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Identification of black bean (*Phaseolus vulgaris* L.) genotypes resistant to anthracnose and rust for Veracruz and Chiapas, Mexico

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ABSTRACT

Objective: to determine the reaction of 53 lines and three varieties of black bean (*Phaseolus vulgaris* L.) to inoculation with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum*, to identify genotypes resistant to rust and anthracnose.

Design/methodology/approach: 10 seedlings of each genotype were inoculated in the greenhouse with a suspension of *U. appendiculatus* uredospores and another 10 with a suspension of *C. lindemuthianum* conidia. At 14 days after inoculation, the reaction of the genotypes to rust was evaluated with a severity scale of 1 to 6, and to anthracnose, with a scale of 0 to 4. The data were analyzed in a completely randomized design with 10 replications per treatment and LSD at 0.05 was applied for the separation of averages.

Results: 41 genotypes showed a hypersensitivity reaction to rust, of which 25 had a reaction value of 2.0, statistically lower than those of controls. In turn, 45 genotypes were resistant to anthracnose, of which 18 had a value of 1.0, statistically similar to that of Negro Jamapa and lower than those of the rest of the genotypes.

Study limitations/implications: due to the diversity of races of both pathogens, the genotypes were inoculated with monopustular isolates of the principal races of *U. appendiculatus* and with monosporic cultures of *C. lindemuthianum*, which occur in the bean crops of Veracruz and Chiapas.

Findings/conclusions: 25 lines resistant to rust and 18 to anthracnose were identified, which stood out for presenting the least damage from these diseases.

Keywords: fungal diseases, genetic resistance.

INTRODUCTION

In the states of Veracruz and Chiapas, Mexico, most farmers grow black bean cultivars (*Phaseolus vulgaris* L.), since it is the one of highest commercial demand (Rodríguez-Licea *et al*., 2010). Sowing landrace genotypes predominates, of unknown origin, low yield and susceptible to fungal diseases, such as rust [*Uromyces appendiculatus* var. *appendiculatus* (Pers.) Unger] and anthracnose [*Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams. Scrib.] (López *et al*., 2012a; Ugalde-Acosta *et al*., 2014), which can cause losses of 20 to 100% of the grain yield (Becerra-Leor *et al*., 1996; López *et al*., 2006). Improved varieties were used, such as Negro Jamapa, released by the INIA (presently INIFAP) almost six decades ago, which is susceptible to rust (Becerra-Leor *et al*., 1996; Rosales *et al*., 2004). Since these diseases are a factor that can be

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limiting to obtain good productivity in the bean crop, studies have been carried out about their chemical control (Becerra *et al*., 1994; 1996; Tosquy *et al*., 2013); however, it has been determined that applying fungicides increases production costs considerably, which is why in order to avoid or reduce the damage from pathogenic organisms, the most viable and economic alternative is sowing varieties with resistance to these diseases and with good adaptation to the environments where bean is sown in Veracruz and Chiapas. In southeastern Mexico, the assessment of reaction of genotypes (lines and varieties) of black beans to diseases is generally performed in field assays, under conditions of natural incidence of pathogens; this process is slow because there is no certainty that adequate conditions will take place for the development of diseases, or else, they can occur in a delayed way and with low severity of infection, in addition to not guaranteeing that the varieties that are generated are resistant or tolerant to the different species of rust and anthracnose that prevail in commercial crops in the states of Veracruz and Chiapas. To accurately evaluate the reaction of the genotypes to the incidence of rust and anthracnose and to have more certainty in identifying breeding lines and generating bean varieties, resistant or tolerant to a larger number of races of these pathogens, it is required for them to be inoculated with spores or monopustular isolates of the principal races of *U. appendiculatus* and with monosporic cultures of *C. lindemuthianum*. These are present in commercial crops in of both states. In the INIFAP's Bean Program for southeastern Mexico, sampling was done between July and December 2018 in the different bean producing zones of the states of Veracruz and Chiapas, where a total of 46 samples of leaves infected with rust and 49 leaves and pods infected with anthracnose were collected. The samples were sent to the Plant Pathology Laboratory of the INIFAP Experimental "Centro de Chiapas", to carry out monopustural isolates of *U. appendiculatus* and develop monosporic cultures of *C. lindemuthianum*, in order to have inoculum of different races available of both pathogens. The objective of this study was to determine the reaction of 53 recombinant lines and three tropical varieties of black bean to artificial inoculation with these two pathogenic agents, in order to identify resistant or tolerant genotypes to rust and anthracnosis.

MATERIALS AND METHODS

To conserve and increase the spores of *U. appendiculatus*, pustules were selected from all the samples received; they were labeled, dried in a botanical press, and preserved in coin paper envelopes. In total 20 samples were attained, each one with sufficient pustules with spores of *U. appendiculatus*. To isolate *C. lindemuthianum*, the pods with symptoms of anthracnose from different samples received were disinfected superficially with sodium hypochlorite at 1.5%; then, the excess hypochlorite was washed with sterilized distilled water and the pods were placed in a moisture chamber for 48 h, to induce sporulation of the fungus. Later, the spores were transferred to three cultivation media: water-agar (WA), potato-dextrose-agar (PDA) and Mathur (dextrose, heptahydrated magnesium sulfate, monopotassium, neopeptone, yeast extract and agar), which allowed the growth and development most fungi (Pastor-Corrales *et al*., 1995; Balardin *et al*., 1997; Castellanos *et al*., 2011). In turn, isolates were made from the leaves infected with this disease of the lesions present in leaf veins; they were superficially disinfected with sodium hypochlorite at 1.5%, the excess hypochlorite was washed with sterilized distilled water, and the isolates were placed in Petri dishes with the growth media mentioned before, until growth of the colonies was observed. The *C. lindemuthianum* isolate was verified through observation of type of spores and colony in the microscope.

Once identified, monosporic cultures were grown through the dilution technique to obtain pure strains (Garrido-Ramírez and Romero-Cova, 1989). For this purpose, an initial spore suspension was prepared in 10 mL of distilled water, from which 1 mL was taken and diluted in 9 mL of distilled water (dilution 10^{-1}). Serial decimal dilutions were prepared from this, taking 1 mL of the 10^{-1} dilution and transferring it into 9 mL of sterilized distilled water, and thus successively, to obtain dilutions of 10^{-2}, 10^{-3}, 10^{-4}, until 10^{-9}. From each dilution, 1 mL was taken and placed in a Petri dish with PDA. Isolated spores were located with the help of a microscope, and when they began to germinate, they were transferred to Petri dishes with PDA, which were incubated at 18 °C from 48 to 72 h, and later transferences were carried out to tubes with PDA; once an adequate growth of the fungus was obtained, sterilized mineral oil was added for their conservation at low temperature. In total, 40 monosporic isolates of *C. lindemuthianum* were developed, which were kept in tubes with PDA medium in an inclined plane, covered with sterilized mineral oil, in refrigeration at 4 °C.

On May 28, 2019, 20 seeds of each one of the 53 lines and three varieties of black bean (used as control) were sown in Styrofoam cups with peat-moss:agrolite (1:1). Once the seedlings emerged, they were kept in a greenhouse. On June 7, 10 plants of each genotype were inoculated with *U. appendiculatus* and the other 10 with *C. lindemuthianum*. For *U. appendiculatus*, the inoculation was carried out with a mixture of uredospores, made up of individual pustules selected from the samples collected, from which a suspension was prepared at a concentration of 2×10^4 uredospores/mL, to which 40 μL/L of Tween 20 (detergent) was added, for a uniform distribution of uredospores. The suspension was dispersed at the bean growth stage V2 (primary leaves), using the Stavely technique (1983), for which a plastic tube of 5 cm length and 12 mm diameter was placed in the manual spraying exit, to direct the suspension to a point on the leaf. The plants inoculated were incubated in a moisture chamber with temperature between 20 and 22 °C for 16 h and later transferred to the greenhouse. The The genotype reaction to rust was evaluated 14 d after inoculation, with the scale of 1 to 6 described by Stavely *et al*. (1983), which is based on the type of pustule, where 1=without visible symptoms of the disease (immune), 2=chlorotic spots (from the loss of chlorophyll) that are necrotized later, without sporulation (hypersensitivity reaction), 3=pustules with sporulation smaller than 300 μm in diameter (resistant), 4=pustules with sporulation of 300 to 500 μm in diameter, sometimes surrounded by chlorotic halos (moderately resistant), 5=pustules with sporulation of 500 to 800 μm in diameter, frequently surrounded by chlorotic halos (moderately susceptible), and 6=pustules with sporulation larger than 800 μm, surrounded by chlorotic halos (susceptible).

For *C. lindemuthianum*, inoculation was carried out at stage V2 (completely open primary leaves), using a manual atomizer, with which a suspension of spores or conidia was sprayed (developed from monosporic isolates of *C. lindemuthianum*) at a concentration of 1.2×10^6 conidia/mL (Castellanos *et al*., 2011). The inoculated plants were kept in a moisture chamber during 48 h and later placed later in greenhouse tables. The genotypes' reaction to anthracnose was evaluated 14 d after inoculation, using the 0 to 4 scale proposed by Garrido-Ramírez and Romero-Cova (1989), where 0=without visible symptoms of the disease, 1=small necrotic lesions on the underside venation of the leaves, 2=medium necrotic lesions on the underside venation of the leaves, 3=large lesions with sporulation, and 4=large disperse lesions with sporulation and plant death. Plants from genotypes with 0, 1 and 2 values considered resistant, while those with 3 and 4 were classified as susceptible. The reaction values of the genotypes to the diseases were subjected to an analysis of in a completely randomized experimental design with 10 replications per treatment. For the separation of averages, the test based on the Least Significant Difference at 5% probability of error (LSD, α=0.05) was applied.

RESULTS AND DISCUSSION

Highly significant differences were detected (<0.01) for both reactions, to *U. appendiculatus* and to *C. lindemuthianum* among treatments (Table 1), which indicates that the bean genotypes showed a different reaction to the inoculation with each one of the pathogens.

Table 1. Square means and significance detected in the reaction variables of bean genotypes to rust and anthracnosis.

Source variation	GL	Reaction to rust	Reaction to anthracnose
Treatments	55	2.767139 **	4.450617 **
Error	504	0.130556	0.134325
Total	559		
CV (%)		15.35	22.14

GL=degrees of freedom; CV=Coefficient of variation; **Highly significant (p≤0.01).

When evaluating the reaction to rust, it was determined that 41 genotypes presented only small necrotic spots without sporulation (grades <2.4), that is, showing a hypersensitivity reaction (Stavely *et al*., 1983). From these, 25 lines (13 derived from the Papaloapan/SEN 46 cross, seven from the Negro Citlali/XRAV-187-3 cross, three from the Jamapa Plus/XRAV-187-3 cross and the ELS-15-55 line) stood out because they presented the lowest damages, with a reaction value of 2.0, statistically similar to the other 11 lines and lower than the rest of the genotypes, including the control varieties Negro, Negro Jamapa and Verdín (Table 2). The last two varieties were moderately susceptible to rust, since they had reaction values higher than 4.0. The susceptibility to rust of the three control varieties has already been documented in other assessment studies (Becerra-Leor *et al*., 1996; Tosquy *et al*., 2012; 2016).

In relation to anthracnose, 45 genotypes showed resistance (Garrido-Ramírez and Romero-Cova, 1989), with 18 recombinant lines standing out (nine from the

Table 2. Reaction of black bean genotypes (*Phaseolus vulgaris* L.) to inoculation with *Uromyces appendiculatus* var. *appendiculatus* and *Colletotrichum lindemuthianum*, causes of rust and anthracnose, respectively.

genotype	rust (scale 1 a 6)†	Classification	anthracnose (scale 0 a 4)‡	Classification
Papaloapan/SEN 46-1-7	2.0 h	RH	1.0 g	R
Papaloapan/SEN 46-1-8	2.0 h	RH	2.6 b	S
Papaloapan/SEN 46-1-10	2.5 ef	R	1.7 cd	R
Papaloapan/SEN 46-2-1	2.3 fgh	RH	2.8	S
Papaloapan/SEN 46-2-2	2.7 de	R	2.6	S
Papaloapan/SEN 46-2-3	2.3 fgh	RH	1.4 def	R
Papaloapan/SEN 46-2-4	3.0 cd	R	2.0 c	R
Papaloapan/SEN 46-2-5	2.4 efg	RH	1.0 g	R
Papaloapan/SEN 46-2-6	2.0 h	RH	1.0 g	R
Papaloapan/SEN 46-2-7	2.4 efg	RH	1.7 cd	R
Papaloapan/SEN 46-2-11	2.3 fgh	RH	1.6 de	R
Papaloapan/SEN 46-3-2	2.5 ef	R	1.0 g	R
Papaloapan/SEN 46-3-5	2.3 fgh	RH	1.0 g	R
Papaloapan/SEN 46-3-7	2.7 de	R	1.4 def	R
Papaloapan/SEN 46-4-5	2.3 fgh	RH	1.5 def	R
Papaloapan/SEN 46-4-8	2.0 h	RH	1.3 efg	R
Papaloapan/SEN 46-4-9	2.1 gh	RH	1.3 efg	R
Papaloapan/SEN 46-4-10	2.3 fgh	RH	2.6 b	S
Papaloapan/SEN 46-6-1	2.4 efg	RH	1.4 def	R
Papaloapan/SEN 46-6-2	2.5 ef	R	2.0 c	R
Papaloapan/SEN 46-6-3	2.0 h	RH	2.7 b	S
Papaloapan/SEN 46-6-4	2.0 h	RH	2.0 c	R
Papaloapan/SEN 46-6-5	2.3 fgh	RH	1.0 g	R
Papaloapan/SEN 46-6-6	2.0 h	RH	1.7 cd	R
Papaloapan/SEN 46-7-1	2.4 efg	RH	2.8 b	S
Papaloapan/SEN 46-7-6	2.0 h	RH	1.4 def	R
Papaloapan/SEN 46-7-7	2.0 h	RH	1.0 g	R
Papaloapan/SEN 46-7-8	2.0 h	RH	1.3 efg	R
Papaloapan/SEN 46-7-9	2.0 h	RH	1.4 def	R
Papaloapan/SEN 46-7-10	2.3 fgh	RH	1.0 g	R
Papaloapan/SEN 46-7-11	2.4 efg	RH	1.7 cd	R
Papaloapan/SEN 46 -7-12	2.0 h	RH	1.0 g	R
Papaloapan/SEN 46-7-13	3.0 cd	R	1.4 def	R
Negro Citlali/XRAV-187-3-1-2	2.5 ef	R	1.0 g	R
Negro Citlali/XRAV-187-3-1-5	2.0 h	RH	1.0 g	R
Negro Citlali/XRAV-187-3-1-6	2.0 h	RH	1.0 g	R
Negro Citlali/XRAV-187-3-1-8	2.0 h	RH	2.0 c	R
Negro Citlali/XRAV-187-3-1-9	2.0 h	RH	1.7 cd	R
Negro Citlali/XRAV-187-3-2-2	2.3 fgh	RH	1.0 g	R
Negro Citlali/XRAV-187-3-2-4	2.5 ef	R	1.7 cd	R
Negro Citlali/XRAV-187-3-2-5	2.0 h	HR	2.7 b	S
Negro Citlali/XRAV-187-3-7-2	3.1 c	R	1.0 g	R
Negro Citlali/XRAV-187-3-14-6	2.0 h	RH	2.0 c	R

Table 2. Continuation.

genotype	rust (scale 1 a 6)†	Classification	anthracnose (scale 0 a 4)‡	Classification
Negro Citlali/XRAV-187-3-14-7	2.3 fgh	RH	1.6 de	R
Negro Citlali/XRAV-187-3-16-7	2.0 h	RH	2.7 b	S
Jamapa Plus/XRAV-187-3-1-2	2.0 h	RH	1.0 g	R
J. Plus/XRAV-187-3-1-8 (Rubí)	2.0 h	RH	2.0 c	R
Jamapa Plus/XRAV-187-3-4-1	2.0 h	RH	1.0 g	R
Jamapa Plus/XRAV-187-3-4-4	2.0 h	RH	1.0 g	R
Papaloapan/SEN 46-5-5	2.0 h	RH	2.6	S
NGO 17-99	2.7 de	R	3.2 a	S
SCN-2	3.8 b	MR	2.0 c	R
ELS-15-55	2.0 h	RH	1.0 g	R
Negro Medellín (TR)	2.5 ef	R	3.4 a	S
Negro Jamapa (TR)	4.2 a	MS	1.2 fg	R
Verdín (TR)	4.5 a	MS	1.6 de	R
Comparador DMS (0.05)	0.3167		0.3213	

TR=Regional control. †Values recorded according to the severity scale of rust described by Stavely *et al.* (1983). ‡Values recorded according to the severity scale of anthracnose proposed by Garrido-Ramírez and Romero-Cova (1989). Genotypes with similar letters in each column are statistically equal according to DMS, α=0.05. R=Resistant. S=Susceptible. RH=Hypersensitivity reaction. MR=Moderately resistant. MS=Moderately susceptible.

Papaloapan/SEN 46 cross, five from the Negro Citlali/XRAV-187-3 cross, three from the Jamapa Plus/XRAV-187-3 cross and the elite line, ELS-15-55), since they minimum symptoms or very small necrotic lesions in the leaf venation, with a reaction value of 1.0, which was statistically similar to other three breeding lines and to the Negro Jamapa variety and lower than the rest of the genotypes; that is, they showed lower symptoms or less damage from this disease. At the same time, the Negro Medellín variety and NGO-17-99 line showed susceptibility to anthracnose, with higher reaction values than 3.0, which were statistically similar to those of nine other recombinant lines (Table 2). In the central mountainous zone of central Veracruz, these genotypes had an intermediate reaction to anthracnose (López *et al*., 2012b; Tosquy *et al*., 2012), while the Verdín variety, freed by INIFAP in 2015 for the states of Veracruz and Chiapas, has shown resistance to this disease (Tosquy *et al*., 2016).

Based on these results, 11 recombinant lines were selected because of their resistance to anthracnose and rust (five from the Papaloapan/SEN-46 cross, three form the Negro Citlali/XRAV-187-3 cross and three from the Jamapa Plus/XRAV-187-3 cross), and also because of their high grain yield and lower interaction with the environment, defined in previous studies of genotype evaluation (Tosquy *et al*., 2019). These are currently being evaluated under field conditions in Veracruz and Chiapas, in environments that favor the incidence of these diseases, to identify the most outstanding ones due to their high yield, wide adaptation and disease resistance, which in the short term can be released as improved varieties for commercial use.

CONCLUSIONS

Forty-one (41) black bean genotypes were identified with hypersensitivity reaction to rust and 45 resistant to anthracnose, of which 25 recombinant lines stood out in the first group and 18 in the second, since they present lower damage from these fungal diseases. Based on the results, eleven recombinant lines were selected that will follow the process of evaluation and selection for disease reaction field conditions, to release one or some of them as high productivity commercial varieties, resistant to rust and anthracnose, and with high adaptation to the environment.

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