

Characterization of the Angular Leaf Spot Resistance Gene Present in Common Bean Cultivar Ouro Negro

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Abstract

Angular leaf spot (ALS), incited by the fungus *Pseudocercospora griseola*, is a major disease affecting common bean. Important resistance sources have been identified and characterized: AND 277, BAT 332, Cornell 49-242, MAR-2, and Mexico 54. *P. griseola* presents high physiological variability. The control of this kind of pathogen requires continuous evaluation, including monitoring and characterization of pathogenic variability, and eventual germplasm introgression of genes in adapted cultivars. Cultivar Ouro Negro is currently being used as an ALS resistance source in Brazil. This work aimed to characterize the ALS resistance gene present in cultivar Ouro Negro in relation to the other five resistance sources. These cultivars were crossed with ‘Ouro Negro’ (allelism tests) and with susceptible cv. Rudá (inheritance studies) and their segregation patterns were evaluated. The inheritance studies and the allelism tests indicate that ‘Ouro Negro’ harbors at least one resistance locus which is distinct from those present in the other resistance sources.

Keywords: allelism test, inheritance studies, *Phaseolus vulgaris*, *Pseudocercospora griseola*

1. Introduction

Angular leaf spot, incited by *Pseudocercospora griseola* (Sacc.) Crous and Braun, is among the fungal diseases causing great damage to common bean growers (Rava, 2002). ALS occurs essentially in all bean growing regions mainly under mild temperatures and high humidity. These conditions, combined with the use of susceptible cultivars, favor the occurrence of the disease, leading to losses that may reach over 80% of the production. The high pathogenic variability of *P. griseola* is an obstacle for the development of resistant cultivars (Nietsche et al., 2001; Sartorato, 2006; Sartorato & Alzate-Marin, 2004; Silva, Souza, Sartorato, & Freire, 2008).

Pastor-Corrales and Jara (1995) characterized 316 isolates of the pathogen from 11 Latin American and 10 African countries, based on a set of differential cultivars and found that 82 were Andean, 193 Mesoamerican and 41 of unknown origin. Although the isolates have shown considerable variation between and within countries, it was possible to conclude that *P. griseola* and the common bean co-evolved. It was also observed that, in general, Mesoamerican isolates are more virulent than those of Andean origin.

Stable resistance to plant pathogens with high physiological variability requires monitoring of isolates and race characterization in addition to continuous germplasm evaluation and eventual introgression of resistance genes in adapted cultivars (Young & Kelly, 1996; Iwo, Itah, & Osai, 2012). To facilitate this process, and to identify new resistance sources, the inheritance of resistance genes needs to be determined. In addition, allelism tests are required to determine possible relationships among these genes, aiming to orient crosses and possible association (pyramiding) of different resistance genes in the same background.

In Brazil, ‘AND 277’, ‘BAT 332’, ‘Cornell 49-242’, ‘MAR-2’, and ‘Mexico 54’ are important ALS resistance sources (Balbi et al., 2009; Nietsche, et al., 2001; Sartorato & Alzate-Marin, 2004; Silva et al., 2008). The resistance present in these genotypes in crosses with susceptible cultivar Rudá was shown to be monogenic and dominant (Caixeta et al., 2003; Carvalho et al., 1998; Ferreira et al., 2000; Nietsche et al., 2000; Sartorato et al.,

2000). However, using allelism tests with these same cultivars Caixeta et al. (2005) demonstrated that the complexity involving the resistance loci in these sources is greater than previously thought.

Cultivar Ouro Negro (Honduras 35) harbors an ALS resistance gene, provisionally designated *Phg-ON*, which has been shown to confer a broad resistance spectrum to ALS (Balbi et al., 2009; Sanglard et al., 2009a; Sartorato, 2006). This cultivar presents desirable agronomic and cooking characteristics, high combining ability and has been used in Brazil as an ALS resistance source (Sanglard et al., 2005; Sartorato, 2006). Ouro Negro is also used as an anthracnose and rust resistance source in Brazil. It is resistant to *Uromyces appendiculatus* races 6, 7, 9, 10, 12, 15, 35, 45, 49, 50, and 59 and to most *Colletotrichum lindemuthianum* races except for race 65 (Faleiro et al., 2001; Ragagnin et al., 2003). This study aimed to characterize the ALS resistance gene present in cultivar Ouro Negro in relation to the other five ALS resistance sources previously characterized.

2. Materials and Methods

2.1 Genetic Material

Seeds of the common bean genotypes used in the present study were obtained from: International Center for Tropical Agriculture (CIAT, Cali, Colombia), Germplasm Bank of the Biotechnology Institute - BIOAGRO (UFV, Viçosa, MG, Brazil), USDA (Beltsville, Maryland, USA) and Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil).

2.2 Inheritance Studies and Allelism Tests

For the inheritance studies, genotypes Ouro Negro, BAT 332, Cornell 49-242, MAR-2 and Mexico 54 were crossed with Rudá (female parent). For the allelism studies, genotypes AND 277, BAT 332, Cornell 49-242, MAR-2 and Mexico 54 were crossed with Ouro Negro (male parent). To ensure that the F_1 seeds were true hybrids, we analyzed the color, shape, size and brightness of the corresponding F_2 seeds. In the case of the cross between 'Ouro Negro' and 'Cornell 49-242', which are phenotypically very similar, the F_1 seeds were submitted to a molecular analysis with marker SF10_{1050a} (Corrêa et al., 2000) according to a procedure described by Alzate-Marin et al. (1996). The F_2 segregating populations were used in artificial inoculations.

2.3 Fungal Races and Inoculum Preparation

The *P. griseola* isolates (races 31.17, 63.19, 63.23, and 63.39) used in the study were provided by the BIOAGRO/UFV Fungal Collection. These races are the same ones used by Caixeta et al. (2005) in allelism tests. The races showing incompatibility with 'Ouro Negro' and the other ALS resistance sources were used in the allelism tests and in the inheritance studies involving susceptible cultivar Rudá. The inoculum consisted of a spore suspension prepared by scraping the surface of 12-day-old fungal colonies grown according to Sanglard et al. (2009b). For inoculation the suspension was diluted to 2.0×10^4 conidia/mL.

2.4 Inoculation and Disease Assessment

Seeds of each genotype were sown in plastic pots of 2.5 L containing a mixture of soil and dry manure at a ratio of 4:1, and NPK (4-14-8) in a proportion of 5 kg per m³ of substrate. Twelve plants of each progenitor and all F_2 plants were inoculated when they were at the V3 stage (14-16 days old) at a density of four plants per pot. Inoculations were performed on the adaxial and abaxial surfaces of the first trifoliate leaf with the aid of a DeVilbiss atomizer no. 15. After inoculation, the plants were transferred to a mist chamber (20 ± 1 °C and relative humidity >95%), where they remained for 48 h, under a 12-h photoperiod. Then the plants were again transferred to the greenhouse, where they remained until they were evaluated. Disease severity was assessed visually at 18 and 21 days after inoculation using a nine-degree severity scale (Van-Schoonhoven & Pastor-Corrales, 1987). Plants with grades ≤ 3 were assumed to be resistant, and those graded 4 or higher, susceptible.

2.5 Statistical Analysis

For the statistical analyses the GENES software (Cruz, 2006) was used. Nine phenotypic ratios were considered: 3:1 (one dominant gene), 15:1 (two dominant independent genes), 9:7 (two dominant complementary genes), 13:3 (two epistatic genes, one dominant and one recessive), 63:1 (three independent dominant genes), 57:7 (one dominant and two complementary genes), 27:37 (three dominant complementary genes), 61:3 (two dominant and one recessive gene), 49:15 (one dominant and two recessive genes). The chi-square test was used to indicate the most probable segregation ratio.

3. Results and Discussion

P. griseola races 63.23 and 63.39 were chosen to be used in the allelism tests because they were incompatible with 'Ouro Negro' and with the other ALS resistance sources used in the crosses (Table 1). These two races were also used in the inheritance studies (Table 2).

The segregation ratio of 3:1 obtained in the crosses between susceptible cultivar Rudá and Ouro Negro confirmed that Ouro Negro harbors at least one gene conferring resistance to *P. griseola* races 63.23 and 63.39 (Table 2). The same conclusion can be inferred from the segregation ratio (3:1) obtained in the crosses Rudá x AND 277, Rudá x BAT 332, and Rudá x MAR-2. In the case of Mexico 54, two resistance genes are involved (segregation ratio of 15:1) (Table 2).

Table 1. Reaction of bean genotypes to different races of *Pseudocercospora griseola*

Genotypes	Races			
	63.23	63.39	63.19	31.17
AND 277	^a R	^b S	R	R
BAT 332	S	R	S	S
Cornell 49-242	R	S	R	R
MAR-2	R	R	R	R
Mexico 54	R	R	R	R
Ouro Negro	R	R	S	S
Rudá	S	S	S	S

^aR: resistant; ^bS: susceptible.

In previous works, cultivar Rudá was crossed with the following resistance sources: AND 277 (Carvalho et al., 1998), MAR-2 (Ferreira et al., 2000), Mexico 54 (Sartorato et al., 2000), Cornell 49-242 (Nietsche et al., 2000) and BAT 332 (Caixeta et al., 2003). The segregation analysis indicated that resistance was monogenic and dominant. However, the use of different crosses and different races showed greater complexity than was previously reported. According to Caixeta et al. (2005) Cornell 49-242 harbors only one dominant gene (*Phg-3*), Mexico 54 harbors three genes (*Phg-2*, *Phg-5*, *Phg-6*), MAR-2 harbors two genes (*Phg-4* and *Phg-5*², an allelic form of *Phg-5*), BAT 332 has the allelic form *Phg-6*², and AND 277 harbors the allelic forms *Phg-2*², *Phg-3*² and *Phg-4*².

Table 2. Segregation of angular leaf spot resistance genes in common bean F₂ populations inoculated with *Pseudocercospora griseola* races 63.23 or 63.39

F ₂ Population	Race	N° of F ₂ plants		Expected ratio		χ^2 value	°P (%)
		^a R	^b S	R	S		
Rudá x Ouro Negro	63.23	226	71	3	1	0.1898	66.3187
Rudá x Ouro Negro	63.39	354	111	3	1	0.3161	57.3943
^d Rudá x AND 277	63.23	145	48	3	1	0.0017	96.6850
Rudá x BAT 332	63.39	396	129	3	1	0.0514	82.0595
Rudá x Mexico 54	63.23	376	28	15	1	0.3194	57.1950
Rudá x Mexico 54	63.39	355	22	15	1	0.1105	73.9551
Rudá x MAR-2	63.23	387	125	3	1	0.0937	75.9462
^e Rudá x MAR-2	63.39	121	37	3	1	0.2109	64.6007
Rudá x Cornell 49-242	63.23	303	98	3	1	0.0673	79.5262
AND 277 x Ouro Negro	63.23	432	25	15	1	0.4739	49.1170
BAT 332 x Ouro Negro	63.39	404	92	13	3	0.0132	90.8413
Mexico 54 x Ouro Negro	63.23	551	8	63	1	0.0627	80.2239
Mexico 54 x Ouro Negro	63.39	376	4	63	1	0.6422	42.2889
MAR-2 x Ouro Negro	63.23	415	24	15	1	0.4593	49.7915
MAR-2 x Ouro Negro	63.39	398	28	15	1	0.0757	78.3150
Cornell 49-242 x Ouro Negro	63.23	396	25	15	1	0.0698	79.1579

^aR: resistant; ^bS: susceptible; ^cP (%): percent probability; ^d data obtained by Carvalho et al. (1998); ^e data obtained by Ferreira et al. (2000).

Based on the assessment depicted in Table 1 race 63.23 was inoculated in four F₂ populations for allelism tests (Table 2). For the F₂ populations AND 277 x Ouro Negro, Cornell 49-242 x Ouro Negro, and MAR-2 x Ouro Negro a typical segregation pattern of two genes (15:1) was obtained, indicating that each of these genotypes contributes with a dominant gene for the resistance reaction. For the population Mexico 54 x Ouro Negro a segregation ratio of 63:1 was obtained, indicating the action of two of the three genes characterized in Mexico 54 for resistance to this race (Caixeta et al., 2005).

Inoculations with race 63.39 produced similar results to those observed with race 63.23. The F₂ populations Mexico 54 x Ouro Negro and MAR-2 x Ouro Negro inoculated with race 63.39 also showed segregation ratios of 63:1 and 15:1, respectively. The F₂ population BAT 332 x Ouro Negro segregated in a proportion of 13:3, which is typical of an inheritance involving two epistatic genes, one dominant and one recessive. The F₂ populations AND 277 x Ouro Negro and Cornell 49-242 x Ouro Negro were not tested with race 63.39 because Cornell 49-242 and AND 277 are susceptible to this race (Table 1). Following the same reasoning the F₂ population BAT 332 x Ouro Negro was not inoculated with race 63.23 because BAT 332 was susceptible to this race (Table 1).

Our results strongly indicate that Ouro Negro harbors one ALS resistance gene or complex gene locus which confers resistance to both races 63.23 and 63.39. This is supported by the observation that inoculation of F₂ population Mexico 54 x Ouro Negro with these two races yielded the same segregation ratio of 63:1. This was also the case for F₂ population MAR-2 x Ouro Negro (segregation ratio of 15:1) (Table 2). Furthermore, inheritance studies (Rudá x Ouro Negro) involving inoculation by these two races revealed a 3:1 segregation pattern typical of a monogenic dominant inheritance (Table 2). In addition, considering the segregation ratios obtained in all five F₂ populations, it can be inferred that at least one ALS resistance locus present in 'Ouro Negro' differs from those present in AND 277, BAT 332, Cornell 49-242, MAR-2, and Mexico 54.

We suggest the 'Ouro Negro' ALS resistance gene is distinct from the other ALS resistance sources characterized so far, and be named *Phg-7*. This classification would be consistent with the work done by Caixeta et al. (2005).

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