Investigating the resistance of common bean germplasm to *Colletotrichum lindemuthianum* and its implications for breeding

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Abstract: *Common bean breeding obtained cultivars with high yield and resistance to diseases. We evaluated anthracnose resistance of genotypes from breeding programs where selection was made mainly to increase grain yield. A germplasm collection of 845 lines were assessed with four of the most frequent Colletotrichum lindemuthianum races found in Brazil (65, 73, 81 and 89). In addition to high reaction variability, we identified 633 lines resistant to at least one race. Most resistant lines have a Carioca grain type. The virulence indexes ranged from 51.7% to 63.2% (races 81 and 73) and resistance indexes ranged from 0% (susceptible) to 100% (resistant). This study presents complete characterization about resistance to anthracnose of a wide collection, provides information about cultivars that can be directly introduced in current breeding programs and shows that breeding focused on increasing grain yield has also been successful in selecting resistant genotypes to anthracnose disease.*

Keywords: Colletotrichum lindemuthianum*, genetic resistance,* Phaseolus vulgaris*, resistance sources*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) breeding programs financed by public resources at universities and research institutes over the past 50 years produced Brazilian cultivars adapted to different environments. Selection aims to obtain gains important for both farmers and consumers, especially grain yield, resistance to diseases, upright plant architecture and grain type (Abreu et al. 2007, Melo et al. 2010, Pereira et al. 2017, Lemos et al. 2020). However, anthracnose, a fungal disease caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi & Cavara, is a limiting factor to achieve high grain yield, leading to economic losses to producers worldwide (Padder et al. 2017).

Breeding to improve grain yield began in the 70's with introduction, evaluation and selection of pure lines followed by hybridization in the 80's and recurrent selection in the 90's (Pereira et al. 2017, Lemos et al. 2020). Anthracnose resistance breeding strategies such as pyramiding, gene transfer between Mesoamerican and Andean gene pools, multilines and recurrent selection have been applied to obtain cultivars with durable resistance (Pereira and Santos 2004, Pereira et al.
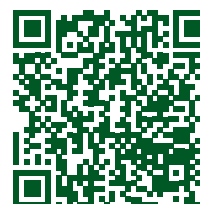
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2004, Costa et al. 2010, Marcondes et al. 2010, Botelho et al. 2011, Costa et al. 2020). Moreover, resistance genes were introduced in adapted lines by crosses with some resistance sources from the set of anthracnose differential cultivars, such as Cornell 49242, TO, TU, AB136, G2333 (Pereira et al. 2004, Lemos et al. 2020).

Common bean/anthracnose pathosystem has revealed many resistance loci identified in different lines (Meziadi et al. 2016) and high genetic and pathogenic *C. lindemuthianum* variability worldwide. In Brazil, the most frequent races are 65, 73, 81 and 89 (Silva et al. 2007, Pinto et al. 2012, Ribeiro et al. 2016, Paulino et al. 2022) and pathogenic variability among strains of the same race of *C. lindemuthianum* has also been reported, challenging the achievement of cultivars with durable resistance (Ishikawa et al. 2011, Ishikawa et al. 2012, Costa et al. 2017).

Characterization and identification of sources of resistance to anthracnose in germplasm collection could provide useful information for the scientific community and use in breeding programs. Therefore, this study aims to assess anthracnose resistance in a common bean germplasm collection against the four most frequent *C. lindemuthianum* races in Brazil and evaluate the impact of breeding to improve grain yield to obtain anthracnose-resistant genotypes.

MATERIAL AND METHODS

Plant and fungal material

We evaluated the reaction of 823, 789, 802 and 815 common bean lines against races 65, 73, 81 and 89 of *C. lindemuthianum*, respectively. Studies have shown that these are the most frequent races found in Brazil (Silva et al. 2007, Pinto et al. 2012, Ribeiro et al. 2016, Paulino et al. 2022). The common bean lines were developed by breeding programs conducted over 50 years at Universidade Federal de Lavras (UFLA) – Lavras, Minas Gerais, Brazil and other federal institutions such as other Brazilian Universities, Research Institutes and the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) and international research institutes. This germplasm collection has been maintained by UFLA, and the genotypes are morphologically classified into seven commercial grain type groups: carioca, jalo, mulatinho, black, purple, red and "other" types. The "other" type includes less frequent grain types, such as pinkish, speckled, yellow, grayish, green, and beige, classified as a single group. The pathogen strains races 65, 73 and 81 belong to the Laboratório de Resistência de Plantas a Doenças of UFLA and race 89 is from BIOAGRO, Universidade Federal de Viçosa (UFV). These strains were previously tested on the set of differential cultivars (Pastor Corrales 1991) to confirm the *C. lindemuthianum* races.

Inoculum preparation procedure

To obtain spores, mycelial discs from strain colonies maintained in M3S medium (Tu 1985) were placed on sterilized bean pods and incubated for 10-15 days. All incubations were placed in a BOD (Biological Oxygen Demand) incubator at 22 ºC and kept in the dark. Inoculum suspensions were prepared by adding 4 mL of sterile distilled water to the tubes and scraping the surface of the bean pods with a platinum loop to release conidia. The suspensions were filtered through a layer of Miracloth (Chicopee Mills, Inc., New York, NY, USA) to remove mycelial fragments, the conidial concentration was quantified in a Neubauer counting chamber, and sterile distilled water was added to the final suspension to reach the concentration of 1.2×10^6 conidia mL^{-1}.

Resistance phenotyping

Seeds of the common bean lines were grown in polystyrene trays of 162 cells containing Multiplant substrate (Terra do Paraíso Ltda. Brazil) under natural greenhouse conditions (25-27 ºC) and manual irrigation. Two replicates of nine plants per plot were used for each cultivar. Cultivars Pérola and Ouro Negro served as susceptible and resistant controls, respectively. After the full development of unifoliolate leaves, seedlings were inoculated with the conidia suspension on both leaf surfaces and stem using a manual sprayer. After inoculation, seedlings were incubated in a moist chamber at 22 °C with 95% relative humidity and 12-hour photoperiod for 72 hours, followed by a seven-day period in a greenhouse at 25 °C and 85% humidity. Plant symptoms were evaluated using a scale from 1 to 9 developed by Schoonhoven and Pastor-Corrales (1987). Average scores were calculated, and common bean lines were classified either as resistant (1 to 3) or susceptible (4 to 9). The resistance index (RI) of each genotype and the virulence index (VI) of each race were

estimated and expressed as percentages (Balardin and Kelly 1998, Vidigal Filho et al. 2007): $RI = \dfrac{100RC}{T}$, where RC = total number of races to which each genotype was resistant and T = total number of races of *C. lindemuthianum* assessed. $VI = \dfrac{100S}{T}$, where S = total number of susceptible genotypes to that race and T = total number of genotypes evaluated (823, 789, 802, 815 to races 65, 73, 81 and 89 respectively).

RESULTS AND DISCUSSION

In a general view, 633 common bean lines were resistant to at least one *C. lindemuthianum* race. We found 221 genotypes resistant to one race, 221 resistant to two races, 113 resistant to three races and 78 resistant to all four races tested (supplementary material). From 802 lines evaluated, 48.25% were resistant to race 81. A total of 815 lines were tested against race 89 and 40.0% were resistant. We found 37.8% resistant lines from 823 evaluated against race 65, and 36.75% from 789 evaluated were resistant to race 73 (Figure 1).

Resistance indexes ranged from 0% to 100% for susceptible and resistant lines to all races assessed, respectively (supplementary material). The virulence indexes were 51.7% (race 81), 60% (race 89), 62.2% (race 65) and 63.2% (race 73), and 512, 499, 415 and 489 lines susceptible to races 65, 73, 81 and 89 were identified, respectively.

A high number of susceptible genotypes and highest VI to all races have also been reported by Balardin and Kelly (1998), Mencalha et al. (2022) and Vidigal Filho et al. (2007). According to Balardin and Kelly (1998), Andean races exhibited higher levels of virulence (VI 53% to 100%) on Andean than on Mesoamerican germplasm (19% to 62%). Mencalha et al. (2022) identified race 81 as the most virulent (VI = 77.5%) and 65 as the least virulent (VI = 65%). We found that race 81 was the least virulent (VI = 51.7%) and race 73 was the most virulent (VI = 63.2%) as reported by Vidigal Filho et al. (2007) to races 81 (VI = 65%) and 73 (VI = 85%).

Information from the germplasm collection such as grain type group, institution of origin and development period were used to make associations in the group of 191 genotypes resistant to at least three races (113 resistant to three races and 78 resistant to four races). Regarding grain type groups, 58.8% of the 191 lines belong to Carioca group (beige with brown stripes), followed by black (18.2%) and 14.0% other grain types (Figure 2). The grain type groups Carioca (55.6%) and black (11.37%) were also the most frequent considering all lines resistant to at least one race (633). Common bean grain type is an important trait to consumers, and preferences vary according to country or region (Costa et al. 2010, De Ron et al. 2016). In this work the greatest number of resistant genotypes have Carioca grain type, which is the most consumed grain type in the country, followed by black bean (Costa et al. 2010, Borém and Carneiro 2015).

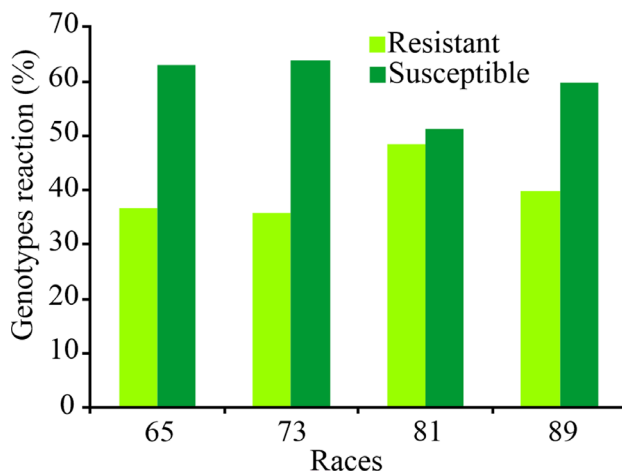Considering the development period, 34.9% of these lines resistant to at least three races were developed

Figure 1. Percentage of common bean genotypes resistant and susceptible to races 65, 73, 81, and 89.
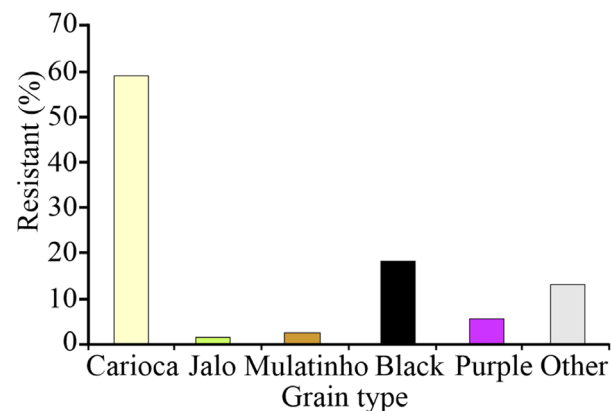

Figure 2. Grain type percentage of the 191 resistant lines to at least 3 *C. lindemuthianum* races. Jalo type of seed has yellowish color, mulatinho is brown without stripes and Other comprises seeds with white, red, pinkish and green color.

between 1970 and 1990, and 65.1% were obtained between 1991 and 2013, showing that most of the resistant genotypes were obtained in the last decades. Considering the institution of origin, common bean breeding program of UFLA developed 38% of these lines and 62% were developed by other institutions.

The common bean lines evaluated in the present study have origins in both Andean and Mesoamerican genetic pool, exhibiting wide genetic variability for many traits. This collection comprises inbreeding lines and cultivars developed during breeding programs with selection for desirable traits such as resistance to diseases, grain type and upright plant architecture. However, most breeding programs were conducted mainly with the objective of increasing grain yield (Lemos et al. 2020). Identification of resistant genotypes in germplasm collections can provide useful information about cultivars that can be directly introduced in current common bean breeding programs (De Ron et al. 2016). In this study, more than 800 genotypes were tested against the four most frequent races of *C. lindemuthianum* in Brazil, and 633 common bean lines resistant to at least one race were identified.

Most of the resistant genotypes were developed in the last decades by UFLA and other public institutions, which indicates the success of common bean breeding programs started around 1970 (Matos et al. 2007, Lemos et al. 2020). Resistance to anthracnose disease was one of the goals of breeding programs; however, at the beginning these programs faced difficulties concerning lack of infrastructure and standardized methodologies of inoculation and evaluation of symptoms. Therefore, common bean breeding programs conducted in Brazil have explored grain yield potential of this crop as the main goal (Ramalho et al. 2005, Matos et al. 2007, Lemos et al. 2020). Most productive genotypes in different environments might have high number of alleles providing adaptability to biotic and abiotic stresses, including resistance to diseases. The high number of resistant genotypes identified in this study indicates that breeding focused on increasing grain yield is effective to select resistant genotypes to anthracnose disease.

Selection for grain yield increasing efficiency to obtain resistant genotypes to the race 65 of *C. lindemuthianum* and race 63-63 of *Pseudocercospora griseola* has been observed in common bean (Pereira et al. 2019). Abreu et al. (2003) showed that under high disease pressure grain yield was an efficient selection criterion to identify resistant genotypes to anthracnose in common bean. The development of BRSMG Talismã cultivar is another example in which evaluation and selection for resistance to diseases were not applied, although it has resistance to several anthracnose and angular leaf spot races (Souza et al. 2005). Furthermore, the Ouro Negro cultivar, developed mainly for high grain yield, was resistant to all anthracnose races evaluated in this work. Besides that, linked loci conferring resistance to rust and angular leaf spot diseases have also been identified in this cultivar (Valentini et al. 2017). It was also found that, if the selection for anthracnose resistance is made in early generations, it is possible to obtain very productive and resistant genotypes in later generations in common bean breeding program (Pádua et al. 2015).

The reaction to angular leaf spot disease of 416 genotypes from the same germplasm collection evaluated in this work was analyzed by Pádua et al. (2022). The common bean lines were tested against the most aggressive *P. griseola* race (63-63) and 67 genotypes were resistant to both anthracnose and angular leaf spot diseases (supplementary material).

Pereira et al. (2004) evaluated 256 lines from a backcrossing program using G2333 cultivar as parental donor and Brazilian cultivars ESAL 696 and CI140 as recurrent parents. Common bean lines were inoculated with races 2047, 1545 and 73. Genotypes resistant to anthracnose, very productive, with carioca grain type and upright architecture were identified. Lines identified with *Co*-4^2 allele might also have *Co*-5, and *Co*-7. Among lines without *Co*-4^2 allele, some of them were identified with a pyramid of *Co*-5 and *Co*-7 and others with *Co*-5 or *Co*-7.

Botelho et al. (2011) evaluated the multiline usefulness to reduce yield losses caused by anthracnose in common bean. Disease progress and grain yield of a mixture of seven lines and the same pure lines individually were compared. The lines had carioca grain type, were agronomically uniform, but showed different patterns of *C. lindemuthianum* resistance. The multiline had grain yield similar to that of the most productive lines and slower disease progress over time compared with susceptible lines. Most of the resistant lines (55.6%) found in this work belong to the carioca grain type group, and the information provided in this work can be useful to select lines and explore multilines and recurrent selection also as strategies to access durable resistance and grain yield.

The high frequency of resistant genotypes to different races in this study indicates that prevalent races of *C. lindemuthianum* in common bean production areas have changed over time in the country since the beginning of

breeding programs. It was also verified in a study that characterized races from 1991 to 2021 in each Brazilian state, providing information that can assist breeding and marker-assisted selection (Paulino et al. 2022). We phenotyped 845 lines from Brazil and abroad to the most frequent races, complementing information for breeding programs to achieve durable resistance in the most consumed commercial grain types such carioca and black.

A set of 40 carioca grain type cultivars recommended by different institutions in Brazil from 1980 to 2013 was also tested against races 65, 73, 81 and 89, and twenty-seven cultivars were susceptible to all races evaluated. However, 12.5, 2.5, 12.5, and 15% were resistant to one, two, three, and four races of anthracnose, respectively pointing out parental sources of resistance to the pathogen (Mencalha et al. 2022).

The search for resistant genotypes in germplasm collections has been conducted by other researchers. In Spain, Ferreira et al. (2008) evaluated 246 landraces and 42 lines derived from breeding programs against five *C. lindemuthianum* races (3, 6, 19, 38, and 102). Three landraces resistant to four races and nine breeding lines resistant to five races were identified. In North Dakota, United States, a collection of 12 commercial cultivars from different market classes of dry beans was screened for resistance to the most frequent races in that area (7, 73, 79, 1153 and 1161), and the authors found that most cultivars were resistant to race 7 and none were resistant to all races evaluated (Goswami et al. 2011).

The results of this study showed that most of the lines were resistant to races of higher binary number and susceptible to races with lower binary number. For example 47.23% of the resistant lines to race 89 were susceptible to race 73, 36.8% were susceptible to race 81 and 40.2% were susceptible to race 65. Moreover, 13.49% of the resistant lines to race 89 were susceptible to 81, 73 and 65. According to the race nomenclature system based on reaction in a set of twelve differential cultivars proposed by Pastor-Corrales (1991), if a genotype is resistant to race 89 it should also be resistant to races 81, 73 and 65 because it carries genes conferring resistance to all virulence factors in these strains. This fact shows that other virulence factors might be present in these races and are not being detected by the set of differential cultivars. Literature has reported that major genes and QTLs are involved in resistance to different races of *C. lindemuthianum* (Oblessuc et al. 2014, Zuiderveen et al. 2016, Costa et al. 2017, Costa et al. 2020, Shafi et al. 2022). A methodology limitation to resistant phenotyping studies is the pathogenic variability among strains of the same race of *C. lindemuthianum*. However, studies have evaluated lines able to identify this variation and complement the results of the standard set of differential cultivars, allowing the search of cultivars with durable resistance (Ishikawa et al. 2011, Ishikawa et al. 2012, Costa et al. 2017).

This study was performed in greenhouse using a highly variable common bean germplasm collection and revealed that common bean breeding lead to anthracnose resistance even when selection is made to improve other traits, such grain yield, grain type and upright plant architecture. This information is important for common bean breeders to identify resistant genotypes to include in crosses in breeding programs and in studies involving *Colletotrichum lindemuthianum/Phaseolus vulgaris* pathosystem, contributing to advancement of knowledge and sustainable agriculture.

DATA AVAILABILTY

The dataset analyzed during the current research is accessible in the CBAB repository, available at https://data.scielo.org/dataverse/brcbab

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