VARIABILITY AMONG PSEUDOCERCOSPORA GRISEOLA ISOLATES BY RAPD MARKERS

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INTRODUCTION: Angular leaf spot disease of common bean (*Phaseolus vulgaris*), caused by *Pseudocercospora griseola*, is one of the most important of this crop in Brazil. Control strategies include, mainly, the development of resistant cultivars. However, a strategy to control and/or reduce the impact of *P. griseola* requires a previous knowledge of the population structure of this fungus. Understanding the pathogenic variability is a fundamental point in breeding program. Therefore, the purpose of this study was to investigate the genetic diversity and population's genetic structure among *P. griseola* isolates collected in Minas Gerais and Goiás state, Brazil.

MATERIALS AND METHODS: 70 isolates of *P. griseola* obtained from naturally-infected common bean cultivars were used in this study. Isolates were collected in the states of Minas Gerais (Ijací, Lambarí, Lavras and Viçosa) and Goiás (Damolândia), Brazil, as shown in Table 1. *P. griseola* isolates were grown in liquid medium for 15 days (110 rpm at 20°C) and the DNA was extracted according to the methodology developed by Raeder & Broda (1985) with minor modifications. The RAPD reactions were carried out with the *primers* OP AN11, OP AP18, OP AQ01, OP AQ02, OP AQ03, OP AQ04, OP AQ08, OP AS03, OP AS04, OP AS05, OP AS06, OP AS07, OP AS08, OP AS11, OP AS15, OP AS19, OP AT19, OP BB06 e OP BB08 and performed in a final volume of 14 µl containing 4 µl water, 35 ng of genomic DNA, 50 µM of each dNTP and 0.4 µM oligonucleotide *primer*, 50 mM Tris-HCl, pH 8.0, 2.0 mM $MgCl_2$, 20 mM KCl, and 0.6 units Taq DNA polymerase. Amplification was programmed for 1 initial desnaturation cycle (94°C for 2 minutes), followed by 38 cycles of 2 minutes at 94°C, 15 seconds at 37°C and 1 minute at 72°C and a final extension step of 2 minutes. Amplicons were separated by electrophoresis, visualized under UV light and photographed with the Kodak EDA – 290 camera. The genetic similarities and clustering analysis were performed by using the Nei and Li coefficient and UPGMA, respectively. The analysis of molecular variance (AMOVA) was also performed.

RESULTS AND DISCUSSION: The *primers* amplified a total of 76 polymorphic bands, with an average of 4.0 polymorphic bands per *primer*. The genetic similarity among the isolates varied from 0.301 to 0.993 with an average of 0.746. The descriptive analyses revealed a tendency of differentiation of isolates by origin areas. The Shannon diversity index revealed that Viçosa, MG, presented the largest genetic diversity, whereas Ijaci, MG, presented the smallest genetic diversity. The total Nei's genetic diversity was partitioned (H_T = 0.3535). The genetic differentiation among the populations was 0.1979 (G_{ST} value). Therefore, 80.21% of the genetic variation observed in this study was due to differentiation within populations. AMOVA demonstrated that 77.51% of the variation was contained within places and 22.49% among places (Table 2). Pairwise comparisons of 76 polymorphic RAPD loci gave disequilibrium values that were all significantly different from zero (Fisher's exact test, $P < 0.05$) for the studied populations, showing that *P. griseola* maintains a genetic structure consistent with asexual reproduction.

Table 1 *Pseudocercospora griseola* isolates (Isol.), counties (C.) and patothypes (Pat.) of Minas Gerais (MG) and Goiás (GO) State, Brazil.

Isol.	C.[1/]	P.	Isol.	C	Pat.	Isol.	C.	Pat.
Ig - 792	DA	63-31	Pg - 16	IJ	63-31	Pg - 52	LM	-
Ig - 799	DA	63-63	Pg - 17	IJ	63-31	Pg - 53	LM	-
Ig - 802	DA	63-63	Pg - 19	LV	63-47	Pg - 54	LM	-
Ig - 806	DA	63-31	Pg - 20	LV	63-63	Pg - 55	LV	-
Ig - 808	DA	63-63	Pg - 21	LV	63-63	Pg - 56	LV	-
Ig - 809	DA	63-31	Pg - 23	LV	63-63	Pg - 57	LV	-
Ig - 822	DA	63-63	Pg - 24	LV	63-63	Pg - 58	LV	-
Ig - 828	DA	63-31	Pg - 25	LV	63-63	Pg - 59	LV	-
Ig - 854	DA	63-31	Pg - 26	LV	63-31	Pg - 60	LV	-
Ig - 860	DA	63-63	Pg - 27	LV	63-31	Pg - 61	LV	
Ig - 865	DA	63-31	Pg - 28	LV	63-63	Pg - 62	LV	-
Ig - 868	DA	63-63	Pg - 31	LV	63-63	Pg - 63	LM	-
Pg - 01	IJ	63-47	Pg - 32	LV	63-31	Pg - 64	LV	-
Pg - 02	IJ	63-15	Pg - 33	LV	63-63	Pg - 65	LV	-
Pg - 03	IJ	63-47	Pg - 34	LV	63-63	Pg - 67	LV	-
Pg - 04	IJ	63-63	Pg - 35	LV	63-63	Pg - 68	LV	-
Pg - 05	IJ	63-31	Pg - 41	LV	63-31	Pg - 69	LV	-
Pg - 06	IJ	63-31	Pg - 45	LV	63-63	Pg - 70	VI	-
Pg - 07	IJ	63-55	Pg - 46	LV	63-63	Pg - 71	VI	-
Pg - 08	IJ	63-15	Pg - 47	LV	63-63	Pg - 72	VI	-
Pg - 09	IJ	63-31	Pg - 48	IJ	63-63	Pg - 73	VI	-
Pg - 10	IJ	63-63	Pg - 50	LM	-	Pg - 74	LM	-
Pg - 12	IJ	63-23	Pg - 51	LM	-	Pg - 75	LM	-
Pg - 15	IJ	63-31						

[1/]County: DA: Damolândia (GO); IJ: Ijací (MG); LM: Lambari (MG); LV: Lavras (MG); VI: Viçosa (MG).

Table 2 Summary of AMOVA for five places of occurrence of *P. griseola*, evaluated by RAPD markers.

S.V.	DF	SS	Variance components	% Total	Φ_{ST}	P
Among places	4	206.593	3.2463	22.49	0.2249	0.000
Within places	65	727.079	11.1858	77.51		
Total	69	933.672	14.4321	100.00		

CONCLUSIONS: The existence of high variability has been demonstrated by RAPD markers. *P. griseola* maintains a genetic structure consistent with asexual reproduction.

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REFERENCE

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