 in Celaya, Guanajuato. 1 and 2 indicate the sowing dates.



In the trial with 16 materials established in July, the incidence of the disease was lower; this was proven because the control varieties were the same in the two trials, JANASA, Azufrasin, and Azufrado Higuera, which showed lower incidence in the second trial (Table 2). This indicates that environmental conditions influence the incidence of the disease since the sowing date of late July and a drought period in mid-August did not favor the early development of halo blight; for the controls of both trials, the same seed source was used.

Table 2. Reaction of 16 bean materials to the natural incidence of halo blight during the rainfed cycle. CEBAJ, Celaya, Guanajuato 2018.

Genealogy	Reaction	Genealogy	Reaction
L6/L11(Mo)-M-7-1-1-1-M	2.7 ¹	(G17421/Az.26) //FME)-1-6-2	2.8
L6/L11(Mo)-M-7-1-1-2-M	3	Univ. Puerto Rico 10	3.2
L6/L11(Mo)-M-6-2-1-M	3	Univ. Puerto Rico 11	3
(G17421/Az26//FME)-1-8-1	1.5	Univ. Puerto Rico 12	3.2
(G17421/Az26//FME)-1-8-2	1.5	Minimi-UACH	2.7
(G17421/Az26//FME)-1-57-1	2.5	Azufrado Higuera (T)	4
(G17421/Az26//FME)-2-6-1-	2.7	Azufrasin (T)	3
(G17421/Az26//FME)-1-10-2	1.5	JANASA (T)	3

¹ = scale of 1 to 9 (Shoonhoven and Pastor-Corrales, 1987), average of 4 repetitions.

In the trial of 16 genotypes (Table 2), there were two outstanding ones with incidence < 3.0, both derived from the same cross: (G17421/AZ 26// FME)-1-8-2 and (G17421/AZ 26// FME)-1-8-1. The cross and selection in the segregating population from which these lines were derived were carried out in the test locality; these genotypes should be inoculated under controlled conditions to verify their resistance. Currently, the production of Azufrado Higuera in Sinaloa is carried out under irrigation and with certified seed, and chemical protection for plants is used against pathogens (Félix-Gastelum *et al.*, 2016).

As for the crosses made to incorporate resistance to Azufrado-type varieties, F₁ and F₂ seed was obtained from the crosses: Aluyori x Flor de Mayo Dolores, Azufrasin x Minimi, Azufrasin x Flor

de Mayo Dolores, Azufrasin x Junio León, San Rafael x Janasa and Raramuri x Janasa. These populations were incorporated into the local improvement program to continue their assessment.

Use of markers associated with *Psph* resistance genes

In the differential varieties, except for Tepari 1072, the presence of the six markers available in the literature was determined (Fourie *et al.*, 2004; Miklas *et al.*, 2009; Miklas *et al.*, 2011) and of these, only three produced the expected amplicons, ST8, B10 and SW13 (Table 3), while R13, SAE 15 and H11 presented different amplicons, which may be due to differences in the genetic background of the loci that these markers label. In fact, two of the last three markers, R13 and H11, mark the same gene that ST8 and its developers (Miklas *et al.*, 2009) indicate that they only work on Andean material and the black and red types of the Mesoamericana race.

Table 3. Presence of STS markers in seven differential varieties for halo blight, *Psph*.

Differential variety	Marker			Resistance to races
	T8	B10	SW13	
Red Mexican UI 13	+	+	-	1, 5, 7, 9
A 52 (ZAA 54)	-	+	-	5
GUA 196B	+	+	+	1, 3, 4, 5, 7, 9
Tendergreen	-	-	+	3, 4
A 43 (ZAA 12)	-	-	+	3, 4
A 53 (ZAA 55)	-	+	-	5
Canadian Wonder	-	-	-	0

The resistance reported in the differential varieties corresponds to the genes *Pse-1* and *Pse-3*; these do not have resistance to races 2, 6, and 8. That is, the markers do not work in the selection against those races; therefore, their usefulness is limited to races 1, 3, 4, 5, 7, and 9 (Miklas *et al.*, 2009). The three markers that showed the expected response allow the identification of bean genotypes resistant to races 1, 3, 4, 5, 7, and 9 (Miklas *et al.*, 2009), which will be useful to incorporate resistance to the varieties of greater demand of the Azufrado and Cacahuete Bola types, but not against races 2 and 6 that have been isolated in Guanajuato and Sinaloa.

Recent studies of Trabanco *et al.* (2014); González *et al.* (2016); Took *et al.* (2017) indicate the presence of different genotypes resistant to race 6, with non-race-specific resistance, which, when combined with race-specific alleles, can result in lasting resistance. In the case of the resistant pinto-type genotype US14HBR6, resistance against race 6 was determined to be monogenic (Duncan *et al.*, 2014). Of the materials studied, Flor de Mayo M38, Pinto Saltillo, and Pinto Lagunero 80 with resistance reaction to race 6 do not have the three markers; it is inferred that they have some other resistance gene.

These results suggest the need to develop new markers to be able to use assisted selection efficiently (Miklas *et al.*, 2009) or base it on inoculation under controlled conditions. Recently, Took *et al.* (2017) pointed out that resistance to race 6 includes both alleles with non-race-specific (quantitative effects) and race-specific effects (qualitative effect), and that the combination of both types of alleles will provide lasting resistance.

Regarding crosses between resistant and susceptible varieties, it was observed that the B10 marker is present in all (resistant and susceptible); that is, it will not be useful for assisted selection in this group of parents (Table 4). The susceptible variety Azufrasin presented two of the three markers; in this case, none of the three markers will be useful for the selection in crosses with this variety. The results of the parents show that markers, especially T8 and SW13, appear heterogeneously in some; that is, in different proportions (Table 4).

Table 4. Presence of three markers associated with two genes of halo blight resistance in individual F1 plants and their parents.

Cross	Marker		
	T8	B10	SW13
F1 Aluyori/Flor de Mayo Dolores	5/10 ¹	10/10	6/10
F1 Azufrasin/Minimi	10/10	10/10	10/10
F1 Azufrasin/ Flor de Mayo Dolores	4/4	4/4	4/4
F1 San Rafael/ Janasa	0/10	10/10	10/10
F1 Rarámuri/Janasa	0/10	7/7	7/7
F1 Aluyori/ Rarámuri	3/4	3/4	3/4
F1 Azufrasin/Andino 28	0/5	5/5	0/5
Rarámuri (Durango race)	0/5	3/5	0/5
Aluyori (Nueva Granada race)	0/9	9/9	0/9
Azufrasin (Nueva Granada race)	8/8	8/8	8/8
San Rafael (Durango race)	0/10	10/10	9/10
Flor de Mayo Dolores (Jalisco race)	10/10	3/10	10/10
Janasa (Nueva Granada race)	0/10	10/10	3/10

¹ = numerator, plants with the marker; denominator, the total number of plants in the test.

This suggests their use to homogenize the presence of these in those genotypes. Likewise, the absence of the corresponding marker in F1 plants of the Aluyori by Flor de Mayo Dolores cross indicates the presence of self-fertilization. Recently, several authors have conducted studies to identify sources of quantitative resistance, considering that this, in combination with race-specific resistance, may be more durable (Trabanco *et al.*, 2014; González *et al.*, 2016). Took *et al.* (2017) mapped alleles of resistance to *PspH* race 6 from different sources and showed evidence of durable resistance by combining non-race-specific alleles with race-specific alleles. They suggest the use of molecular markers for the improvement of resistance, especially of the non-race-specific allele.

The varieties Flor de Mayo M38 and Pinto Saltillo have been used extensively in crosses with similar materials; they should be used in crosses with materials of the Cacahuete Bola and Azufrado types. Selection in segregating populations should be made through inoculation with the races present in the region for which the varieties are being developed. In the case of the Bajío region, a region with consecutive rainfed and irrigated cultivation cycles, the possibility of maintaining healthy seeds of susceptible varieties, such as Azufrado and Cacahuete Bola, is limited and is compromised by the reuse of grain as seed by producers.

Conclusions

Through inoculation with two isolates of *Pseudomonas syringae* pv *phaseolicola*, five varieties resistant to race 2 and eight to race 6 were identified. In field trials, *PspH* severely damaged materials of the Azufrado type (Nueva Granada race, Andino Pool) established in the field under rainfed conditions, damage related to the inherent susceptibility of the materials and the climatic conditions of high moisture and temperate temperature that favored the development of the disease. Selection with the use of available molecular markers may be functional against *PspH* races less aggressive than races 2 and 6.

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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 August 2023
Date accepted: 01 October 2023
Publication date: 02 December 2023
Publication date: November 2023
Volume: 14
Issue: 8
Electronic Location Identifier: e3274
DOI: 10.29312/remexca.v14i8.3274

Categories

Subject: Articles

Keywords:

Keywords:

Pseudomonas syringae pv. *phaseolicola* (Burkh)

Phaseolus vulgaris L.

resistance.

Counts

Figures: 1

Tables: 4

Equations: 0

References: 22

Pages: 0