CROP PRODUCTION AND MANAGEMENT
https://doi.org/10.1590/1678-4499.20220225 Article

Differential responses of *Phaseolus* spp. against Black node disease (*Boeremia noackiana*)

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Received: Nov. 14, 2022 | **Accepted:** Feb. 14, 2023

Section Editor: Robson Di Piero

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How to cite: Ligarreto, G., Garzón Gutiérrez, L. N. and Pimentel Ladino, C. C. (2023). Differential responses of *Phaseolus* spp. against Black node disease (*Boeremia noackiana*). Bragantia, 82, e20220225. https://doi.org/10.1590/1678-4499.20220225

ABSTRACT: Black node disease is one of the most limiting biotic stresses affecting bean production in the Andean countries. The objective of the study was to evaluate the degree of resistance to *Boeremia noackiana* against genotypes of *Phaseolus vulgaris*, *Phaseolus polyanthus*, and their interspecific crosses using two disease evaluation methods (pathogenicity test in the greenhouse and *in vitro*) to contribute the generation knowledge towards the use of durable resistance. Black node disease severity was assessed using a descriptive scale and the leaf detached method under greenhouse and *in-vitro* conditions. The results of the study confirmed the isolate Ascochyta ASC 001 pathogenicity, allowing the different genotypes to be discriminated. A contrasting resistance response was confirmed for the two methodologies used to estimate severity. Accession G35575 (*P. polyanthus*) was the most resistant whereas NCB 226 (*P. vulgaris*) was the most susceptible. Regional cultivars Simijaca and Cabrera, together with Cargamanto Blanco and Bacata, showed fluctuations in their reaction to the disease from intermediate to susceptible. Resistance was confirmed in the interspecific crosses of the ASC series, which can be an alternative in breeding programs for durable resistance in common beans. The percent leaf area affected was identified as a variable that can be easily assessed, and the processing and analysis of digital images in vitro avoided the use of destructive sampling. This technique is a fast, useful, and economical tool for this type of studies, as it provides the possibility of accelerating the selection of genotypes with resistance.

Key words: *Boeremia noackiana*, *Phaseolus vulgaris*, *Phaseolus polyanthus*, plant breeding, interspecific hybrids.

INTRODUCTION

Black node disease is a severe fungal alteration affecting beans cultivated in regions with cold to moderate temperatures (15–25°C), continuous rainfall, and high relative humidity (80–100%). In America, Black node disease is commonly found in cold climate regions located at more than 1,500 m above sea level. In Colombia, it is especially prevalent in the Departments of Antioquia, Boyacá, Cauca, Cundinamarca, and Nariño and many areas of Ecuador and Peru as well (Blair et al. 2011). However, the disease occurs in a wide range of crops under field conditions with an incidence of up to 100% at advanced degrees of severity (Garzón et al. 2011).

The sexual phase of *Boeremia* has not been reported for any of the species related to this disease on common bean (*Phaseolus vulgaris*) (Aveskamp et al. 2010). The symptoms correspond to dark-brown lesions, that developed concentric zones 10 to 30 mm in diameter and contained small black pycnidia which appear initially on leaves but could be dispersed on other organs such as peduncles, petiole, nodes, and pods; after a while, the lesions turn brown since necrosis occurs in the tissue (Schwartz 1982, Bardas et al. 2008). This is a seed-borne disease, although crop debris could act as inoculum from one crop to the next (Schwartz 1982). Among the control measures are the use of innocuous seed, elimination of crop

debris and host weeds, as well as the crop rotation and chemical controls based in fungicides (Holm et al. 1977, Schwartz 1982). However, the most efficient strategy would be the use of cultivars resistant to the disease, with the introgression of genes from *Phaseolus polyanthus* and *Phaseolus coccineus* (Schwartz 1982).

The biggest problem to be considered in plant breeding programs is that no source of resistance has been identified within the *P. vulgaris* species (primary gene pool). Therefore, it is necessary to examine the secondary gene pool, since *P. polyanthus* possesses such resistance. This would allow us to get one step closer towards the obtention of resistant cultivars with commercial characteristics (Gill and Myek 2008).

In beans, Black node disease caused by *Boeremia* spp. was considered as of little agronomic interest until 1991, when studies by Centro Internacional de Agricultura Tropical (CIAT) (Singh et al. 1991) classified it as a disease of difficult management. Additionally, efforts to develop commercial *P. vulgaris* materials resistant to the disease have been limited by the absence of genotypes with this trait (Blair et al. 2014, Raatz and Studer 2016). Black node disease is also one of the most limiting diseases for the cultivation of beans in Colombia. Efficient control of the disease has been difficult due to the continuous use of susceptible varieties. This has resulted in a decrease in yields and the development of variants of the pathogen (Gallego et al. 2010, Blair et al. 2014).

Schmit and Baudoin (1992) reported 12 *P. vulgaris* genotypes with an intermediate level of resistance to Black node disease after inoculation at the phenological stages of pre-flowering, flowering, and pod filling. However, when these genotypes were evaluated against Colombian isolates, they were found to be susceptible (Blair et al. 2011, Blair et al. 2014).

Phaseolus polyanthus accessions were evaluated by CIAT using a descriptive severity scale (Schoonhoven and Pastor-Corrales 1987), in which:

- 1 = plants without visible symptoms;
- 3 = plants with 2% of affected leaf area;
- 5 = plants with several lesions that cover up to 5% of the leaf area with sporulation;
- 7 = plants with damage close to 10% of the leaf area with sporulation and also affecting the pods, branches and stems;
- 9 = plants with large lesions and sporulation, which cover about 25% or more of the leaf area, causing premature defoliation and large leaf openings, as well as necrosis in other tissues.

This group is composed of accessions G 35182 (Guatemala 1076), G 35336, G 35337, G 35359, G 35372, G 35373, G 35415, G 35441, G 35461, G 35473, and G 35481. These accessions originated in Guatemala, Colombia, and Mexico. However, CIAT reports that attempts to confer resistance to *P. vulgaris* have had little success mainly because interspecific crosses have been recalcitrant and because of susceptibility in specific environments (Beebe and Pastor-Corrales 1991).

In Colombia, 23 lines with intermediate resistance were identified from four populations of interspecific beans against four isolates of *Boeremia noackiana* from Cauca, Antioquia, Cundinamarca, and Santander. These tests included accessions from CIAT's bean gene bank, commercial varieties such as Bola Roja and Cargamanto Rojo, FEIN and ASIN populations (derived from interspecific crosses between *P. vulgaris* × *P. polyanthus*), ASR populations (sources identified at the International Phaseolus Information System-APHIS) and ASC populations (ASC 160 and ASC 162) from the Andean and Mesoamerican bean breeding programs of CIAT (Garzón et al. 2011).

Diagrammatic scales are the most used tools to determine the reaction to the disease according to the percentage of leaf area affected (Pastor-Corrales et al. 1995). However, the evaluator's capacity and experience are sources of variation in this type of scale. Nevertheless, in the last two decades evaluation methodologies in beans have been explored with reliable results. One of these is known as the detached leaf technique, used in studies with pathogens such as *Colletotrichum lindemuthianum* (Sacc. and Magn.) Lams. Scrib., *Phaeoisariopsis griseola* (Sacc.) Ferr, *Sclerotinia sclerotiorum* (Lib.) de Bary., and *Macrophomina phaseolina* (Tassi) Goid. (Mendoza et al. 2001, Bañuelos-Balandrán and Mayek-Pérez 2008). Also, as part of this same bean project, the use of the logarithmic interval diagrammatic scale was developed by Miranda et al. (2021).

Therefore, the objective of this study was to evaluate the degree of resistance to *B. noackiana* in 11 genotypes that include Andean common bean (*P. vulgaris*), Mesoamerican secondary gene pool (*P. polyanthus*), and interspecific crosses (*P. vulgaris* × *P. polyanthus*) using two-resistance assessment techniques, to contribute to the knowledge of gene donor sources for the development of bean cultivars with durable resistance.

MATERIALS AND METHODS

Plant genotypes

The germplasm used is described in Table 1; genotypes classified as resistant and susceptible were obtained from CIAT's Bean Breeding Program, Colombia. The reaction to the disease caused by *B. noackiana*, with values of 1 for leaves and 2 for pods, was classified as resistant according to Schoonhoven and Pastor-Corrales (1987). Genotypes ASC (*P. vulgaris* × *P. polyanthus*) and G35575 (*P. polyanthus*) were the sources of resistance, and Cal 96 and NCB 226 (*P. vulgaris*) were the susceptible controls with disease reaction values between 7 and 8. Improved cultivars for resistance to anthracnose Bacata (shrubby habit) and Sutagao (climbing habit), Simijaca and Cabrera (regional cultivars with red grains), and Cargamanto Blanco (Cblanco) were obtained from the Legume Program at Universidad Nacional de Colombia, and selected for their characteristics of large grain, color, and shape with commercial acceptance.

Table 1. Genotypes used in the different pathogenicity tests under greenhouse and *in-vitro* conditions.

Species	Genotype	Type of grain	Source program
Phaseolus vulgaris × *Phaseolus polyanthus*	ASC 144	Red speckled	CIAT[1]
	ASC 160	Pinto	CIAT
	ASC 162	Dark red	CIAT
Phaseolus polyanthus	G35575 *	Non-commercial	CIAT
Phaseolus vulgaris	Cal 96 *	Red speckled	CIAT
	NCB 226	Black	CIAT
	Bacata	Red kidney	UNC
	Cabrera	Red round shape	UNC[2]
	Cargamanto Blanco (Cblanco)	Cranberry	UNC
	Simijaca	Red round shape	UNC
	Sutagao	Red round shape	UNC

*Genotypes not evaluated under *in-vitro* conditions; [1]Bean Breeding Programs of Centro Internacional de Agricultura Tropical (CIAT), Colombia; [2]Legume Program at Universidad Nacional de Colombia (UNC), Bogotá.

Selection of fungal isolates

Isolate ASC 001 was selected from a Colombian collection consisting of 611 monosporic or monoconidial isolates obtained from a CIAT collection. The origin of the ASC 001 isolate was Popayan (Cauca, Colombia), collected in 1996, without registering the bean cultivar of the collection, and it was chosen because it is one of the most used in previous studies and because of its high virulence (Blair et al. 2011, Garzón et al. 2011).

Pathogenicity test in the greenhouse

The pathogenicity test was carried out during the second half of 2018 on 11 genotypes of the genus *Phaseolus* under greenhouse conditions at CIAT facilities in Palmira, Colombia. Six plants or experimental units were evaluated per genotype. These plants were randomly arranged in a humidity chamber of 5.2 m × 2.1 m, placing them under controlled conditions with average temperature of 23°C and average relative humidity of 90%. The seeds per genotype were sown in two pots with a mixture of soil and previously sterilized rice husk, three seeds were placed in each 3-kg pot.

Plants were inoculated by foliar spray at a concentration of 1.2×10^6 conidia·mL^{-1} (CIAT, 2011). The isolate ASC 001 was grown on potato dextrose agar (PDA) medium and incubated at a temperature between 19–20°C in darkness for 14 days until sporulation was abundant. Then, the conidial suspension was prepared by filtering through sterile gauze. Inoculation was carried out at 17 days after sowing (DAS) during afternoon hours, on both the upper and underside of leaves (Hanson et al. 1993, Castellanos et al.). The plants were kept in the humidity chamber in the greenhouse for eight days, maintaining

relative humidity greater than 90%. Four observations of disease severity were recorded at eight, 10, 12, and 14 days after inoculation (DAI), corresponding to stage V3 (first trifoliate leaf).

Disease severity was recorded in each plant using CIAT's scale (Schoonhoven and Pastor-Corrales 1987). Values of 1 to 9 were used, in which 1 to 3 correspond to a resistance reaction, 4 to 6 to an intermediate reaction, and 7 to 9 are considered susceptible materials. Additionally, the first fully expanded trifoliate leaf was selected to determine the leaf area affected (LAA), and percentage of leaf area affected (PA). Variable LAA were processed under the area under the disease progress curve (AUDPC) methodology by Campbell and Madden (1990), considering four samplings over time. These measurements were taken on leaves attached to the plant.

To estimate the leaf area, the length (cm) and width (cm) of each leaflet that made up the trifoliate leaf were determined separately, and the LAA was estimated by measuring the length (cm) and width (cm) of each of the lesions. Additionally, data of 50 fully expanded healthy trifoliate leaves and 50 trifoliate leaves with lesions at different stages were recorded, all these measurements taken on detached leaves to the plant, and photographed with a Panasonic Lumix DMC-FZ40 camera to calculate their total area (TA) and LAA based on the analysis of digital images using the ImageJ software (ImageJ, NIH, Bethesda, ML, United States of America). With this information, linear regressions were applied to calculate TA and PA (Ferreira and Rasband 2012).

Resistance testing under *in-vitro* conditions

The *in-vitro* test was carried out at the Plant Pathology Laboratory of the Faculty of Agricultural Sciences, at Universidad Nacional de Colombia, Bogotá, during the first semester of 2019. Due to seed unavailability, tests were performed only in nine of the 11 genotypes (Table 1). Six experimental units or plants were evaluated per genotype. Seeds were sown under controlled conditions with the average temperature of 18.6°C and average relative humidity of 70%. Three-kg pots were used with a mixture of soil and previously sterilized rice husk. Due to the time of emission of the first trifoliate leaf, seeds were sown 11 days before growing the fungal isolate.

The *in-vitro* test was set up following the detached leaf methodology proposed by Bañuelos-Balandrán and Mayek-Pérez (2008). First trifoliate leaves from 25-day-old plants were manually collected and taken to the laboratory. The plant tissue was placed in individual plastic humidity chambers, using absorbent paper at the bottom, and applying 50 mL of sterile distilled water. A grid was placed on the paper to avoid direct contact of the leaves with water. The treatments consisted of humidity chambers with trifoliate leaves inoculated with a 5-mm agar disc versus humidity chambers containing leaves with agar discs free of the pathogen. In the first case, agar discs with mycelium and reproductive structures of isolate ASC 001 were placed on the center of each of the leaflets of the trifoliate leaf. Each leaflet was considered as one of the six replicates per genotype and was used to determine the LAA (measured in mm2). Disease reaction was recorded at four, six, and eight DAI, and the images were analyzed using the ImageJ software (ImageJ, NIH, Bethesda, ML, United States of America) (Ferreira and Rasband 2012).

Experimental design and data analysis

A completely randomized design was used in the greenhouse and *in-vitro* experiments. For the second case, the treatments were set up in a factorial arrangement, in which the first factor was the genotypes and the second factor corresponded to inoculation (inoculated and inoculum-free). Data transformation was carried out using sqrt(x+0.5) for the variables LAA and AUDPC, and arcsin(sqrt(x)) 100^{-1} was used for the variable PA. Additionally, Duncan's mean comparison test was performed ($p < 0.05$).

RESULTS

Pathogenicity test of genotypes of the genus Phaseolus under greenhouse conditions

Highly significant differences were found for the variables LAA, TA and PA in the factor genotypes when evaluated under greenhouse conditions at eight, 10, 12, and 14 DAI (Fig. 1). The disease severity based on the descriptive scale from

the evaluation of 11 genotypes against the Black node disease, using isolate ASC 001 revealed that genotypes G35575, ASC 144, and ASC 162 obtained reaction values of 2, 2 and 3, respectively, and were categorized as resistant. Genotypes ASC 160 (reaction 4), Simijaca (reaction 5), and Cabrera (reaction 6) showed an intermediate response, and genotypes Bacata, CAL 96, Cblanco, Sutagao (reaction 7), and NCB 226 (reaction 8) were categorized as susceptible (data not shown in this study).

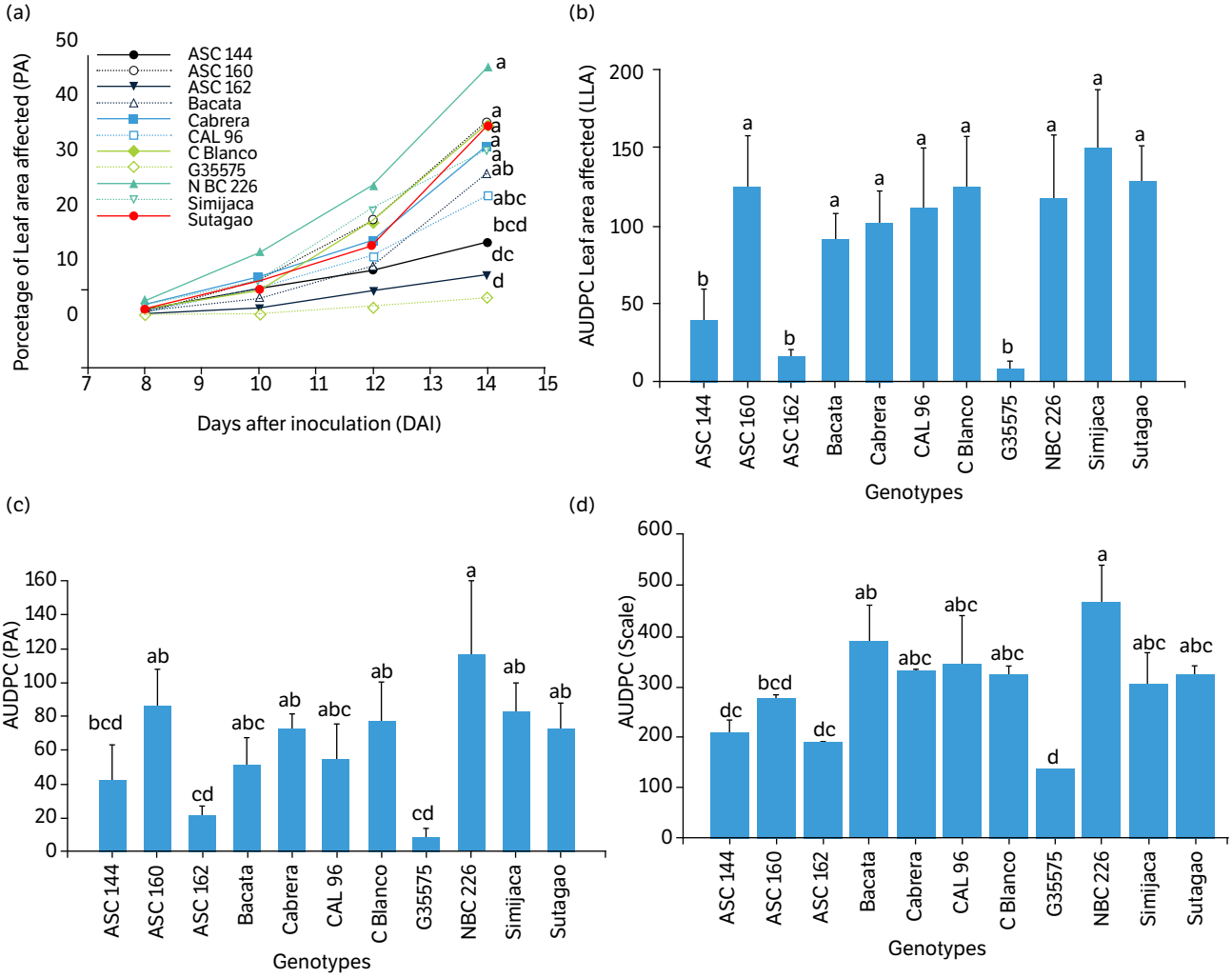

Figure 1. Variables of disease reaction in *Phaseolus* genotypes inoculated with isolate ASC 001 under greenhouse conditions at eight, 10, 12, and 14 days after inoculation (DAI). (a) Percentage of leaf area affected (PA), (b) area under the disease progress curve (AUDPC–LAA) calculated with the leaf area affected in cm^2, (c) AUDPC–PA calculated with the percentage of PA, (d) area under the disease progress curve (AUDPC) of the severity scale. Means with letters in common do not show significant differences (Duncan's test $p < 0.05$). In Fig. 1a the differences were analyzed at 14 DAI.

Figure 1a shows that under greenhouse conditions there is a slow increase of the disease. From the 10th DAI, there is an exponential growth of the PA of the genotypes caused by *B. noackiana*. At 14th DAI, the genotypes G35575, ASC 162 and ASC144 recorded the lowest severity of the disease without statistical differences between them. The same behavior was observed for the AUDPC–LAA calculated from LAA (Fig. 1b) and AUDPC–PA calculated from PA (Fig. 1c), in which these same genotypes showed the lowest averages of severity. In contrast, the other eight genotypes, including ASC 160, obtained high averages of AUDPC. The variables PA, LAA, and AUDPC in Fig. 1 showed the same behavior for the AUDPC calculated with the severity data in the bean genotypes inoculated with isolate ASC 001 (Fig. 1d). The genotypes G35575, ASC 162, and ASC144 showed the lowest averages of *B. noackiana* severity. The difference in the use of this scale occurred with genotype ASC 160 since it does not differ statistically from the three-resistant genotypes or the intermediate reaction genotypes such as Bacata, Cabrera, CAL 96, Cblanco, Simjijaca, and Sutagao. However, differences were observed between ASC 160 and the susceptible genotype NBC 226.

In-vitro pathogenicity test of Phaseolus genotypes

Highly significant differences were found among the nine genotypes for the variables LAA and AUDPC of the LAA in the in-vitro pathogenicity test. The three ASC resistant genotypes showed LAA values of less than 110 mm^2, while the other genotypes of medium reaction and susceptible to *B. noackiana* showed an LAA greater than 370 mm^2. Genotype Sutagao recorded the highest LAA, with a value greater than 710 mm^2 (Fig. 2a).

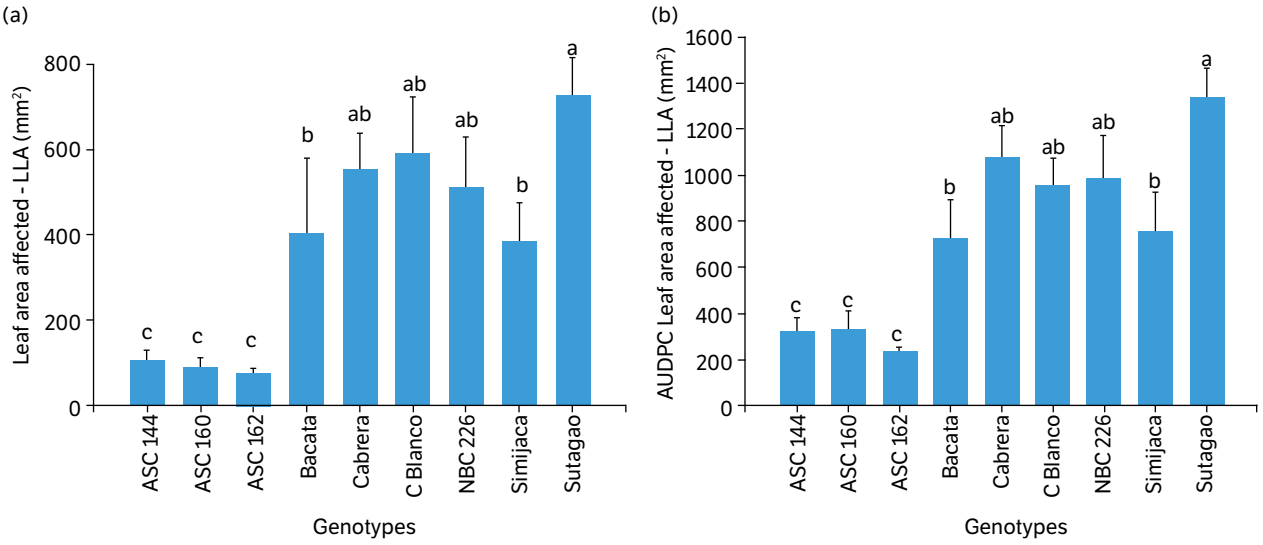

Figure 2. Variables of disease reaction in *Phaseolus genotypes* inoculated with isolate ASC 001 under *in-vitro* conditions. (a) Leaf area affected at eight days after inoculation, (b) area under the disease progress curve (AUDPC–LAA) calculated from the leaf area affected at two, four, six, and eight days after inoculation. Means with letters in common do not show significant differences (Duncan's test $p < 0.05$).

The genotypes showed slight variations in the AUDPC–LAA (Fig. 2b) compared to LAA (Fig. 2a). Among the resistant genotypes, ASC 160 recorded the highest value without significant differences with ASC 144 and ASC 162, which obtained the lowest value of AUDPC of 220 mm^2. This group of resistant genotypes is followed by Bacata with AUDPC–LAA value of 708 mm2 and Simijaca with 754 mm^2. The highest average of AUDPC–LAA was for Sutagao, with 1,353 mm^2. Genotypes Cabrera, Cblanco, and NBC 226 recorded intermediate values between genotypes Simijaca and Sutagao, without statistical differences between them and genotype Sutagao (Fig. 2b).

DISCUSSION

Several studies in search for resistance to Black node disease in beans have been focused for identifying the sources of desirable genes in *P. polyanthus* since it has not been possible to find it in *P. vulgaris* (Schmit and Baudoin 1992, Blair et al. 2011). However, studies with cultivars (*P. vulgaris*) grown in the high tropics are very scarce (Blair et al. 2011). Therefore, this is one of the first contributions towards the search for resistance in common bean genotypes in Colombia.

The results of this study confirmed that the isolate ASC 001 is highly virulent to common bean, which showed various levels of disease reaction among genotypes from resistant to susceptible. Significance differences in disease reaction to *B. noackiana* were observed among the genotypes for both the use of the descriptive scale and estimation of affected leaf area (LAA or PA). Our research found that accession G35575 (*P. polyanthus*) was the most resistant to the disease, and NCB 226 (*P. vulgaris*) was the most susceptible. Regional cultivars Simijaca and Cabrera, together with Bacata, showed their reactions to the disease from susceptible to intermediate.

Coincidences were observed in the degree of infection caused by Black node disease for most of the genotypes when comparing the use of CIAT's descriptive scale with the records of severity data under greenhouse conditions and the detached

leaf method *in vitro*. This is contrary to the reports of Bade and Carmona (2011), that visual methods for estimating severity, such as evaluation scales for pathogens, are of low repeatability, imprecise, and less reliable. Different reaction values were obtained for genotype ASC 160 when using both methods as it showed high severity of the disease under greenhouse conditions, but exhibited a reaction of resistance to the pathogen under *in-vitro* conditions. This demonstrated the importance of combining methodologies to increase the reliability of the assessment.

When the AUDPC was estimated from variables LAA and PA, LAA was inferred to be a better estimator of the reaction to Black node disease since the leaf blade area can mask susceptibility or resistance, causing characterization problems (Olaya et al. 1996). Similar findings were reported in the case of CAL 96, whose total leaf area was significantly greater than that of the other genotypes, but the size of the lesions was equal to or greater than that of other genotypes with apparent higher susceptibility. In contrast, ASC 144, ASC 160, ASC 162, and G35575, of smaller trifoliate leaf size, may appear to be more susceptible.

According to Blair et al. (2014), LAA is a technique inherited quantitatively that is easy to evaluate. Additionally, the processing and analysis of digital images *in vitro* may prevent the use of destructive sampling (Bock et al. 2008), which allows conserving a higher number of individuals and harvesting seed from plants. They are also a useful, fast, and inexpensive tool for this type of study, which offers the possibility of accelerating the selection of genotypes with possible resistance.

The variation of data between both methodologies for the pathogenicity test, in greenhouse and *in vitro*, may be due to the possibility of a fluctuation in temperature. In the laboratory, temperatures were stable and optimal for the pathogen (Blair et al. 2011) with a minimum temperature of 18°C and a maximum of 21°C, that guarantee high infection and development of the disease. On the other hand, the greenhouse temperature had a higher value, with a minimum of 20°C and a maximum of 26°C. This fluctuation could have affected the development of the pathogen, which has been reported for a large number of fungi. However, in our study, the environmental conditions in the greenhouse were within the growth ranges of the disease (Castellanos et al. 2011).

The comparison between *in-vitro* and field tests is often discussed. For instance, Higuera (1991) does not recommend the evaluation of resistance to the bean pathogen *Macrophomina phaseolina* in the laboratory due to the lack of association with field data. In contrast, Amand and Wehner (1995) promote laboratory tests since they provide consistent data between methodologies for evaluating the reaction of cucumber to different fungi. Bañuelos-Balandrán and Mayek-Pérez (2008) reported the high correlation between five inoculation methods for *M. phaseolina* pathogenicity values in common beans, which included cotyledonal leaves, seeds, and seedlings.

The evaluation of a complete bean plant is complex due to the number of trifoliate leaves and the plant architecture. These factors limit data collection especially when using visual scales that are helpful to detect monogenic resistance given their qualitative nature. Therefore, *in-vitro* methods can be considered for estimating resistance to Black node disease because their results can be representative of the reaction under greenhouse conditions that is closer to the response under field conditions. Additionally, these methods can detect quantitative resistance.

Most of the Andean and Mesoamerican gene pools of common beans are susceptible to Black node disease (*B. noackiana*) (Blair et al. 2014). This matches our findings that Andean cultivars grown at high altitudes in Colombia such as Cblanco, Cabrera, and Sutagao were also susceptible. Resistance is confirmed in the secondary gene pool (*P. polyanthus*), and its interspecific crosses of the ASC series. This can be utilized as an alternative for a common bean breeding program via backcrosses or molecular breeding to obtain resistance against the Black node disease, conserving desirable genes of plant architecture, adaptation to cold conditions, and characteristics of the commercial grain.

CONCLUSION

The estimation of the affected foliar area through the greenhouse and in-vitro pathogenicity tests allowed knowing that the cultivars (*P. vulgaris*) Bacata and Simijaca present an intermediate reaction to the pathogen *B. noackiana*, so they can be used with the secondary gene pool (*P. polyanthus*) in sustainable genetic improvement programs for Black node disease.

The methodologies for pathogenicity tests in greenhouse and *in vitro* can estimate the leaf area affected, avoiding grouping plants with different levels of severity into the same categories. Although the methods based on leaf affected area require

more logistics, resources, and staff to be carried out than descriptive scale evaluations in the field, they are more reliable, giving the opportunity to use the data in quantitative resistance studies.

AUTHORS' CONTRIBUTION

Conceptualization: Garzón Gutiérrez, L. N. and Ligarreto, G.; **Methodology:** Garzón Gutiérrez, L. N. and Ligarreto, G.; **Investigation:** Pimentel Ladino, C. C. and Ligarreto, G.; **Data curation:** Pimentel Ladino, C. C.; **Formal analysis:** Pimentel Ladino, C. C. and Ligarreto, G.; **Funding Acquisition:** Garzón Gutiérrez, L. N.; **Writing – Original Draft:** Pimentel Ladino, C. C., Garzón Gutiérrez, L. N. and Ligarreto, G.; **Writing – Review and Editing:** Ligarreto, G.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

FUNDING

Ministry of Science, Technology and Innovation of Colombia
Grant no. 110271351710 046-2016.

ACKNOWLEDGMENTS

Not applicable.

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