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Embrapa Meio-Norte, Teresina, PI, Brazil

Pathogenic Variability of Isolates of *Pseudocercospora griseola*, the Cause of Common Bean Angular Leaf Spot, and its Implications for Resistance Breeding

Kaesel Jackson Damasceno e Silva[1], Elaine Aparecida de Souza[2], Aloisio Sartorato[3] and Cassius nonato de Souza Freire[2]

Authors' addresses: [1]Embrapa Meio-Norte, Avenue Duque de Caxias, 5650, 64006-220 Teresina, PI, Brazil; [2]Laboratório de Resistência de Plantas a Doenças e Genética Molecular, Departamento de Biologia, Universidade Federal de Lavras – UFLA, PO Box 37, 37200-000 Lavras, MG, Brazil; [3]Embrapa Arroz e Feijão, PO Box 179, Santo Antônio de Goiás, GO, Brazil (correspondence to Kaesel Jackson Damasceno e Silva. E-mail: kaesel@cpamn.embrapa.br)

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Abstract

The pathogenic variability was evaluated of 48 *Pseudocercospora griseola* isolates collected in the State of Minas Gerais, Brazil. Isolates were inoculated to a set of 12 international differential cultivars in a greenhouse. Ten pathotypes (55-15, 63-7, 63-15, 63-23, 63-25, 63-27, 63-31, 63-47, 63-55 and 63-63) were identified, showing the great pathogenic variability of this fungus in Minas Gerais State. Pathotypes 55-15, 63-15, 63-25 and 63-27 had not previously been reported in the State. Of the 48 isolates, all except pathotype 55-1547 induced a compatible reaction with all cultivars from the Andean group. Isolates were highly pathogenic in both Andean and Mesoamerican cultivars, thus being classified as Mesoamerican pathotypes. Pathotype 63-63 was the most widespread, and overcame the resistance genes present in all differential cultivars.

Introduction

Common bean (*Phaseolus vulgaris* L.) is susceptible to several pathogens, including *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, previously known as *Phaeoisariopsis griseola*, which causes angular leaf spot (ALS) disease. This disease is responsible for significant crop damage in Brazil and can result in yield losses of up to 70%, depending on the susceptibility of the cultivars, environmental conditions and the time of the outbreak of the disease (Sartorato, 2004).

Control strategies mainly include foliar spraying with fungicides which, however, can seriously reduce profitability and threaten the environment (Sartorato, 2004; Miklas et al., 2006) and the development of resistant cultivars. Consequently, the development of resistant cultivars is pivotal to any effective, economical and environmental-friendly strategy used to control ALS.

Guzmán et al. (1995) and Pastor-Corrales and Jara (1995) provided evidence suggesting the coevolution of *P. vulgaris* and *P. griseola*. Knowledge of the host–pathogen interaction is essential for the development of adequate strategies to obtain ALS-resistant cultivars.

Genetic resistance to *P. griseola* can be monogenic and/or oligogenic (Mahuku et al., 2004; Miklas et al., 2006; Amaro et al., 2007). Due to the great pathogenic variability, a combination of genes from different resistance sources is needed to provide broad resistance to an array of pathotypes prevalent in a region (Miklas et al., 2006).

Constant evaluation of pathogenic variability and the identification of new resistant genes are of crucial importance for the development of adequate pathogen-resistant cultivars. Variability has been studied using a standard differential series with 12 cultivars proposed by CIAT (Pastor-Corrales and Jara, 1995), and divided into two sets (Mesoamerican and Andean), with six cultivars each.

Genetic diversity in *P. griseola* was determined in Minas Gerais State by Nietsche et al. (2001). Thirteen different pathotypes were identified among the 30 isolates studied, and the most frequently found pathotypes were 31-21, 31-23, 63-39, 63-55 and 63-23. Sartorato (2002) studied 51 isolates of *P. griseola* from five Brazilian states and observed the occurrence of seven different pathotypes (31-23, 55-31, 63-15, 63-23, 63-31, 63-39 and 63-63). In Brazil, Nietsche et al. (2002) detected high variability, totalling 26 different pathotypes among 73 isolates. Great genetic variability was also detected in other parts of the world (Mahuku et al., 2002a; b; Pastor-Corrales et al., 2004; Wagara et al., 2004; Orozco and Araya, 2005; Stenglein et al., 2005; Stenglein and Balatti, 2006). The occurrence of pathotype 63–63, which has a compatible reaction to

all differential cultivars, was mentioned in all papers cited above, which implies in a constant search for new resistance sources.

Our objective was to evaluate the pathogenic variability of 48 isolates of *P. griseola* collected in Minas Gerais State, Brazil.

Materials and Methods

Phaeoisariospsis griseola pathotype identification

Fungal isolates A collection of 48 isolates of *P. griseola* was obtained from naturally infected bean leaves and pods collected from experimental fields (Universidade Federal de Lavras Breeding Programme) in the counties of Lavras and Ijaci, in the state of Minas Gerais, MG, Brazil, while two isolates were collected in Alterosa, MG (Table 1). After single-spore isolation, cultures were transferred to Eppendorff tubes and kept at 4°C.

Spores for inoculation were obtained by culturing the fungus on bean leaf-dextrose-agar medium (Silveira, 1967) in a camera B.O.D. chamber (Fanem, São Paulo, SP, Brazil) at 24 ± 2°C. After approximately14 days, inoculum was prepared by adding 5–10 ml of sterile distilled water to each plate and scraping the surface of culture. The spore suspension so obtained was filtered through a double layer of cheese-cloth to remove the mycelial mass. The inoculum concentration was adjusted to 2×10^4 conidia/ml.

Pathotypes identification A set of 12 differential cultivars (Pastor-Corrales and Jara, 1995), plus cv. Rosinha G-2 (susceptible) and cv. AND 277 (resistant), were used to classify *P. griseola* pathotypes (Table 1). Seeds of differential cultivars were sown in aluminium pots at a density of five seeds per pot containing 2.0 kg of soil.

The first trifoliate leaf from each differential cultivars was inoculated (on both sides) at the V_3 development stage (CIAT, 1987). The inoculated plants were incubated in a moist chamber (>95% of relative humidity, for 48 h with a 16-h photoperiod) and then transferred to a greenhouse.

Disease reactions were scored 14–18 days after inoculation according to the 1–9 descriptive scale (CIAT, 1987), described as follows: 1, plants no symptoms; 3, plants with 5–10% of the leaf area with lesions; 5, plants with 20% of the leaf area with lesions and sporulation; 7, plants with up to 60% of the leaf area with lesions and sporulation, associated with chlorosis and necrotic tissues; 9, 90% of the leaf area with lesions, frequently associated with early loss of the leaves and plant death. Plants rated 1–3 were considered resistant (incompatible reaction), whereas plants with scores 4 or higher were considered susceptible (compatible reaction). When inoculated, plants that showed symptoms but no sporulation, were transferred to a moist chamber for 20–24 h. After this period, plants with non-sporulating lesions were considered resistant.

Pathotypes were classified according to the methodology proposed by the I Taller International Sobre la Mancha Angular del Frijol, at CIAT in 1995, and described by Sartorato (2004).

Table 1
Origin and pathotype of *Pseudocercospora griseola* isolates

Isolate	Cultivar	County	Pathotype
Pg-01	CV-13	Ijací	63-47
Pg-02	CV-78	Ijací	63-15
Pg-03	ESAL 507	Ijací	63-47
Pg-04	Z-22	Ijací	63-63
Pg-05	CV-78	Ijací	63-31
Pg-06	MAI – 8-13	Ijací	63-31
Pg-07	LH-10	Ijací	63-55
Pg-08	CI – 257	Ijací	63-15
Pg-09	ERIPARSA	Ijací	63-31
Pg-10	ERIPARSA	Ijací	63-63
Pg-11	ERIPARSA	Ijací	63-63
Pg-12	RC-I-3	Ijací	63-23
Pg-13	MAI-6-10	Ijací	63-55
Pg-14	ANLAV-51	Ijací	63-7
Pg-15	ESAL 502	Ijací	63-31
Pg-16	–	Ijací	63-31
Pg-17	–	Ijací	63-31
Pg-18	–	Ijací	63-63
Pg-19	–	Lavras	63-47
Pg-20	–	Lavras	63-63
Pg-21	–	Lavras	63-63
Pg-22	–	Lavras	63-31
Pg-23	Batatinha	Lavras	63-63
Pg-24	RC[a]	Lavras	63-63
Pg-25	RC	Lavras	63-63
Pg-26	Batatinha	Lavras	63-31
Pg-27	RC × Talismã	Lavras	63-31
Pg-28	Jalo	Lavras	63-63
Pg-29	Jalo	Lavras	63-31
Pg-30	Jalo	Lavras	63-63
Pg-31	Jalo	Lavras	63-63
Pg-32	Jalo	Lavras	63-31
Pg-33	F1 (PA3)	Lavras	63-63
Pg-34	RC	Lavras	63-63
Pg-35	RC	Lavras	63-63
Pg-36	RC	Lavras	63-63
Pg-37	RC	Lavras	63-63
Pg-38	Carioca	Alterosa	63-27
Pg-39	Jalo	Lavras	63-15
Pg-40	Small White	Lavras	55-15
Pg-41	Mulatinho Vagem Roxa	Lavras	63-31
Pg-42	CIII-R-3-19	Alterosa	63-63
Pg-43	Talismã	Lavras	63-63
Pg-44	Talismã	Lavras	63-25
Pg-45	Talismã	Lavras	63-63
Pg-46	Talismã	Lavras	63-63
Pg-47	Talismã	Lavras	63-63
Pg-48	Talismã	Ijací	63-63

[a]RC, Progenies from angular leaf spot recurrent selection programme.

Results and Discussion

Identification of *P. griseola* pathotypes

Isolates had different patterns of virulence when inoculated on 12 differential cultivars of *P. griseola,* and were classified into 10 pathotypes (Table 2). These results confirm the high variability of *P. griseola* and are in agreement with studies conducted elsewhere (Mahuku et al., 2002a; Nietsche et al., 2002; Sartorato, 2002, 2004; Orozco and Araya, 2005).

Nietsche et al. (2002) and Orozco and Araya (2005) observed wide pathogenic variability among the isolates of *P. griseola*, and identified a different pathotype

Table 2
Pathotype identification and reaction of differential cultivars to the isolates of *Pseudocercospora griseola* collected in Minas Gerais State

	Differential cultivars												
	Andean[a]						Mesoamerican[b]						
Pathotype	2^0	2^1	2^2	2^3	2^4	2^5	2^0	2^1	2^2	2^3	2^4	2^5	Number of isolates
Lavras													27
55-15	+[c]	+	+	–[d]	+	+	+	+	+	+	–	–	1
63-15	+	+	+	+	+	+	+	+	+	+	–	–	1
63-25	+	+	+	+	+	+	+	–	–	+	+	–	1
63-31	+	+	+	+	+	+	+	+	+	+	+	–	6
63-47	+	+	+	+	+	+	+	+	+	+	–	+	1
63-63	+	+	+	+	+	+	+	+	+	+	+	+	17
Ijací													19
63-07	+	+	+	+	+	+	+	+	+	–	–	–	1
63-15	+	+	+	+	+	+	+	+	+	+	–	–	2
63-23	+	+	+	+	+	+	+	+	+	–	+	–	1
63-31	+	+	+	+	+	+	+	+	+	+	+	–	6
63-47	+	+	+	+	+	+	+	+	+	+	–	+	2
63-55	+	+	+	+	+	+	+	+	+	–	+	+	2
63-63	+	+	+	+	+	+	+	+	+	+	+	+	5
Alterosa													2
63-27	+	+	+	+	+	+	+	+	–	+	+	–	1
63-63	+	+	+	+	+	+	+	+	+	+	+	+	1
Total	48	48	48	47	48	48	48	47	46	44	40	28	48

[a]2^0, Don Timóteo; 2^1, G11796; 2^2, Bolón Bayo; 2^3, Montcalm; 2^4, Amendoin; 2^5, G5686.
[b]2^0, Pan 72; 2^1, G2858; 2^2, Flor de Mayo; 2^3, Mexico 54; 2^4, BAT 332; 2^5, Cornell 49–242.
[c]Compatible reaction (+).
[d]Incompatible reaction (–).

for each of the three isolates studied. Similar results were reported by Mahuku et al. (2002a) and Sartorato (2002, 2004) who observed, on average, the occurrence of one pathotype for each two and seven isolates respectively. Studies by Mahuku et al. (2002a) and Orozco and Araya (2005) found larger variability than those conducted by Sartorato (2002, 2004) probably due to the great diversity of the sampled places and to the hosts from both gene pools (Mesoamerican and Andean).

Different patterns of virulence were observed in isolates collected at the same location. For example, six pathotypes were identified in the county of Lavras (Table 2). This result confirmed the data previously reported by Nietsche et al. (2002) and Sartorato (2002, 2004).

Likewise, Sartorato (2004) verified the existence of high pathogenic variation of *P. griseola* isolates from two locations in the State of Goiás, GO, Brazil (Damolândia and Inhumas). Ten distinct pathotypes were identified in each of these locations.

Pathotype 63-63 was the most widespread it was detected in all counties studied. The wide distribution of the variability of *P. griseola* was confirmed to occur worldwide. Jara et al. (2001) verified the occurrence of 120 pathotypes in 22 countries and, among the pathotypes identified, 71 were discovered specifically in Brazil. In Brazil, Nietsche et al. (2002) and Sartorato (2002) also reported the wide distribution of this pathogen.

Pathotype 63-15 was found in Lavras–MG and in the county of Ijací–MG. Pathotypes 55-15 and 63-25 were identified, exclusively, in the county of Lavras–MG. Pathotype 63-27 occurred exclusively in the county of Alterosa–MG. Patothypes 55-15, 63-15, 63-25 and 63-27 had not been previously reported in Minas Gerais State. Furthermore, this is the first report on the occurrence of pathotypes 55-15, 63-25 and 63-27 in Brazil.

All the pathotypes (63-7, 63-15, 63-23, 63-25, 63-27, 63-31, 63-47, 63-55 and 63-63) identified in this study, except pathotype 55-15, induced compatibility reactions to all Andean cultivars (Table 2), and were classified as of the Mesoamerican group. Highly pathogenic isolates in both differential cultivars gene pools (Andean and Mesoamerican) were observed. In Minas Gerais State, most of the farmers cultivate Carioca type grains, favouring strong directional selection on the pathogen population. The occurrence of isolates from Mesoamerican origin has also been demonstrated in Brazil (Nietsche et al., 2001, 2002; Sartorato, 2002, 2004; Vital, 2006).

Pathogen–host coevolution affects resistance gene deployment strategies (McDonald and Linde, 2002; Miklas et al., 2006). Dynamic processes that affect plant pathogen populations can reduce the effectiveness of resistant genes to allow the change of genes in a population; the introduction of genes in a population through gene flow; the random change in the allele frequency of a population by genetic drift and the predominance of genotypes due to more adapted individual selection (Mizubuti, 2002).

Pyramiding resistance alleles from both gene pools can be an efficient control strategy, considering that ALS genetic resistance is monogenic (Mahuku et al., 2002a; Sartorato, 2004; Miklas et al., 2006). However,

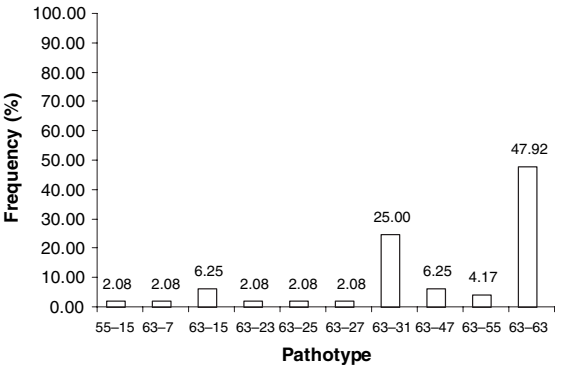

Fig. 1 Frequency of *Pseudocercospora griseola* pathotypes in Minas Gerais, Brazil

inheritance of this trait is more complex. Taking into account that the ALS genetic resistance is a quantitative trait, different strategies should be prioritized. Recurrent selection is a good alternative, because it provides an increasing number of favourable resistance alleles to the same lineage (Ramalho et al., 2001).

The most frequent pathotypes found in Lavras and Ijaci were 63-31 and 63-63 (Fig. 1), with 25% and 47.9% respectively. In Lavras-MG and in Ijací-MG, pathotypes 63-63 and 63-31 were the most frequent respectively. Similar results were obtained by Nietsche et al. (2002), Sartorato (2002, 2004) and Sartorato and Alzate-Marin (2004) These pathotypes presented wide adaptation to different regions, generated by the free grain trade within the state. In addition, the reutilization of grains as seeds increased the probability of contamination in crop production fields. The high frequency of the pathotype 63-63 we observed poses a risk due to a wide pathogenicity spectrum, revealing the need for a continuous search for new ALS resistance (Nietsche et al., 2001; Sartorato, 2004).

Nietsche et al. (2002) observed larger frequency of the pathotype 63-39 (29.41%) than pathotypes 63-31 and 63-63 in Lavras–MG. We did not observe the presence of this pathotype (63-39), stressing the importance of carrying out periodic observations in production fields, since each place has unique cultivar management characteristics and specific environmental conditions. Results of Nietsche et al. (2002) and those observed in the present work suggest a change in pathogen population structure.

To determine whether the pattern of infection of the differential cultivars by *P. griseola* isolates is a general pattern, a comparison was made between infection patterns from all pathotypes reported in Minas Gerais state in the last years (Fig. 2; Nietsche et al., 2001; Sartorato, 2002; Nietsche et al., 2002). Results showed small changes in the pattern of infection in the differential cultivars, altering population structure of *P. griseola* fungus. Among all comparisons made between our results and those of others, the largest percentages of compatible reactions were those reported for the isolates used in the present study.

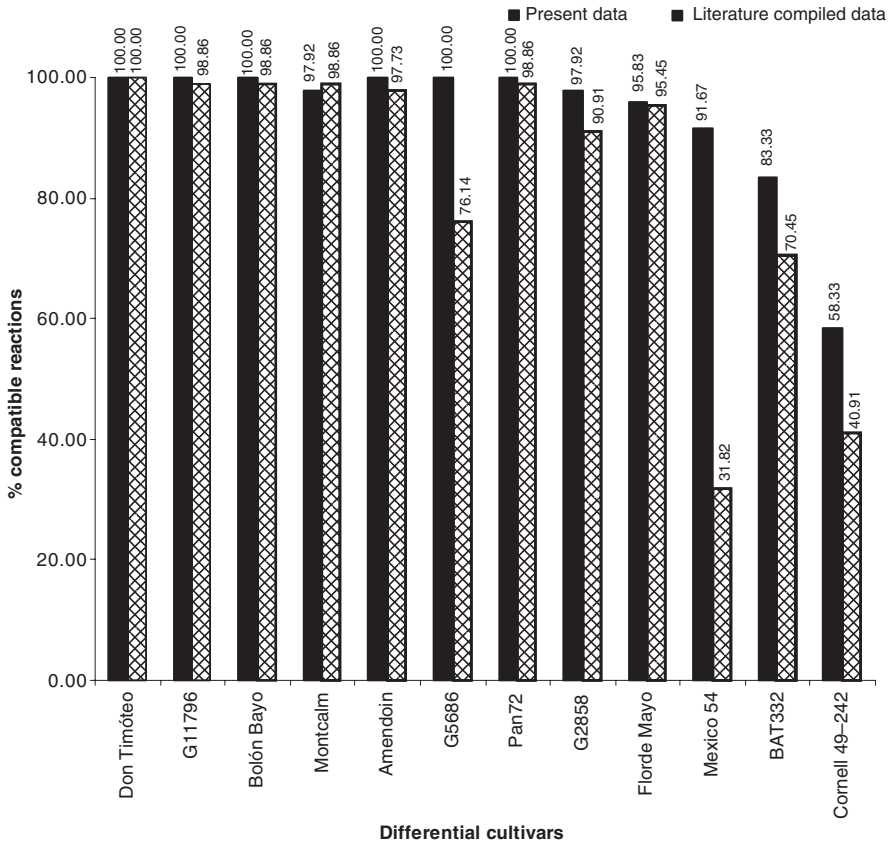

Fig. 2 Percentage of compatible reactions between differential cultivars and evaluated isolates: present data and literature compiled data since 2001 in Minas Gerais

Figure 2 shows that the differential cultivars BAT 332 (*Phg-6*^{2}) and Cornell 49-242 (*Phg-3*) are important sources of resistance for a breeding programme to control ALS. The association of alleles present in these two resistant sources could become an alternative for ALS control. McDonald and Linde (2002) suggest possibilities (pyramiding resistance alleles, disruptive selection and genes rotation) that can change the way selection operates on the pathogen population. According to these authors, the most common alternative is the pyramiding of resistance alleles.

Selection has been the most studied evolutionary force and probably the most easily managed factor in agroecosystems. When a resistance gene becomes widely distributed, strong directional selection occurs, causing an increase in the frequency of the virulent mutant until the resistance gene is broken (McDonald and Linde, 2002). Major selective forces may be imposed by the degree of specialization in host–pathogen interactions, control measures or more general environmental constraints (Mahuku et al., 2002a). These factors generate differences in the distribution of genotypic and phenotypic variations among plant pathogen populations that can lead to high genetic variation. Any of these, alone or in combination, may be interacting to give rise to new pathotype, leading to high levels of genetic diversity (Mahuku et al., 2002a).

A large variability among *P. griseola* isolates has been demonstrated, emphasizing the great potential of this fungus to generate variability. Information gained from this study has significant implications for regional ALS resistance breeding and resistance gene deployment.

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References

Amaro GB, Abreu AFB, Ramalho MAP, Silva FB. (2007) Phenotypic recurrent selection in the common bean (*Phaseolus vulgaris* L) with carioca-type grains for resistance to the fungi *Phaeoisariopsis griseola*. *Genet Mol Biol* **30**:584–588.

CIAT. *Standard System the Evaluation of Bean Germoplasm* [van Schoonhoven A, Pastor-Corrales MA. (compilers)], Cali, Colômbia, CIAT, 1987, 54 pp.

Guzmán P, Gilbertson RL, Nodari R et al. (1995) Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). *Phytopathology* **85**:600–607.

Jara C, Castellano G, Mahuku G. (2001) Estado actual y proyección de la investigación relacionada com la mancha angular del frijol (*Phaeoisariopsis griseola*). *Fitopatol Colomb* **25**:1–6.

Mahuku GS, Henríquez MA, Muñoz J, Buruchara RA. (2002a) Genetic variability within *Phaeoisariopis griseola* from Central America and its implications for resistance breeding of common bean. *Plant Pathol* **51**:594–604.

Mahuku GS, Henríquez MA, Muñoz J, Buruchara RA. (2002b) Molecular markers dispute the existence of the afro-andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. *Phytopathology* **92**:580–589.

Mahuku G, Montoya C, Henríquez MA, Jara C, Teran H, Beebe S. (2004) Inheritance and characterization of angular leaf spot resistance gene present in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene. *Crop Sci* **44**:1817–1824.

McDonald BA, Linde C. (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* **124**:163–180.

Miklas PN, Kelly JD, Beebe SE et al. (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* **147**:105–131.

Mizubuti ESG. (2002) O vértice – biologia de populações e manejo de doenças. *Fitopatol Bras* **27**:37.

Nietsche S, Borém A, de Carvalhos GA et al. (2001) Genetic diversity of *Phaeoisariopsis griseola* in the State of Minas Gerais, Brazil. *Euphytica* **17**:77–84.

Nietsche S, Borém A, Alzate-Marin AL et al. (2002) Variabilidade genética da patogenicidade de *Phaeoisariopsis griseola* no Brasil. *Summa Phytopathol* **28**:331–335.

Orozco S, Araya CM. (2005) Variabilidade patogênica de *Phaeoisariopsis griseola* na Costa Rica. *Fitopatol Bras* **30**:589–593.

Pastor-Corrales MA, Jara C. (1995) La evolucion de *Phaeoisariopsis griseola* com el frijol comum en America Latina. *Fitopatol Colomb* **19**:15–22.

Pastor-Corrales MA, Aggarwal VA, Chirwa RM, Buruchara RA. (2004) Andean beans with resistance to angular leaf spot and virulence diversity of *Phaeoisariopsis griseola* in Southern and Eastern Africa. *Annu Rep Bean Improv Coop* **47**:129–130.

Ramalho MAP, Abreu AFB, dos Santos JB. Melhoramento de espécies autógamas. In: Nass LL, Valois ACC, de Melo IS, Valadares-Inglis MC. (eds), *Recursos genéticos e melhoramento de plantas*., Rondonópolis, Brazil, Fundação MT, 2001, pp. 201–230.

Sartorato A. (2002) Identification of *Phaeoisariopsis griseola* pathotypes from five states in Brazil. *Fitopatol Bras* **27**:78–81.

Sartorato A. (2004) Pathogenic variability and genetic diversity of *Phaeoisariopsis griseola* isolates from two counties in the State of Goias, Brazil. *J Phytopathol* **152**:385–390.

Sartorato A, Alzate-Marin AL. (2004) Analysis of the pathogenic variability of *Phaeoisariopsis griseola* in Brazil. *Annu Rep Bean Improv Coop* **47**:235–236.

Silveira GA*Evaluación de la resistencia de frijol a la mancha angular: algunos aspectos fisiológicos de Isariopsis griseola Sacc. y patogenicidad de algunas cepas colectadas en Costa Rica*. Master Thesis, Turrialba, Costa Rica, Instituto Interamericano de Ciencias Agrícolas da OEA, . (1967) 60 pp.

Stenglein SA, Balatti PA. (2006) Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. *Physiol Mol Plant Pathol* **68**:158–167.

Stenglein SA, Fermoselle GE, Balatti PA. (2005) Pathogenic and molecular studies of *Phaeoisariopsis griseola* in Argentina. *Annu Rep Bean Improv Coop* **48**:92–93.

Vital WM*Phaeoisariopsis griseola: caracterização fisiológica, fontes de resistência e reação do feijoeiro*. Master Thesis, Campinas, Brazil, Instituto Agronômico de Campinas, . (2006) 49 pp.

Wagara IN, Mwang'ombe AW, Kimenju JW, Buruchara RA. (2004) Occurrence and distribution of Andean, Afro-Andean and Mesoamerican pathotypes of *Phaeoisariopsis griseola* in Kenya. *Annu Rep Bean Improv Coop* **47**:239–240.