Article

Detection of genetic markers associated with resistance to pathogens in ayocote beans from Puebla, Mexico

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Abstract

Ayocote beans (*Phaseolus coccineus* L.); 2n= 2x= 22) is a legume native to the highlands of Mexico, it is important in human nutrition and detection of improvement characteristics, it shows resistance to pathogens and certain abiotic factors that cause losses in the bean crop. With the premise of identifying *Phaseolus* germplasm with multiple resistance to biotic and abiotic adverse factors, this work was carried out with the objective of this work was to determine the resistance or susceptibility to two important pathogens: common blight [*Xanthomonas axonopodis* pv. *phaseoli* (Smith)] and anthracnose [*Colletotrichum lindemutianum* Sacc. & Magn (anamorph *Glomerella lindemuthiana* Shear)] based on the identification of sequences characterized by amplified regions (SCARs). The study included 117 accessions collected at different sites in Puebla, Mexico (region called Carso Huasteco), which were analyzed with 10 SCAR markers (six markers for common blight: SAP6, BAC6, SU91, LG5, R7313, R4865, four for anthracnose: SAS13, SBB14, SAB3, SH18) at the Center for Genomic Biotechnology in Reynosa, Tamaulipas. The SAS13 and SBB14 anthracnose resistance markers are present more frequently (89 and 74%), followed by the common blight resistance markers BAC6 and SU91 (74 and 42%). The germplasm of Zacapoaxtla and Tlatlauquitepec had a higher frequency of SCARs (Zacapoaxtla: 90% and 100% for SAS13 and SBB14; Tlatlauquitepec: 94% and 56%). Accessions with five SCARs could be used as a source of resistance to diseases in *Phaseolus* and early and late genotypes to flowering and physiological maturity, as well as variables in testa color, pod size and seed, but no association was detected between the presence of SCARs with the morphology of ayocote beans.

Keywords: *Colletotrichum lindemuthianum*, *Phaseolus* pathogens and *Xanthomonas axonopodis* pv. *Phaseoli,* Carso Huasteco.

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Introduction

Ayocote beans (*Phaseolus coccineus* L.) in Mexico are mainly used as food and even in regions of Spain, the Netherlands and the United Kingdom, their consumption has replaced beans (*P. vulgaris* L.) (Rodiño *et al*., 2006). Of the domesticated species of the genus *Phaseolus* spp., the ayocote bean is the second species in economic importance (Al Hassan *et al*., 2016). Traditional ayocote bean cultivation occurs on a small scale (Schwember *et al*., 2017). In Central America, this bean is grown almost exclusively in association with corn (*Zea mays* L.) (Vargas-Vázquez *et al*., 2007).

Since beans are an important element in the self-supply of rural populations, the variation in forms of cultivation, use and forms of preparation for consumption is wide. Common blight is a disease with wide distribution in Mexico due to monoculture and the continuous exchange and reuse of grain as a seed, factors that increase the accumulation of the primary inoculum and the dispersion of the causative bacteria (Acosta-Gallegos *et al*., 2013). For its part, anthracnose also shows wide distribution in our country and can be a devastating disease if susceptible varieties are planted and, in addition, favorable climatic conditions occur for their development (Rodríguez-Guerra *et al*., 2006).

The ayocote bean has outstanding characteristics that can be exploited in programs of genetic improvement of the common bean such as: vigorous radical system, numerous floral knots, long peduncles, also stands out for its resistance to viral diseases such as the common mosaic virus and the virus of the golden mosaic; bacterial diseases such as halo blight (*Pseudomonas syringae* Van Mall) and common blight (*Xanthomonas axonopodis* pv *phaseoli* Smith.) (Duncan *et al*., 2006; Ruiz-Salazar *et al*., 2016), as well as fungal diseases such as anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magn) (teleomorph *Glomerella lindemuthiana* Shear)] (Ruiz-Salazar *et al*., 2016), presents resistance to abiotic factors such as low temperature and salinity (Rodiño *et al*., 2006; Al Hassan *et al*. 2016).

The use of simple and/or multiple genetic resistance to diseases of economic importance in beans is supported by the constant search for new sources of resistance, their incorporation into cross-breeding, evaluation and selection programs of segregating germplasm in field conditions and controlled; and obtaining germplasm with resistance to these adverse factors, which also shows high grain yields and with stability in the yield and quality of the superior grain (Acosta-Gallegos *et al*., 2007; Anaya-López *et al.*, 2015a).

The genetic improvement identifies and selects genotypes based on phenotypes and the subsequent introgression of the desired characters for the development of superior germplasm. Thus, virtually all important agronomic features have been improved: grain yield and quality; phenology; resistance to diseases, pests, drought or salinity. The process can last for several years depending on the genetics of the trait and the response to the selection. Molecular marker assisted selection (SAMM) could reduce process time and costs (Bernardo, 2008; Collard and Mackill, 2008; Xu and Crouch, 2008).

SAMM quickly identifies germplasm that may contribute to the improvement of cultivated species of *Phaseolus* spp. (Svetleva *et al*., 2003). Unlike countries such as the United States of America, where SAMM supports and accelerates the incorporation of genes in the germplasm of interest

(Miklas *et al*., 2006), in Mexico, only one variety released under this scheme is reported ('Dalia') (Acosta-Gallegos *et al*., 2014), with resistance to BCMV and BMCNV (common bean and black root mosaic virus or necrosis). Duncan *et al*. (2012) indicated; however, using a single SCAR is not enough to provide sufficient resistance to a pathogen, suggesting two or more SCARs under a scheme of resistance gene pyramidation (Anaya-López *et al*., 2015a, b).

The objective of this investigation was to determine the resistance or susceptibility to two important *Phaseolus* pathogens: common blight [*Xanthomonas axonopodis* pv. *phaseoli* (Smith)] and anthracnose [*Colletotrichum lindemutianum* Sacc. & Magn (anamorph *Glomerella lindemuthiana* Shear)] based on the identification of sequences characterized by amplified regions (SCARs).

Materials and methods

Extraction of DNA

The present work was carried out in the Plant Biotechnology Laboratory of the Center for Genomic Biotechnology of the National Polytechnic Institute (CBG-IPN) in Reynosa, Tamaulipas where 117 accessions of *P. coccineus* L. collected in the Carso Huasteco of Puebla subprovince were analyzed, more five accessions used as control group (Table 1), DNA extraction was carried out according to the protocol Doyle and Doyle (1987).

Table 1. Origin and number of samples of the populations of *Phaseolus* spp. studied.

Species	Number of accessions	Location	State
P. coccineus	38	Zacapoaxtla	Puebla
	3	Zacatlan	
	18	Tlatlauquitepec	
	7	Nauzontla	
	1	Teteles de Avila Castillo	
	4	Zoquiapan	
	1	Huauchinango	
	6	Chignahuapan	
	3	Ahuacatlan	
	2	Xochiapulco	
	14	Market of Zacapoaxtla	
	4	Market of Cuetzalan	
	6	Market of Tlatlauquitepec	
	6	Market of Ciudad Serdan	
	4	Atempan	
P. glabellus, *P. vulgaris* (Pinto Villa and Pinto Zapata) and *P. coccineus* (*P. coccineus* type and Blanco Tlaxcala)	5	Texcoco	State of Mexico

SCAR marker amplification

From the sequences reported by the bean improvement program (BIC, 2010), ten SCAR markers were selected, of which six detect gene sequences that confer resistance to common blight (SAP6, BAC6, SU91, LG5, R7313 and R4865), and four to anthracnose (SAS13, SBB14, SAB3 and SH18) in beans (Table 2). The amplifications were carried out using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems®) according to the indications cited for each of the SCAR markers (BIC, 2010).

Table 2. Information on SCAR markers evaluated in ayocote beans.

SCAR	Resistance gene	Pb$^{\Delta}$	Sequence	Reference
			SCARs for anthracnose	
SAS13	Co-4^2	950	F-CAC GGA CCG AAT AAG CCA CCA ACA R-CAC GGA CCG AGG ATA CAG TGA AAG	Young *et al*. (1998); Kelly *et al*. (2003)
SBB14	Co-4^2	1150	F-GTG GGA CCT GTT CAA GAA TAA TAC R-GTG GGA CCT GGG TAG TGT AGA AAT	Awale y Kelly (2001); Kelly *et al*. (2003)
SAB3	Co-5	400	F-TGG CGC ACA CAT AAG TTC TCA CGG R-TGG CGC ACA CCA TCA AAA AAG GTT	Vallejo y Kelly (2001)
SH18	Co-4	1100	F-CCA GAA GGA GCT GAT AGT ACT CCA CAA C R-GGT AGG CAC ACT GAT GAA TCT CAT GTT GGG	Awale y Kelly (2001); Kelly *et al*. (2003)
			SCARs for common blight	
SAP6	QTL in GL 10 (GN#1 sel. 27)	820	F-GTC ACG TCT CCT TAA TAG TA R-GTC ACG TCT CAA TAG GCA AA	Miklas *et al*. (2000)
BAC6	QTL in GL 10 (GN#1 sel. 27)	1250	F-TAG GCG GCG GCG CAC GTT TTG R-TAG GCG GCG GAA GTG GCG GTG	Jung *et al*. (1999)
SU91	QTL in GL 8 (XAN 159)	700	F-CCA CAT CGG TTA ACA TGA GT R-CCA CAT CGG TGT CAA CGT GA	Pedraza *et al*. (1997)
LG5	QTL in GL 6 (XAN 159)	900	F-GCA GGG TTC GAA GAC ACA CTG G R-GCA GGG TTC GCC CAA TAA CG	Yu *et al*. (2000)
R7313	QTL in GL 8 (OAC 88-1)	700	F-ATT GTT ATC GTC GAC ACG R-AAT ATT TCT GAT CAC ACG AG	Bai *et al*. (1997); Beattie *et al*. (1998)
R4865	QTL in GL 8 (OAC 88-1)	950	F-TCC AAA GCC ATT CTA GTT R-CAG CTA CTT TCA AAC TGG G	Bai *et al*. (1997); Beattie *et al*. (1998)

$^{\Delta}$pb= base pairs of the expected product; GL= linkage group.

Visualization and statistical analysis

The products of each amplification were stained with 1 µL of SYBR Gold® and separated on 1% agarose gel, at 80 V and 50 mA. Each gel was then visualized with UV light and photo-documented (KODAK Digital Science 1D; Rochester, NY, USA). The revision of gels allowed to construct a binary matrix where 1= presence and 0= absence of band (amplicon) and that included all the amplified SCARs in each genotype. The data analysis was carried out with the Statistica program

(StatSoft ™) version 7 (StatSoft Inc. 2004) and basically consisted of the calculation of frequencies, percentages and averages of occurrence of SCAR markers detected by location and type of marker generated.

Results and discussion

In this study, the SCARs with the highest amplification frequency were SAS13 and SBB14 with 89 and 74%, respectively, in the total accessions evaluated. The germplasm of Zacapoaxtla and Tlatlauquitepec, presented the detection frequencies of SCARs of resistance to anthracnose (34 of 38 accessions in Zacapoaxtla and 17 of 18 in Tlatlauquitepec for SAS13; 30 of 38 and 10 of 18 for SBB14), which suggests that these Accessions are candidates for use as parents in the genetic improvement of *Phaseolus*.

To date there were no reports of the identification of anthracnose resistant germplasm in *P. coccineus* L. by detecting SCARs such as SAS13 and SBB14, since these have only been used in *P. vulgaris*, but if SCARs of common blight resistance such as SAP6, BAC6 or SU91 (Ruiz-Salazar *et al*. 2016). Of the ten SCAR genomic sequences tested, only seven amplified PCR products in the ayocote bean germplasm evaluated.

The SCARs SAB3, SH18 and R7313 did not amplify in any accession. The SAS13 and SBB14 sequences were followed, in terms of higher amplification frequency by BAC6 (74%) and SU91 (42%), SCARs related to common blight resistance (Table 3). The locations with the highest number of accessions with SCARs for common blight correspond to Zacapoaxtla and Tlatlauquitepec (25 of 38 accessions and 11 of 18 accessions for BAC6; for SU91 17 of 38 accessions and 6 of 18 accessions, respectively) (Table 3). The highest proportion of SCAR markers in Zacapoaxtla and Tlatlauquitepec germplasm coincides with the case of common blight, coupled with the identification of SCARs associated with resistance to virosis (common mosaic virus and golden mosaic virus) and angular spot (*Phaeoisariopsis griseola*) (Ruiz-Salazar *et al*. 2016).

Table 3. SCAR amplification frequencies in ayocote beans from 16 locations in Puebla, Mexico.

Location	n	Anthracnose		Common blight					$\bar{X}$
		SAS13	SBB14	SAP6	BAC 6	SU91	LG5	R4865	
Zacapoaxtla	38	34	30	0	25	17	2	15	17.5
Zacatlan	3	2	3	0	0	0	3	1	1.2
Tlatlauquitepec	18	17	10	0	11	6	2	5	7.2
Nauzontla	7	6	5	0	5	2	3	4	3.5
Teteles de Avila Castillo	1	1	1	0	1	1	0	0	0.5
Zoquiapan	4	4	4	0	3	3	0	0	2
Huauchinango	1	1	1	0	1	1	1	1	0.8
Chignahuapan	6	6	5	0	3	1	0	0	2.1
Ahuacatlan	3	3	1	0	3	1	0	1	1.2

Location	n	Anthracnose		Common blight					$\bar{X}$
		SAS13	SBB14	SAP6	BAC 6	SU91	LG5	R4865	
Xochiapulco	2	2	1	0	1	1	0	0	0.7
Market of Zacapoaxtla	14	14	13	1	12	5	2	6	7.5
Market of Cuetzalán	4	1	4	0	2	2	0	2	1.5
Market of Tlatlauquitepec	6	6	5	0	6	5	0	2	3.4
M. Serdán	6	6	2	0	3	0	0	1	1.7
Control	5	3	3	0	4	3	1	0	2
Atempan	4	3	3	0	2	4	0	1	1.8
Total	122	109	91	1	82	52	14	37	-

n= number of accessions by location; $\bar{X}$= average SCAR amplified by location.

Several accessions were detected that have at least five resistance SCARs, which highlights the importance of these accessions for genetic improvement in common beans. This germplasm is more likely to be used as a source of multiple resistance to diseases in *Phaseolus* (Rodríguez-Guerra *et al*., 2006).

In spite of the high detection frequencies of SCARs in ayocotes of specific locations of the Carso Huasteco de Puebla, it should be considered that in addition to the ratification of the effective resistance in field conditions, said resistance may sometimes behave unstable, due to the high specificity of the interaction between the genotypes of the pathogen and the host (Rodríguez-Miranda and Rosas-Sotomayor, 2010).

However, it is notable to identify germplasm that has a high frequency of SCARs associated with resistance to two important diseases in central Mexico, which suggests that high selection pressures occur due to favorable environmental conditions for the higher incidence of diseases and inoculum. primary and greater pathogenicity (Francisco-Francisco *et al*., 2013; Rodríguez-Guerra *et al*., 2006) have forced farmers in the region to empirically select for disease resistance and, consequently, have achieved an increase in the frequency of resistance genes in them.

Mexico is considered the center of origin and diversity of beans and also the pathogens that cause its diseases exhibit wide pathogenic diversity, such as anthracnose (Rodríguez-Guerra *et al*., 2006) or common blight (Prudencio-Sains *et al*., 2008), unfortunately, in the case of common blight only the existence of such variability is assumed, but there is no updated information on its quantity and distribution in the beans regions of Mexico (Navarrete and Acosta, 2000; Prudencio-Sains *et al*., 2008).

In the case of anthracnose there is a more complete perspective, since more than 100 patotypes are recorded in the world, with at least 54 presents in Mexico (Rodríguez-Guerra *et al*., 2006). The wide genetic variability of the causative agents of both diseases hinders the development of resistant varieties. Also, it is common to observe that varieties with resistance in one region are susceptible in another (Acosta-Gallegos *et al*., 2013).

Sources of genetic resistance to common blight have been identified in *P. vulgaris, P. acutifolius* and *P. coccineus*. However, most resistance genes are inherited as quantitative character loci (QTL) and therefore exhibit variation in their genetic effects because they are influenced by the environment (Kelly *et al*., 2003; Miklas *et al*., 2006; Duncan *et al*., 2007). For anthracnose, a group of 21 genotypes with diverse characteristics were identified in terms of their genetic base, grain color, growth habit and response to different anthracnose patotypes in Mexico (González-Chavira *et al*., 2004).

Among them, Pinto Villa and Bayo Mecentral 90 stand out, commercially released with resistance to anthracnose and which have been cultivated in different regions and conditions in Mexico (Rodríguez-Guerra *et al*., 2006). Most studies have focused on the identification of molecular markers linked to genes with major (monogenic) effects in anthracnose (Miklas, 2002). For example, SCAR SAS 13 for anthracnose is linked to the Co-4^2 resistance gene that is located in linkage group 8, while SU91 is a QTL genomic region associated with common blight resistance and is also located in the group of linkage 8.

Then both markers could be more effective in improving by being more likely to co-segregate together in segregating bean populations. Emmalea and Kelly (2004) detected resistance to 33 of 34 *C. lindemuthianum* patotypes from nine countries in the Americas with the use of SCAR SAS13, because Co-4^2 was first detected in the differential variety Tu and also, in G-2333 The latter, also called 'Colorado de Teopisca' is originally from Chiapas, Mexico, was resistant to 380 anthracnose isolates from eleven Latin American countries because it has the Co-4^2, Co-5 and Co-7 genes (Pastor-Corrales *et al*., 1994; Vallejo and Kelly, 2009).

The SAP6, SU91 and LG5 SCARs, despite being located in different linkage groups, can be applied in the search for resistance to common blight. Ibarra-Perez and Kelly (2005) associated SU91 with resistance in the field, demonstrating the advantages that SAMM could provide to detect resistance to diseases in *Phaseolus*. SAMM, in the development of improved germplasm with disease resistance, has been successful because it allows indirect selection of desirable characteristics compared to conventional techniques.

Park and Yu (2004) identified SCAR SAS13 (linked to the Co-4^2, 0.39 cM gene) in the H4514 Navy, H4628 Dark Red Kidney, H4642 Navy and H4836 Dark Red Kidney bean lines, obtaining an increase in anthracnose resistance 43% (H4642 Navy), compared to H4628 (29%), H4514 (7%) and H4836 (7%), which were susceptible. Mukeshimana and Kelly (2003) studied Rwanda common bean germplasm and only detected SCAR SH18 on the RWV167 line.

Meanwhile, Miklas (2002) recorded low levels of resistance to common blight in ayocote beans, but high levels of resistance in tepary beans (*P. acutifolius* A. Gray) with SCAR R7313, which did not amplify in the germplasm of this job. According to Márquez *et al*. (2007), said SCAR has only been detected in the XAN159 line and in Tepary beans. Given the economic importance of common blight, lines that have broad spectra of resistance to said pathogen should be developed in the short term.

Accessions with up to five or six SCAR sequences include early and late accessions to flowering and maturity, as well as various testa colors, pod and seed size, so that no relationship was found between increased presence of SCARs with any particular morphological characteristic of the ayocote bean germplasm of Carso Huasteco (Prudencio-Sains *et al*., 2008; Ruiz-Salazar *et al*., 2016). Bitocchi *et al*. (2017) suggest that domestication acts on increasing functional diversity in particular loci, which probably control traits related to expansion and adaptation to new agroecological conditions (Table 4).

Table 4. Characteristics of ayocote bean accessions with five or six genomic sequences that confer resistance to anthracnose and common blight.

Accession	Location	Days to flowering	Days to maturity	Seed color	Weight 10 seeds (g)	Pod length (cm)
8 449	Tlatlauquitepec	52	111	White	5.45	9.49
8 210		77	131	Beige	4.7	-
8 213		54	126	Dark violet	5.1	8.3
8 446		39	101	White, lilac, violet, black	7.46	9.66
8 506	Zacapoaxtla	62	115	Beige	4.79	10.05
8 762		46	139	Violet	5.6	8.4
8 452		50	120	Beige, violet, white and lilac	7.38	8.9
8 104		46	126	Yellow mustard	5.5	10.4
8 193	Cd. Serdan	78	131	Beige	2.8	8.32
9 237	Atempan	69	120	Dark violet	4	7.81

In the medium and long term, there are several options for the genetic improvement of beans in Mexico, assisted by Biotechnology. In principle, the strategy of handling two or more SCARs must be maintained at the same time or, better yet, emphasize resistance gene pyramid strategies (Anaya-López *et al*., 2015a, b). Also, it should not be overlooked that the efficiency of SAMM can be affected by the degree of dominance of the marker, the type of linkage between molecular markers and traits of interest, the need to develop progeny tests to corroborate resistance and changes in specificity of markers (commercial types of grain and/or genetic collections, for example) (Bello *et al*., 2014).

Finally, biotechnology progresses day by day, new molecular marker strategies are developed, which in the case of Mexico must be evaluated and validated. It is work with new generation sequencing strategies that take advantage of different types of polymorphisms, the combination of segregating analysis and their transcriptional profiles, the analysis of '*in silico*' segregators and the structuring of high performance platforms for integral genomic analysis (BeanCAP , Langebio) (Bello *et al*., 2014; Bolger *et al*., 2014; Mukeshimana *et al*., 2014; Viteri *et al*., 2014; Saburido-Álvarez and Herrera-Estrella, 2015).

Conclusions

The ayocote bean germplasm of Carso Huasteco de Puebla analyzed with SCAR type markers presented regions associated with genes of resistance to common blight (*X. axonopodis* pv. *phaseoli*) and anthracnose (*C. lindemuthianum*), where the accessions of Zacapoaxtla and Tlatlauquitepec are those with the highest frequency of these markers.

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