AGRONOMY SCIENCE AND BIOTECHNOLOGY

RESEARCH ARTICLE

Enhanced understanding of anthracnose resistance in Michigan Dark Red Kidney common bean cultivar

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ABSTRACT

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, is a major disease affecting the common bean (*Phaseolus vulgaris* L.), potentially causing yield losses of up to 100%. Utilizing resistant cultivars is the most effective approach for disease control. This study aimed to characterize the genetic resistance of two cultivars, TU and Michigan Dark Red Kidney (MDRK), to anthracnose. Inheritance tests were performed on $F_{2:3}$ families from the TU (R) × AND 277 (S) cross using *C. lindemuthianum* race 3, and on $F_{2:3}$ families from the crosses TU (R) × Kaboon (S) and TU (R) × Perry Marrow (S) inoculated with race 39. Additionally, inoculation of $F_{2:3}$ families from the MDRK × TU cross with race 1545 revealed that MDRK's resistance to this race is conditioned by two dominant genes (*Co-1* allele on Pv01 and another allele on Pv04). Segregation results from inheritance tests using $F_{2:3}$ families with the TU resistant cultivar fitted to a 1RR:2RS:1SS ratio, indicating the presence of a single dominant gene in the TU cultivar. Both the Mesoamerican TU and the Andean MDRK cultivars represent valuable sources of resistance to *C. lindemuthianum* and can be incorporated into common bean breeding programs to enhance disease resistance.

Keywords: *Phaseolus vulgaris* L., *C. lindemuthianum,* genetic resistance.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an economically significant legume species that has been cultivated for over 7,000 years. Its two primary domestication centers are the Mesoamerican and Andean regions, which can be differentiated by several distinct traits, such as phaseolin types, molecular markers, morphological features, and isoenzymes (Gepts et al., 1988; Singh et al., 1991; Haley et al., 1994; Beebe et al., 2000; Ansari et al., 2004). Common beans are among the most significant legumes for human consumption (Messa et al., 2019; Coêlho et al., 2020; Carvalho et al., 2021; Chimenez-Franzon et al., 2022; Ferreira et al., 2023; Silva et al., 2023). However, their productivity can be severely impacted by anthracnose, a disease caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara. Regarded as the primary disease affecting this crop, anthracnose can lead to substantial yield losses, potentially resulting in up to a 100% reduction in production (Borges et al., 2012).

The pathogen exhibits high genetic variability, and no resistance genes within the host are effective against all known races of the pathogen (Mahuku et al., 2002; Rey et al., 2005; Paulino et al., 2022). The extensive pathogenic variability of *C. lindemuthianum* may be attributed to the co-evolution of the fungus in relation to its host, as well as the potential for mutations and parasexual and genetic recombination (Araya, 2003; Mahuku and Riascos, 2004).

The emergence of new virulence races in pathogens has resulted in reduced or complete loss of yield in previously resistant commercial cultivars (Pastor-Corrales et al., 1995; Gonçalves-Vidigal et al., 2011, Paulino et al., 2022). Therefore, the development of new sources of durable resistance is highly desirable for effective breeding efforts. Gene pyramiding for durable resistance to diseases caused by highly variable pathogens can be greatly facilitated by marker-assisted selection. The development of highly accurate molecular markers that are closely linked to important disease-resistance genes enables the pyramiding of these genes into single cultivars with broad-spectrum resistance (Vaz Bisneta & Gonçalves-Vidigal, 2020).

The most effective and sustainable strategy to control anthracnose is the use of resistant cultivars, which can reduce yield losses without the negative environmental impact of fungicide application (Gonçalves-Vidigal et al., 2020). However, the implementation of resistance is challenged by the recurrent emergence of virulence phenotypes in the pathogen population, commonly referred to as races of C. lindemuthianum (Mahuku et al., 2002; Sousa et al., 2014).

These virulence races can render previously resistant cultivars susceptible to anthracnose, leading to significant yield losses. The frequent emergence of new races makes it difficult to develop and maintain resistant cultivars (Paulino et al., 2022). Therefore, there is a constant need to identify new sources of resistance and develop breeding strategies that can overcome the challenges posed by the emergence of new races. The use of resistant cultivars is a sustainable and environmentally friendly approach to managing anthracnose. It can reduce the use of fungicides, which can have negative impacts on human health and the environment. Developing cultivars with durable resistance to multiple races of *C. lindemuthianum* can provide long-term solutions to this disease, ensuring the sustainability of common bean production.

Given the significance of studying Andean resistance genes in conjunction with Mesoamerican resistance genes, this study aimed to investigate the genetic resistance of cultivars TU (Mesoamerican) and MDRK (Andean) against races 3, 39, and 1545 of *C. lindemuthianum*.

MATERIAL AND METHODS

The experiment was conducted between August 2016 and January 2017 at the Common Bean Breeding and Molecular Biology Laboratory of the Center for Applied Research to Agriculture (Nupagri) at the Universidade Estadual de Maringá (UEM) in Maringá, Paraná, Brazil.

Plant Material and Inheritance Test

The TU cultivar, originating from the Mesoamerican region, is one of the 12 anthracnose differentiating cultivars described by Pastor-Corrales (1991) and carries the *Co-5* anthracnose resistance gene. The MDRK cultivar, an Andean variety also evaluated in the same study, is another of the 12 anthracnose differentiating cultivars and possesses the *Co-1* anthracnose resistance gene.

The inheritance resistance studies were conducted on four different crosses. First, 16 $F_{2:3}$ families derived from TU (R) × AND 277 (S), totalling 150 plants, were inoculated with race 3. Second, 10 $F_{2:3}$ families from TU (R) × Kaboon (S), encompassing 63 plants, were tested with race 39. Third, 18 $F_{2:3}$ families from TU (R) × Perry Marrow (S), totalling 163 plants, were assessed with race 39. Lastly, 56 $F_{2:3}$ families from the Michigan Dark Red Kidney (R) × TU (S) cross, comprising 509 plants, were inoculated with race 1545 of *C. lindemuthianum*.

The F_2 generation seeds, obtained from the aforementioned cultivar crosses, were sown in 5 dm3 plastic pots filled with a pre-fertilized mixture of soil material and organic substrate. After the seedlings emerged, they were maintained in a greenhouse with controlled lighting and irrigation conditions until the pods matured and dried completely, at which point they were harvested to obtain the $F_{2:3}$ seeds.

The parent, differential, and $F_{2:3}$ seeds were sown in plastic trays and kept in a greenhouse until the seedlings fully developed their first trifolium. After this stage, the seedlings were acclimatized in a controlled environment and then inoculated with spores from various races of *C. lindemuthianum* (Cárdenas et al., 1964).

Pathogenesis assays

The *C. lindemuthianum* races employed in this research were 3, 39, and 1545, sourced from Nupagri's mycology collection. Monosporic cultures were prepared at the Common Bean Breeding and Molecular Biology Laboratory. Race 3, originating from the Andes, has been identified in various countries, including Peru, Ecuador, Argentina, Colombia, the United States, the Dominican Republic, India, Spain, and Brazil (Balardin et al., 1997; Falconi et al., 2003; Ansari et al., 2004; Mahuku and Riascos, 2004; Sharma et al., 2007; Ferreira et al., 2008; Muth & Liebenberg, 2009; Uchôa et al., 2015). Race 3 of *C. lindemuthianum* was used in the inheritance test of the $F_{2:3}$ families TU (R) × AND 277 (S).

Race 39, of Mesoamerican origin, has been identified in India and the Dominican Republic (Sharma et al., 1999; Ansari et al., 2004; Mahuku and Riascos, 2004). This race was used in the inheritance test with the $F_{2:3}$ families derived from the crosses TU (R) × Perry Marrow (S) and TU (R) × Kaboon (S). Race 1545 has been identified in countries such as Honduras, Guatemala, Nicaragua, Costa Rica, and Colombia (Rava et al., 1994; Balardin et al., 1997; Awale et al., 2008) and was used in this study for the inheritance test involving Michigan Dark Red Kidney (R) × TU (S) families.

Monosporic cultures of each *C. lindemuthianum* race used in this study were prepared in a young green common bean pod medium and incubated at 25°C for 14

days. The inoculation of the parents and $F_{2:3}$ families from each cross was conducted separately. The inoculated plants were maintained in a fog chamber for 72 hours at 20 ± 2°C, with a controlled light cycle of 12 hours of illumination at 680 lux and 12 hours of darkness. After the incubation period, the trays were transferred to an appropriate environment with a temperature of 22 ± 2°C under artificial light, where they remained until evaluations began (Cárdenas et al., 1964).

Symptom visual evaluation for each plant was performed approximately ten days after inoculation, using the severity scale proposed by Pastor-Corrales et al. (1995). With the Mendelian segregation data of the phenotypes R (resistance) and S (susceptibility), genetic-statistical analysis was conducted using the Genes Program software (Cruz, 2013) and applying the Chi-square test (χ^2).

RESULTS AND DISCUSSION

Resistance inheritance test

The results of the inheritance tests, *the* Chi-square tests (χ^2), and the probability analyses of the gene presence hypothesis are presented in table 1.

Table 1. Inheritance test in $F_{2:3}$ families, using races 3, 39 and 1545 *of Colletotrichum lindemuthianum*.

Parental cross	Race	Phenotype of parents		Segregation observed			Segregation expected			χ^2	*p* value
				RR	RS	SS	RR	RS	SS		
TU × AND 277	3	R	S	4	9	3	4	8	4	0.375	0.829
TU × Kaboon	39	R	S	2	6	2	2.5	5	2.5	0.4	0.818
TU × PM	39	R	S	6	8	4	4.5	9	4.5	0.666	0.716
TU × MDRK	1545	S	R	22	29	5	25	28	6	0.933	0.626

*PM: Perry Marrow; MDRK: Michigan Dark Red Kidney.

The TU cultivar carries the resistance gene *Co-5*, which confers resistance to races 3 and 39 of C. lindemuthianum, as used in this study (Young et al., 1998). The cultivar AND 277 possesses the gene *Co-1*^{4} (Gonçalves-Vidigal et al., 2011); however, this gene does not confer resistance to the aforementioned pathogen races.

The resistance inheritance test conducted on 16 families, comprising 150 plants obtained from the cross between TU × AND 277 cultivars and using *C. lindemuthianum* race 3, revealed a segregation proportion of 1RR:2RS:1SS ($\chi 2$ = 0.375 and p = 0.829), indicating the presence of a single dominant gene in the TU cultivar (Table 1).

Similar results were obtained by Trabanco et al. (2015) when inoculating $F_{2:3}$ family plants from the SEL 1308 × MDRK cross with *C. lindemuthianum* race 3. The authors observed a segregation ratio of 1RR:2RS:1SS, indicating that cultivar SEL 1308 carries a dominant gene responsible for conferring resistance to *C. lindemuthianum* race 3. The results may be related to those obtained in Campa et al. (2009) study, where race 3 spores were inoculated in plants originating from the TU × MDRK differentiating cultivar cross. Resistance of cultivar TU to race 3 was identified, suggesting the presence of a resistant gene in the cultivar. In contrast, the

differentiating cultivar MDRK showed susceptibility.

The resistance inheritance test was also conducted on two other $F_{2:3}$ populations inoculated with C. lindemuthianum race 39. In the first inheritance test, the $F_{2:3}$ TU (R) × Kaboon (S) population comprised 10 families and 63 evaluated plants. The observed segregation was fitting the 1RR:2RS:1SS ratio. This result demonstrates that resistance to *C. lindemuthianum* race 39 in the TU differentiating cultivar is conditioned by a single dominant resistance gene (Table 1). Although cultivar Kaboon contains the *Co-1*^{2} gene (Melotto and Kelly, 2000), it does not confer resistance to race 39 and displays susceptibility.

The second inheritance test with *C. lindemuthianum* race 39 involved 18 $F_{2:3}$ families from the TU × Perry Marrow (PM) cross, totaling 163 evaluated plants. In this study, the segregation fit the 1RR:2R/S:1SS ratio ($\chi 2 = 0.666$; $p = 0.716$), indicating that resistance to race 39 is conferred by a dominant gene (Table 1).

The Andean cultivar Perry Marrow carries the *Co-1*^{3} gene (Melotto and Kelly, 2000); however, this gene does not confer resistance to race 39, as it exhibits susceptibility to this race. This result suggests that a single dominant gene in the TU cultivar is responsible for resistance to race 39.

Campa et al. (2009) obtained similar results when inoculating F_3 families from the TU × MDRK differentiating cultivar cross with *C. lindemuthianum* race 39 spores. In this resistance inheritance study, the segregation also fit the 1RR:2RS:1SS ratio, confirming the presence of only one dominant gene for this race in the TU cultivar, as demonstrated in the current study.

The inheritance test was also conducted on 56 families from the MDRK (R) × TU (S) cultivar cross, with a total of 509 $F_{2:3}$ plants inoculated with *C. lindemuthianum* race 1545. The observed segregation in the $F_{2:3}$ families was 22RR:29RS:5SS, fitting the 7RR:8RS:1SS proportion with a probability of 0.626. This segregation suggests the action of two dominant genes responsible for the resistance reaction to race 1545, present in the differentiating cultivar MDRK, as the differentiating cultivar TU is susceptible to this race (Table 1).

Campa et al. (2009) confirmed the resistance of the cultivar MDRK to race 1545 of C. lindemuthianum, which is conferred by two independent dominant genes. The authors inoculated F_3 families derived from the TU × MDRK crossing and found that the results fit a ratio of 7:8:1, indicating the presence of two independent dominant genes. Additionally, molecular analysis revealed that one of the genes is linked to the MARKER $OF10_{350}$ (located in Pv01) and the other to Pv-ctt001 (located in the Pv04 group and corresponding to the Co-3/*Co-9* locus.

According to Gonçalves-Vidigal et al. (2016), our laboratory conducted additional experiments that revealed the STS marker g2303 has one polymorphic DNA fragment of 350bp in the Andean cultivars MDRK, AND 277, and Jalo EEP558, and another of 340 bp in the Mesoamerican cultivars Mexico 222 (*Co-3*), BAT 93 (*Co-3*^{3}), and Ouro Negro (*Co-3*^{4}) (see Figure 1).

The g2303 marker is located at position 3,356,300 bp on chromosome Pv04, which has a total length of 45,960,019 bp (according to Phaseolus Genes). By comparing the sequences of the fragments, we found that the MDRK cultivar's fragment is positioned at 3,356,179 to 3,356,483 bp on chromosome Pv04.

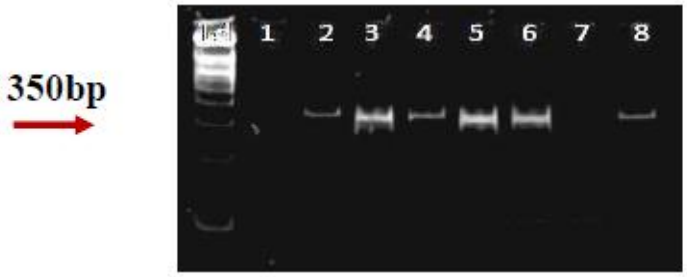

Figure 1. Electrophoretic analysis of the amplification products obtained for the g2303 marker. Lanes: Ld, 100bp ladder; 1, Corinthiano; 2, AND 277; 3, BAT 93; 4, Michigan Dark Red Kidney; 5, Ouro Negro; 6, Mexico 222; 7, Rudá; 8, Jalo EEP558. The arrow left indicates the 350 bp and the right 340bp DNA band present in Andean and Mesoamerican cultivars, respectively.
Source: Gonçalves-Vidigal et al. (2016).

The STS marker g2303 was previously identified on chromosome Pv04, located at position 3,356,300 base pairs (bp). Upon comparison of the fragment sequences, we discovered that the fragment corresponding to g2303 in the MDRK cultivar is positioned between 3,356,179 and 3,356,483 bp on chromosome Pv04. This finding underscores the potential utility of this marker for marker-assisted selection in bean breeding programs, targeting both the Andean and Mesoamerican gene pools.

CONCLUSIONS

In conclusion, the study found that resistance to races 3 and 39 of *C. lindemuthianum*, present in the TU differentiating cultivar, is controlled by only one dominant gene. Meanwhile, the cultivar Michigan Dark Red Kidney exhibited two dominant resistance genes (*Co-1* allele on Pv01 and another allele on Pv04) for race 1545 of *C. lindemuthianum*.

Understanding the presence of resistance genes in Andean and Mesoamerican cultivars is crucial for further research on pyramiding genes from different cultivars. This approach could potentially increase the resistance spectrum of common bean cultivars to the causative agent of anthracnose. The use of resistant cultivars to pyramid genes could provide a more sustainable and effective approach to managing anthracnose in common bean crops. This study contributes to the development of new breeding strategies that can help reduce the negative impact of anthracnose on common bean production.

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