Genetic resistance to *Colletotrichum lindemuthianum* in the Andean cultivar Jalo Pintado 2 of common bean

Angélica AT Frias[1], Maria C Gonçalves-Vidigal[1,*], Danielle SY Nanami[1], Sandra AL Castro[1], Pedro S Vidigal Filho[1] and Giselly F Lacanallo[1]

[1]Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. *Corresponding author: mcgvidigal@uem.br

ABSTRACT

The anthracnose caused by fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is the most widespread disease and economically important fungal disease of common bean (*Phaseolus vulgaris* L.). The use of resistant cultivars is considered as one of the most effective methods in controlling this disease. The present study had as aim to characterize the genetic resistance of the Andean common bean cultivar Jalo Pintado 2 to the *C. lindemuthianum* through inheritance and allelism tests. The experiment was conducted under greenhouse conditions at Laboratório de Melhoramento do Feijão Comum e de Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at Universidade Estadual de Maringá, Paraná, Brazil. The results of the F_2 population from the crossing 'Jalo Pintado 2' (R) × Cornell 49-242 (S), inoculated with race 73 of *C. lindemuthianum*, adjusted to the ratio of 3R: 1S, demonstrating the action of a dominant gene in the cultivar Jalo Pintado 2. The allelism tests evidenced that the gene in the 'Jalo Pintado 2' is independent from those previously characterized: *Co-1, Co-2, Co-3, Co-3[4], Co-4, Co-4[2], Co-4[3], Co-5, Co-6, Co-11, Co-12, Co-13, Co-14, Co-15* and *Co-16*. This gene is also independent from those genes not yet named present in Paloma, Perla and Amendoim Cavalo cultivars. The authors propose the *Co-18* symbol to designate the new gene for resistance to anthracnose.

Key words: Allelism tests, anthracnose, inheritance of resistance, *Phaseolus vulgaris*.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a species of worldwide economic interest (Beebe et al., 2000). Domesticated for more than seven thousand years in two independent centers: Mesoamerican and Andean, this crop is a major source of carbohydrates, protein, fiber, vitamins and iron to human diet (Ansari et al., 2004; Gioia et al., 2012).

Besides its nutritional importance, the common bean is characterized as the most widely cultivated species of the genus *Phaseolus*, representing about 95% of the production worldwide of this genus (Gonçalves-Vidigal et al., 2013). However, diseases triggered by various fungi, viruses and bacteria can affect the productivity of the crops (Schwartz and Pastor Corrales 1989). Among the fungal diseases, anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is among the major diseases that affect bean crops in Brazil and worldwide (Pastor-Corrales and Tu 1989; Rava et al., 1994; Gonçalves-Vidigal et al., 2009, 2012). In favorable survival and development conditions, this pathogen can cause losses of up to 100% in the production of susceptible genotypes (Pastor-Corrales and Tu 1989; Barcelos et al., 2011).

Various strategies have been used to control anthracnose, however, the use of resistant cultivars is characterized as one of the most effective and economical methods (Zaumeyer and Thomas 1957; Singh et al., 1991; Mahuku et al., 2008; Ishikawa et al., 2008; Sousa et al., 2014). Faced the advantages of using resistant cultivars, there is a need for constant search for new sources of genetic resistance to *C. lindemuthianum* (Mahuku et al., 2002).

Currently, 20 resistance genes to anthracnose and four allelic series have been identified (Gonçalves-Vidigal et al., 2012; Trabanco et al., 2015; Sousa et al., 2015). Each of these genes confers resistance to specific races of C. lindemuthianum. However, certain genes have greater broad spectrum of resistance to this pathogen controlling multiple races (Balardin and Kelly, 1998; Kelly and Vallejo 2004). Studies conducted by Vidigal Filho et al. (2007) noted that some genotypes collected in Paraná showed spectrum of resistance to multiple races. Among these genotypes the Andean cultivar Jalo Pintado 2, presented 50% of resistance index, being resistant to races 9, 31, 65, 73, 95 and 453 of *C. lindemuthianum*. Experiments previously conducted in this study demonstrated that 'Jalo Pintado 2' also showed resistance to races 2, 7 and 2047 of this pathogen. This cultivar has no information about the anthracnose resistance inheritance and possible existing allelic reactions. In this light, this study aimed to characterize the genetic resistance of common bean Andean cultivar Jalo Pintado 2 to *C. lindemuthianum.*

MATERIAL AND METHODS

Plant materials

The present study was conducted at the Laboratório de Melhoramento do Feijão Comum e de Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at Universidade Estadual de Maringá, Paraná, Brazil. As shown in Figure 1, cultivar Jalo Pintado 2 is classified as belonging to the Andean gene pool common bean, and it was collected North West regions of the state of Paraná in Southern Brazil (Vidigal Filho et al., 2007). This cultivar has a determinate growth habit (Type I), large seeds with average 100-seed weight of 35.80 g, with a cylindrical shape, and beige color with red stripes. Based on these characteristics, this cultivar is classified as belonging to the Nova Granada race of common bean (Singh et al., 1991). 'Jalo Pintado 2' was evaluated for anthracnose resistance with 18 races of *C. lindemuthianum* and it exhibited resistance to races 2, 9, 31, 65, 73, 95, 449, 453, 1545 and 2047 (Vidigal Filho et al., 2007).

Figure 1. Cultivar Jalo Pintado 2: A - Large seeds, cream coloration with red stripes; B - Pink Flower; and C - Green pods with red streaks.

In order to characterize the resistance genes to anthracnose present in the cultivar Jalo Pintado 2, F_2 segregating populations derived from crosses between the cultivar Jalo Pintado 2 and differentials: Michigan Dark Red Kidney (MDRK), Cornell 49-242, Mexico 222, PI 207262, TO, TU, AB 136, G 2333 and Michelite were developed. Apart from this, crosses were also made between 'Jalo Pintado 2' and other cultivars that have resistance genes to *C. lindemuthianum*, as follows: Ouro Negro, Jalo Vermelho, Jalo Listras Pretas, Pitanga, Corinthiano, Crioulo 159, Amendoim Cavalo, Paloma and Perla. All cultivars used in this study were obtained from the common bean Germplasm Bank of Nupagri.

We sowed seeds of cultivars and differentials in plastic pots (capacity 5 dm3), containing peat-based substrate + fertilizer (NPK) in conditions of greenhouse, with daily irrigation. Nitrogen fertilization was used during the phenological period V_1, using ammonium sulphate (50g of ammonium sulphate 2 liters of water-1) by applying 250 mg of N in 50 mL of water vaso-1. The Potassium fertilization was performed in the R5 period, in the form of potassium chloride to promote the growth of plants.

During the flowering period, we performed hybridizations mainly in the morning hours (7:00 to 8:30 am) and late afternoon (4:00 to 6:00 pm), using sterile tweezers, with the cultivar Jalo Pintado 2 used both as female and male parental, subsequently identifying every cross.

Sowing populations

F_1 generation seeds from the crosses between the cultivars were sown in pots containing soil mix previously fertilized and sterilized. The F_1 plants were kept in greenhouse conditions to harvest the F_2 seeds, to be sown later, leading to obtain the F_2 generations. F_2 seeds were sown in plastic trays containing peat-based substrate. Approximately 100 seeds of each cross were seeded in order to obtain greater certainty of results, except in the crosses between cultivars Jalo Pintado 2 × PI 207262; Jalo Pintado 2 × TO; Jalo Pintado 2 × G 2333; Jalo Pintado 2 × Perla, using 103, 112, 157 and 103 seeds, respectively. The trays were kept in a greenhouse until the full emergence of the first trifoliate. Subsequently, they were acclimated in a controlled environment for about an hour and then proceeded to the inoculation.

C. lindemuthianum races

The races of *C. lindemuthianum*, used in the study were: 2, 65, 73 and 2047, all of them obtained from mycology collection of Nupagri and inoculum prepared in the Laboratório de Melhoramento do Feijão Comum e de Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura. Race 2 of Andean origin, was inoculated in F_2, from the

crosses between the cultivars Jalo Pintado 2 × Michelite and Jalo Pintado 2 × Mexico 222.

Race 65 was used in inoculations of F_2 populations of crosses between the cultivar Jalo Pintado 2 and cultivars: Cornell 49-242, PI 207262, TO, TU and Jalo Vermelho. The occurrence of Race 65, described by Rava et al. (1994), is widespread and considered one of the most common races in bean producing states of Brazil.

Race 73 of *C. lindemuthianum* was used in the inoculation of F_2 populations from the crosses 'Jalo Pintado 2' × 'Michigan Dark Red Kidney'; 'Jalo Pintado 2' × AB136; 'Jalo Pintado 2' × 'Ouro Negro'; 'Jalo Pintado 2' × 'Jalo Listras Pretas' and 'Jalo Pintado 2' × 'Pitanga', all involved cultivars that have resistance to this race of the pathogen. However, this race was also used for inoculation of the cross between 'Jalo Pintado 2' × Cornell 49-242, and this cultivar is susceptible to that race. This race has a high frequency in the state of Paraná (Thomazella et al., 2002).

The race 2047 was used in the F_2 populations of crosses between the cultivars Jalo Pintado 2 × G2333, Jalo Pintado 2 × Corinthiano, Jalo Pintado 2 × Crioulo 159, Jalo Pintado 2 × Amendoim Cavalo, Jalo Pintado 2 × Paloma and Jalo Pintado 2 × Perla, all cultivars involved have resistance to race 2047.

Inoculum preparation

The inoculum preparation followed the methodology proposed by Cárdenas et al. (1964), which consists in the multiplication of the spores of each *C. lindemuthianum* race in test tubes containing sterile pods (autoclaved twice for 20 minutes at 120°C) partially immersed in a water-agar medium. The subculture of the fungus in the pods was made in a laminar flow properly sterilized and incubated in BOD (Biochemical Oxygen Demand) at 20 ± 2°C for 14 days for subsequent inoculation.

Inoculation and incubation

After the period necessary for the development of the fungus, it pulled out of the pods from each tube, with the aid of a sterile tween 20 to a recipient containing distilled water autoclaved that was then filtered through a double layer of gauze, originating a spore suspension. For each race of the pathogen, we performed five counts with hemacytometer (Neubauer-Preciss camera). After counting, we adjusted the spore suspension to a concentration of 1.2 x 106 spores mL^{-1} of autoclaved distilled water.

The inoculation of the spore suspension in plants proceeded with an electric air compressor De Vilbiss type, number 15, from the adaptation of the method employed by Cárdenas et al. (1964). The inoculated plants were kept in a mist chamber for 72 hours at 20 ± 2°C with controlled lighting (12 hours 680 lux . 12 hours dark lighting-^{1}). After the incubation period, the trays were transferred to temperature with tables 22 ± 2°C, under artificial light, where they remained until the evaluations.

We first used this methodology on 12 differential cultivars to anthracnose in order to confirm the phenotypes of virulence of the races 2, 65, 73 and 2047 (Pastor-Corrales 1988; Mahuku and Riascos 2004). After confirming it, the races were inoculated in their F_2 populations of each cross, for further evaluation of the results.

Resistance inheritance test

The resistance inheritance test was applied in the F_2 population from the cross between the cultivars Jalo Pintado 2 × Cornell 49-242, in order to check how many genes act in the resistance reaction. At that cross, the cultivar Jalo Pintado 2 shows resistance to race 73 of *C. lindemuthianum*, while differential cultivar Cornell 49-242 is susceptibility.

Allelism test

The allelism test was conducted in the crosses where both cultivars showed resistant reaction to races 2, 65, 73 and 2047. With this test it was possible to evaluate the independence of the gene present in cultivar Jalo Pintado 2 from the genes previously characterized.

Symptoms evaluations

We performed visual assessment of symptoms of each seedling approximately ten days after inoculation using the severity scale proposed by Pastor-Corrales et al. (1995). The notes were assigned to the first trifoliate leaves, with values ranging from 1 to 9 on individual plants to evaluate the symptoms induced by the physiological strains. Plants that showed notes 1 to 3 (no symptoms or only with few lesions midrib of the leaves) were considered as resistant (R) and those with grades 4 to 9, were considered susceptible (S).

Statistical analysis

Based on the data obtained by Mendelian segregation of resistance and susceptibility phenotypes, using the computer resource Genes program (Cruz 2016), this study conducted a genetic-statistical analysis by applying the chi-square test (χ^2).

RESULTS AND DISCUSSION

Resistance inheritance test

The segregation analysis of 73 resistant and 27 susceptible plants in the F_2 population set the ratio of 3R: 1S (Table 1). It indicates that the action of a dominant gene present in the cultivar Jalo Pintado 2, once the gene of Mesoamerican cultivar Cornell 49-242 (*Co-2*) does not act in this reaction, because this cultivar is susceptible to the race in study.

Table 1. Inheritance of resistance of the cultivar Jalo Pintado 2 inoculated with race 73 of *C. lindemuthianum* (Maringá, PR, 2013).

Cross	Race	F2 Generation		x^2	*P* Value
		Observed Ratio	Expected Ratio		
		R:S	R:S		
Jalo Pintado 2 × Cornell 49-242	73	73:27	3:1	0.213	0.644

The results obtained in this study was similar to those results described by Gonçalves-Vidigal and Kelly (2006), by inoculating race 73 of *C. lindemuthianum* in the F_2 population derived from the cross between Widusa × Cornell 49-242 cultivars. The authors observed a segregation ratio of 3R: 1S (x^2 = 0.002; p = 0.96), concluding that the cultivar Widusa has a dominant allele, which confers resistance to race 73 of *C. lindemuthianum*, which is called *Co-15*. Similarly, Gonçalves-Vidigal et al. (2009), evaluating the F_2 population of the cross between the cultivars Jalo Listras Pretas × Cornell 49-242, obtained segregation of 3R: 1S (x^2 = 0.023; p = 0.88), indicating that resistance to race 73 is conferred by an independent dominant gene, present in the cultivar Jalo Listras Pretas, named as *Co-13*.

The resistance inheritance study with race 73 of *C. lindemuthianum*, is very important for breeding programs, since this breed has a high occurrence in Brazil and especially in the state of Paraná (Thomazella et al., 2002; Ribeiro et al., 2016). By comparing the result obtained for the cultivar Jalo Pintado 2 with the other Andean cultivars (Michigan Dark Red Kidney, Perry Marrow, Kaboon, Widusa, Jalo Vermelho, Jalo Listras Pretas, Corinthiano and Pitanga), whose genes are resistant to anthracnose identified, it is clear that only cultivate Jalo Vermelho presents susceptibility to race 73 of *C. lindemuthianum* (Vidigal Filho et al., 2007).

Based on the data obtained, the chi-square test showed that the resistance of the cultivar Jalo Pintado 2 to *C. lindemuthianum* is monogenic, which means, the resistance in the cultivar Jalo Pintado 2 is conferred by an Andean dominant gene.

Allelism test

Table 2 shows the allelic relations between the resistance gene to anthracnose present in the cultivar Jalo Pintado 2 and the other resistance genes previously characterized in eight Andean cultivars namely Michigan Dark Red Kidney, Jalo Vermelho, Jalo Listras Pretas, Pitanga, Corinthiano, Amendoim Cavalo, Paloma and Perla; and the 10 Mesoamerican cultivars Mexico 222, Michelite, Cornell 49-242, TO, PI 207262, TU, AB 136, Ouro Negro, G 2333, Crioulo 159. In allelism study conducted in F_2 population derived from the cross between Jalo Pintado 2 × Michelite, segregation set the ratio 15R: 1S (p = 0.978) when inoculated with race 2. This result shows the action of two independent dominant genes for resistance to anthracnose, one of them present in Michelite (*Co-11*) and another one in the cultivar Jalo Pintado 2.

The monogenic inheritance of differential cultivar Michelite was characterized by Gonçalves-Vidigal et al. (2007), by inoculating the races 8 and 64 of *C. lindemuthianum* in 14 F_2 populations from the cross with Michelite. The authors noted the presence of a dominant gene independent of the previously characterized *Co-1, Co-2, Co-3, Co-4, Co-5, Co-6, Co-35, Co-9* and $Co\text{-}3^4$ alleles, and this called *Co-11*.

The segregation achieved with the cross between the cultivars Jalo Pintado 2 × Mexico 222, inoculated with the race 2 of *C. lindemuthianum* was 98R: 2S, adjusting the ratio 63R: 1S, with a probability of 0.724. This segregation indicates the action of three dominant genes for resistance reaction to that race, two of these genes present in cultivar Mexico 222 (*Co-3* and *Co-?*) and another one in Jalo Pintado 2. Coelho et al. (2013) also found the evidence of the presence of two resistance genes in cultivar Mexico 222, by inoculating the race 2 of *C. lindemuthianum* in a F_2 population derived from the cross between Crioulo 159 × Mexico 222, with a probability of 0.83.

In the F_2 generations derived from the crosses between the cultivar Jalo Pintado 2 and cultivars Cornell 49-242 (p = 0.974), TO (p = 1), PI 207262 (p = 0.858), TU (p = 0.621), AB 136 (p = 0.942) and Jalo Vermelho (p = 0.917), inoculated with the race 65, segregations set to the ratio 15R: 1S.

Table 2. Allelism test in F_2 population of the cross between the common bean cultivars inoculated with races 2, 65, 73 and 2047 of *C. lindemuthianum* (Maringá, PR, 2013).

Crosses	Race	Resistance Genes	F_2 Generation				
			Observed Plants		Expected Ratio		*P* Value
			R	S	R:S	χ^2	
JP 2 × Mexico 222	2	*Co-3, Co-?*	98	2	63:1	0.124	0.724
JP 2 × Michelite	2	*Co-11*	89	6	15:1	0.001	0.978
JP 2 × Cornell 49-242	65	*Co-2*	61	4	15:1	0.001	0.974
JP 2 × TO	65	*Co-4*	105	7	15:1	0.000	1.000
JP 2 × PI 207262	65	*Co-4*^{3}	97	6	15:1	0.031	0.858
JP 2 × TU	65	*Co-5*	94	5	15:1	0.243	0.621
JP 2 × AB 136	65	*Co-6*	108	7	15:1	0.005	0.942
JP 2 × Jalo Vermelho	65	*Co-12*	94	5	15:1	0.010	0.917
JP 2 × MDRK[a]	73	*Co-1*	91	6	15:1	0.001	0.979
JP 2 × Ouro Negro	73	*Co-3*^{4}	94	6	15:1	0.010	0.917
JP 2 × JLP[b]	73	*Co-13*	80	5	15:1	0.019	0.888
JP 2 × G 2333	2047	*Co-4*^{2}	147	10	15:1	0.003	0.950
JP 2 × Pitanga	2047	*Co-14*	111	7	15:1	0.020	0.886
JP 2 × Corinthiano	2047	*Co-15*	87	6	15:1	0.006	0.936
JP 2 × Crioulo 159	2047	*Co-16*	93	5	15:1	0.220	0.638
JP 2 × A. Cavalo[c]	2047	NN[d]	94	6	15:1	0.010	0.917
JP 2 × Paloma	2047	NN[d]	90	6	15:1	0.000	1.000
JP 2 × Perla	2047	NN[d]	97	6	15:1	0.031	0.858

[a]Michigan Dark Red Kidney; JLP; [b]Jalo Listras Pretas; [c]Amendoim Cavalo; and [d]Not Named Gene.

As shown in Table 2, it can be seen that the rate of segregation indicates the action of two independent dominant genes, acting in resistance to anthracnose in each cross. Therefore, it is observed that this dominant gene in 'Jalo Pintado 2' differs from *Co-2, Co-4, Co-4*^{3}, *Co-5, Co-6* and *Co-12*, respectively.

These results are consistent with those obtained by Gonçalves-Vidigal et al. (2012), which also observed segregation 15R: 1S, in allelic studies in populations derived from crosses between 'Pitanga' and cultivars Cornell 49-242 (p = 0.96), TU (p = 1), AB 136 (p = 0.92) and Jalo Vermelho (p = 0.59), by inoculating the races 64 and 65 of *C. lindemuthianum*. However, by inoculating the 64 race at the cross of 'Pitanga' × PI 207262, observed a segregation 63R:1S (p = 0.77), due to the performance of three resistance genes, two of them present in PI 207262 and another in Pitanga. Although this cultivar has two resistance alleles: *Co-3*^{3} and *Co-4*^{3}, but only *Co-4*^{3} confers resistance to Race 65 of *C. lindemuthianum*. This fact explains ratio 15R:1S, characterized in F_2 population derived from a cross between the cultivar Jalo Pintado 2 x PI 207262.

The ratio 15R:1S (p = 0.979), presented in the F_2 generation of crossing 'Jalo Pintado 2' × 'Michigan Dark Red Kidney', when inoculated with race 73, indicates the segregation of two independent dominant genes with each other. In studies with the cultivar Michigan Dark Red Kidney, Gonçalves-Vidigal et al. (2009) also obtained segregation 15R:1S by inoculating 73 race in F_2 populations of crosses between cultivars Jalo Listras Pretas × MDRK, with a probability of 0.62. Resistance to race 73 of *C. lindemuthianum* in the Andean cultivar Michigan Dark Red Kidney, is conferred by the *Co-1* gene (McRostie 1919), which is one of the first resistance genes characterized to anthracnose.

The race 73 of *C. lindemuthianum* was inoculated in the F_2 populations of crosses between 'Jalo Pintado 2' × 'Ouro Negro' and 'Jalo Pintado 2' × 'Jalo Listras Pretas'. Segregation of 15R:1S (p = 0.917) obtained at the cross of 'Jalo Pintado 2' × 'Ouro Negro', which indicates monogenic interaction of resistance in cultivar Jalo Pintado 2. This interaction was also observed in the cross between the cultivars Jalo Pintado 2 × Jalo Listras Pretas with probability of 0.888. Thus, it reinforces the hypothesis that the existing gene in the cultivar Jalo Pintado 2 is independent of previously identified genes in the cultivars Ouro Negro (*Co-3*^{4}) and Jalo Listras Pretas (*Co-13*).

The F_2 generations from the cross between 'Jalo Pintado 2' and cultivars G 2333 and Pitanga, presented segregation ratio of 15R:1S, indicating the action of two dominant genes for resistance to race 2047 of *C. lindemuthianum*, at each crossing.

This fact highlights the independence of this gene present in the cultivar Jalo Pintado 2 in relation to genes for *Co-4*^{2} present in G 2333 and *Co-14* present in Pitanga, with the probability of 0.950 and 0.886, respectively. Although cultivar G 2333 presents three resistance genes (*Co-4*^{2}, *Co-5*^{2}, and *Co-3*^{5}) to anthracnose (Young et al., 1998; Vallejo and Kelly 2009), only the *Co-4*^{2} gene provides resistance to race 2047 of *C. lindemuthianum.*

The allelic study of F_2 populations derived from crosses between the cultivars Jalo Pintado 2 × Corinthiano and Jalo Pintado 2 × Crioulo 159, has shown segregation 15R:1S, with a probability of 0.936 and 0.638 respectively. This segregation again points the independence of the gene present in 'Jalo Pintado 2', which differs from the *Co-15* gene present in cultivar Corinthiano (Sousa et al., 2015) and *Co-16* present in cultivar Crioulo 159 (Coimbra-Gonçalves et al., 2016).

Finally, we also used the 2047 race in the allelic test, inoculating it in the F_2 generation derived from crosses between the cultivars Jalo Pintado 2 × Amendoim Cavalo, Jalo Pintado 2 × Paloma and Jalo Pintado 2 × Perla data from chi-square tests set up at the ratio 15R:1S. These results indicate that the resistance in Jalo Pintado 2 is conditioned by a dominant gene, which segregates independently of the genes in cultivars Amendoim Cavalo, Paloma and Perla, the latter being identified to date, but not yet named (Nanami et al., 2014; Castro et al., 2014).

CONCLUSION

The segregation observed in allelic tests showed that the cultivar Jalo Pintado 2 presents an independent dominant gene from those previously characterized: *Co-1, Co-2, Co-3, Co-3*^{4}*, Co-4, Co-4*^{2}*, Co-4*^{3}*, Co-5, Co-6, Co-11, Co-12, Co-13, Co-14, Co-15* e *Co-16*, and also from the genes present in Paloma, Perla and Amendoim Cavalo cultivars. The authors propose the *Co-18* symbol to name the anthracnose resistance gene in 'Jalo Pintado 2'. This gene can contribute to future common bean breeding programs and commercial cultivars, with the purpose of increasing the spectrum of resistance to anthracnose.

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