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Identification of a novel source of resistance to angular leaf spot disease of common bean within the secondary gene pool

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Abstract

Phaeoisariopsis griseola (Sacc.) Ferr., the agent of angular leaf spot disease of common bean, is a highly variable pathogen for which resistance gene diversification is required. This study analysed genetic resistance to this disease within genotypes of three *Phaseolus* species. Twenty-nine genotypes of *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus polyanthus* were inoculated with 54 isolates of *Phaeoisariopsis griseola*. The genetic resistance was estimated according to the symptom intensity observed for each plant genotype–pathogen isolate combination. Globally, genotypes of the common bean secondary gene pool were resistant to a higher number of isolates than common bean varieties. Interactions between plant genotypes and pathogen isolates suggested vertical resistance genes within *P. vulgaris*, as well as within *P. coccineus* and *P. polyanthus*. The 'NI666' accession (*P. coccineus*) showed resistance to all the fungal isolates inoculated while the variety 'Aroana' (*P. vulgaris*) was susceptible to most of the isolates. Interspecific hybridization between these two genotypes gave F_1 hybrid plants which showed resistance to angular leaf spot disease.

Key words: *Phaeoisariopsis* (*Isariopsis*) *griseola* — *Phaseolus coccineus* — *P. polyanthus* — *P. vulgaris* — angular leaf spot — resistance — secondary gene pool

The angular leaf spot (ALS) disease of common bean caused by *Phaeoisariopsis griseola* (Sacc.) Ferr. (*Isariopsis griseola* Sacc.) (Cardona-Alvarez and Walker 1956) induces yield losses which can reach 80% under severe conditions of infection (Schwartz et al. 1981).

Many control strategies, including chemical applications (Bhardwaj et al. 1994), cultural practices (Correa et al. 1989) and genetic resistance, (Schwartz et al. 1982) can be used for the management of ALS. Genetic resistance appears to be the most appropriate method for the subsistence agriculture prevailing in developing countries because of the limited finances of the farmers. The main drawback of this method is possible resistance breakdown (Brown 1994) caused by pathogen adaptation to the host resistance (Fry 1982, Chen et al. 1993, McDermott 1993).

Some evidence of pathogenic variability in *Phaseolus griseola* have been shown based on pathogenicity in differential cultivars (Alvarez-Ayala and Schwartz 1979) and on RAPD patterns (Guzmán et al. 1995). Within 54 *P. griseola* isolates inoculated on 29 plant genotypes, we revealed 53 different virulence profiles (Busogoro et al. 1999). Despite the absence of known sexuality, *P. griseola* has to be considered as a highly variable pathogen and ALS disease management by genetic resistance must take into account this diversity. In fact, in India, Srivastava et al. (1995) identified different resistant lines in common bean, which appeared susceptible after 2 or 3 years, suggesting a possible resistance breakdown related to pathogen adaptation. In Africa, Aggarwal et al. (1996) described a *Phaseolus vulgaris* line showing resistance to ALS in Tanzania, Zimbabwe and South Africa, while it was susceptible in Uganda.

Thus, diversification of resistance genes and a rational strategy of resistance gene deployment are necessary for disease management. In this respect, possible sources of resistance to ALS within other *Phaseolus* species are not well known. Resistance genes to ALS were mainly reported within the *P. vulgaris* species (Correa et al. 1989, Beebe and Pastor-Corrales 1991). Only the study of Singh and Saini (1980) describes resistance to ALS identified within a *P. coccineus* genotype. Species within the secondary gene pool (*P. coccineus* and *P. polyanthus*) are already known to have interesting agronomic traits such as resistance to *Ascochyta* leaf spot and golden mosaic virus (Schmit et al. 1993).

The objective of this study was to find novel sources of resistance to ALS by analysing genotypes which belong to the secondary gene pool of *P. vulgaris*.

Materials and Methods

Plant genotypes: Inoculation tests were performed on 29 plant genotypes (Table 1) containing 17 varieties of *P. vulgaris*, six accessions of *P. coccineus* and six accessions of *P. polyanthus*. Among these genotypes, only two wild accessions of *P. coccineus* ('NI1108' and 'NI819') are not used as current cultivars.

Pathogen isolates: A collection of 54 *P. griseola* isolates was obtained from naturally infected bean leaves and cultured on V8 juice agar medium (per litre: 200 ml V8, 3 g $CaCO_3$ and 18 g agar) kept in the dark at $\pm 20°C$. Colonies produced from single spores were conserved in a cold room (4°C) as pure isolates for inoculation tests. Forty-four isolates originated from Central African countries (Burundi, Rwanda, Zaire and Kenya) while 10 isolates were collected in Brazil and Colombia (Table 2).

Inoculation tests: Conidia were harvested from 12-day-old cultures grown on V8 agar medium, suspended in distilled water and adjusted to a concentration of 2×10^4 conidia/ml. Inoculations were performed on the first trifoliate leaves by spraying the inoculum to run-off under a pressure of 2.8 kg/cm^2 until saturation.

Inoculated plants (three per each 'isolate × genotype' combination) were incubated for 4 days in a humid chamber (relative humidity 95%) at 25°C with a 16-h photoperiod. Plants were then maintained in greenhouse and symptom intensity was scored according to the visual scale

Table 1: List of *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus polyanthus* genotypes evaluated for their resistance to angular leaf spot disease

Number	Genotype	Species	Origin
1	'BAT76'	*P. vulgaris*	ISABU[1]
2	'Calima'	*P. vulgaris*	ISABU
3	'Aroana'	*P. vulgaris*	ISABU
4	'A340'	*P. vulgaris*	ISABU
5	'A345'	*P. vulgaris*	ISABU
6	'A285'	*P. vulgaris*	ISABU
7	'A410'	*P. vulgaris*	ISABU
8	'A140'	*P. vulgaris*	ISABU
9	'Prelude'	*P. vulgaris*	Used in Belgium
10	'BAT1647'	*P. vulgaris*	CIAT[2]
11	'Seafarer'	*P. vulgaris*	CIAT
12	'Cornell49242'	*P. vulgaris*	CIAT
13	'Montcalm'	*P. vulgaris*	CIAT
14	'A339'	*P. vulgaris*	CIAT
15	'G5686'	*P. vulgaris*	CIAT
16	'BAT332'	*P. vulgaris*	CIAT
17	'Pompadous Checa'	*P. vulgaris*	CIAT
18	'N115'	*P. coccineus*	Rwanda
19	'NI16'	*P. coccineus*	Rwanda
20	'NI1108'	*P. coccineus*	Mexico[3]
21	'NI819'	*P. coccineus*	Mexico[3]
22	'NI229'	*P. coccineus*	Zaire
23	'NI666'	*P. coccineus*	Porto Rico
24	'NI429'	*P. polyanthus*	Costa Rica
25	'NI519'	*P. polyanthus*	Mexico
26	'NI1208'	*P. polyanthus*	Colombia
27	'NI1010'	*P. polyanthus*	Colombia
28	'NI1011'	*P. polyanthus*	Colombia
29	'NI373'	*P. polyanthus*	Venezuela

[1] ISABU = Institut des Sciences Agronomiques du Burundi.
[2] CIAT = Centro Internacional de Agricultura Tropical.
[3] Wild genotypes.

defined by the CIAT (1992) 16 days after inoculation. The percentage of infected leaf area was evaluated and scored from 1 (if no visible lesion was observed) to 9 (if more than 25% of the leaf area was covered by disease lesions).

Interspecific crossing: The genotype 'Aroana', a highly susceptible variety of *P. vulgaris*, was hybridized with genotype 'NI666', an accession of *P. coccineus*, the *P. vulgaris* variety being used as the female parent. The pollen was harvested from one plant of the genotype 'NI666'. The hybrid status of plants obtained after this intercrossing process was evaluated based on morphological traits (germination type and stem pigmentation) and molecular patterns. For molecular characterization, random amplified polymorphic DNAs (RAPDs) were performed with the OPK9 primer (Operon Technologies, USA) on total DNA extracted according to the method of Doyle and Doyle (1990) from plants of the two parental genotypes and one plant obtained after interspecific crossing. Amplification reactions were performed in a thermocycler (Biometra TRIO-Thermoblock) where a first DNA denaturation at 94°C for 3 min was followed by 45 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C. A final elongation step was carried out at 72°C for 10 min. The reaction mixture was a 50 μl final volume containing 5 μl of the polymerase chain reaction (PCR) reaction buffer 10 × concentrated (Boehringer Mannheim, Germany), 0.4 μM OPK9 primer, 4 mM $MgCl_2$, 200 μM of each dNTP, 50 ng of total DNA and 1 unit of *Taq* polymerase (Boehringer Mannheim). Electrophoretic analysis of amplified products was performed in a 1.5% agarose gel stained by ethidium bromide and observed under UV light. Six plants, randomly chosen among the nine progenies obtained by the interspecific crossing, were inoculated with the isolate KGM6.

Data analysis: Symptom intensity, scored according to the CIAT (1992) scale , allowed the plant reaction type (RT) to be determined: resistance (R if symptom score $\leqslant 3$), partial resistance (PR if $3 <$ symptom score $\leqslant 6$) and susceptibility (S if $6 <$ symptom score $\leqslant 9$). In cases of susceptibility or partial resistance reaction of plant genotypes, isolates were considered as virulent while in cases of resistance reaction, they were considered as avirulent. Plant genotypes were compared according to their respective number of avirulent isolates.

These RT were recorded in a matrix and analysed by hierarchical cluster analysis using the average linkage method with the statistical program SYSTAT version 5. The distances between plant genotypes, which were calculated as the percentage of isolates causing dissimilar reaction types, were shown in a dendrogram derived from this analysis.

Results

The genotype 'NI666', an accession of *P. coccineus*, was resistant to all the inoculated isolates while genotype 'Aroana', a variety of *P. vulgaris*, was susceptible to 48 pathogen isolates. Three categories of RT (resistance, partial resistance and susceptibility) were observed on some other plant genotypes according to the inoculated isolates. For example, the genotype 'BAT76' appeared resistant with isolate KGR1 and partly resistant with isolate KGM1, but susceptible with the isolate KGM2. This interaction profile was revealed for common bean genotypes as well as for the secondary gene pool genotypes.

Table 3 shows the reaction type of each plant genotype inoculated with the different fungal isolates. Cluster analysis allowed the genotypes to be grouped according to their respective reactions after inoculation by the 54 isolates (Fig. 1). The greatest distance on the dendrogram (63%) allowed separation of most of the *P. vulgaris* genotypes from the accessions of *P. coccineus* and *P. polyanthus* and the recognition of two main groups of genotype. One of these groups contains only *P. vulgaris* material

Table 2: List of *Phaeoisariopsis griseola* isolates inoculated on the 29 plant genotypes for the evaluation of the genetic resistance

Number	Isolate	Origin Country	Location	Collection date	Sent by
1	KGM1	Burundi	Muhingira	January 1994	ISABU
2	KGM2	Burundi	Muhingira	January 1994	ISABU
3	KGM3	Burundi	Muhingira	January 1994	ISABU
4	KGM4	Burundi	Muhingira	January 1994	ISABU
5	KGM5	Burundi	Muhingira	January 1994	ISABU
6	KGM6	Burundi	Muhingira	January 1994	ISABU
7	KGM7	Burundi	Muhingira	January 1994	ISABU
8	KGM8	Burundi	Muhingira	January 1994	ISABU
9	KGR1	Burundi	Rubagabaga	January 1994	ISABU
10	KGR2	Burundi	Rubagabaga	January 1994	ISABU
11	KGR3	Burundi	Rubagabaga	January 1994	ISABU
12	KGR4	Burundi	Rubagabaga	January 1994	ISABU
13	KGR5	Burundi	Rubagabaga	January 1994	ISABU
14	KGR6	Burundi	Rubagabaga	January 1994	ISABU
15	NMM1	Burundi	Murama	January 1994	ISABU
16	NMM2	Burundi	Murama	January 1994	ISABU
17	NMM3	Burundi	Murama	January 1994	ISABU
18	KF1	Kenya	Unknown	April 1994	University of Nairobi
19	FK4	Kenya	Unknown	April 1994	University of Nairobi
20	KK1	Burundi	Kabuye	July 1994	ISABU
21	KK2	Burundi	Kabuye	July 1994	ISABU
22	KK3	Burundi	Kabuye	July 1994	ISABU
23	KK4	Burundi	Kabuye	July 1994	ISABU
24	KK5	Burundi	Kabuye	July 1994	ISABU
25	KK6	Burundi	Kabuye	July 1994	ISABU
26	RN1	Colombia	Rio Negro	February 1994	CIAT
27	RN2	Colombia	Rio Negro	February 1994	CIAT
28	RN4	Colombia	Rio Negro	February 1994	CIAT
29	RN8	Colombia	Rio Negro	February 1994	CIAT
30	RN10	Colombia	Rio Negro	February 1994	CIAT
31	BR1	Brazil	Goiás	December 1995	EMBRAPA[1]
32	BR2	Brazil	Ceará	December 1995	EMBRAPA
33	BR3	Brazil	Minas Gerais	December 1995	EMBRAPA
34	BR4	Brazil	Espírito Santo	December 1995	EMBRAPA
35	BR5	Brazil	Pernambuco	December 1995	EMBRAPA
36	RDA1	Rwanda	Unknown	April 1996	University of Rwanda
37	RDA2	Rwanda	Unknown	April 1996	University of Rwanda
38	RDA3	Rwanda	Unknown	April 1996	University of Rwanda
39	RDA6	Rwanda	Unknown	April 1996	University of Rwanda
40	RDA7	Rwanda	Unknown	April 1996	University of Rwanda
41	BGA2	Burundi	Gitega	April 1996	University of Burundi
42	BGA4	Burundi	Gitega	April 1996	University of Burundi
43	BGA5	Burundi	Gitega	April 1996	University of Burundi
44	ZA1	Zaire	Unknown	May 1996	University Kinshasa
45	ZA2	Zaire	Unknown	May 1996	University Kinshasa
46	ZA3	Zaire	Unknown	May 1996	University Kinshasa
47	ZA4	Zaire	Unknown	May 1996	University Kinshasa
48	ZA5	Zaire	Unknown	May 1996	University Kinshasa
49	ZA6	Zaire	Unknown	May 1996	University Kinshasa
50	ZA7	Zaire	Unknown	May 1996	University Kinshasa
51	ZA8	Zaire	Unknown	May 1996	University Kinshasa
52	ZA9	Zaire	Unknown	May 1996	University Kinshasa
53	ZA10	Zaire	Unknown	May 1996	University Kinshasa
54	ZA11	Zaire	Unknown	May 1996	University Kinshasa

[1] EMBRAPA: Empresa Brasileira de Pesquisa Agropecuaria.

(12 genotypes) among which the cultivar 'Aroana' appeared susceptible to the largest number of isolates. The second group contains all the accessions of *P. coccineus* and *P. polyanthus*, including 'NI666', which appeared resistant to all the inoculated isolates. Only five genotypes of *P. vulgaris* were grouped together with the most resistant genotype, 'NI666'.

Figure 2 compares the frequency of genotypes belonging to *P. vulgaris* or to the secondary gene pool according to the number of avirulent isolates per genotype. Globally, genotypes of *P. coccineus* and *P. polyanthus* appeared resistant to a larger number of *P. griseola* isolates than genotypes belonging to *P. vulgaris*. The mean number of avirulent isolates per genotype was equal to 37 for the secondary gene pool genotypes and 20 for the *P. vulgaris* genotypes.

Genotype 'NI666', which appeared resistant to all 54 isolates, was crossed with the susceptible cultivar 'Aroana'. The nine plants resulting from intercrossing appeared to be intermediate between the two parental genotypes in terms of morphological traits, such as germination type (epigeal for 'Aroana' and hypogeal for 'NI666') and stem pigmentation (greenish for 'Aroana' and reddish for 'NI666'). The intermediate character of these plants was confirmed by molecular analysis. The RAPD pat-

Table 3: Interactions between plant genotypes and pathogen isolates

Isolate Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
1	I	S	S	R	S	I	I	R	R	S	S	I	S	I	R	S	I	I	I	S	I	S	S	I	R	R	I	S	I	I	S	I	I	I	I	I	I	I	R	R	I	S	I	R	R	I	R	R	R	R	R	R	R	I
2	R	R	R	R	R	R	R	S	I	R	R	I	I	R	R	R	R	S	R	R	I	I	R	I	R	R	I	R	R	R	S	S	R	I	R	I	R	R	R	R	S	S	I	R	S	R	S	S	S	R	I	R	I	S
3	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	I	S	I	S	S	S	R	S	S	S	S	S	S	S	I	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S
4	S	I	I	R	I	S	I	R	S	I	S	R	I	R	R	I	I	R	R	R	R	I	I	R	I	I	I	I	R	I	S	I	R	R	S	R	R	R	R	R	I	I	R	S	R	R	R	R	R	R	R	R	R	S
5	S	I	S	R	I	S	S	S	S	S	S	S	I	R	R	I	I	R	I	S	S	S	I	I	I	I	I	S	I	I	R	R	I	R	S	R	R	S	S	R	S	R	S	S	R	S	I	R	R	R	R	R	R	S
6	S	S	S	R	S	S	S	S	S	S	S	S	R	S	I	S	I	R	I	S	S	I	S	I	I	R	I	I	I	R	R	S	S	R	R	R	I	I	I	R	R	S	I	R	S	R	I	R	S	R	R	R	R	S
7	I	S	I	R	I	S	S	S	S	S	S	S	S	S	R	S	I	I	I	I	S	I	S	S	S	S	I	S	S	I	R	S	R	R	I	S	S	R	S	R	I	R	I	S	S	S	S	R	I	R	R	I	I	S
8	R	I	I	R	I	S	S	S	R	S	S	S	I	S	R	S	R	S	I	S	S	I	I	S	R	S	S	S	S	R	I	S	R	I	R	S	I	R	S	R	I	S	I	I	S	R	S	R	R	R	R	I	S	S
9	R	I	I	R	S	I	S	S	S	S	I	S	R	I	R	S	I	R	R	I	I	I	I	I	S	I	I	S	S	I	R	S	R	I	R	I	I	I	I	I	S	S	S	S	R	S	S	R	R	I	I	I	S
10	I	I	I	R	I	I	I	S	R	I	S	S	R	I	R	S	I	R	R	I	I	S	I	I	I	I	I	I	I	R	I	S	S	R	R	R	R	I	R	R	R	S	I	S	S	I	S	R	I	R	R	R	R	I
11	I	R	R	R	I	S	S	S	S	S	S	S	S	S	I	I	S	I	S	S	S	S	S	S	S	S	S	R	S	I	R	I	S	R	S	S	R	I	S	R	S	S	S	S	S	S	S	S	S	R	R	S	S	S
12	R	S	I	R	I	R	I	I	I	S	I	S	I	I	R	I	I	I	I	S	S	R	I	S	R	S	S	I	S	R	R	R	R	R	R	S	I	S	I	R	S	S	S	S	S	I	S	S	S	R	R	S	I	S
13	R	R	I	R	I	R	R	S	R	R	R	S	R	I	R	I	R	S	R	I	R	S	R	R	R	R	I	I	R	I	R	R	R	R	R	R	R	R	R	R	S	S	R	I	S	R	R	S	R	R	I	R	I	S
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16	S	S	S	R	I	I	I	I	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	I	R	R	R	R	R	R	R	S	S	R	R	S	R	R	S	S	S	S	S	S	R	R	R	S	I	I	S
17	R	I	R	R	I	S	S	S	S	S	R	S	R	R	R	I	I	I	I	I	R	R	R	R	R	R	I	R	I	I	R	R	R	R	R	S	I	I	R	R	R	R	S	R	S	S	S	I	S	I	R	I	I	S
18	R	R	R	R	R	S	S	S	I	S	R	R	I	I	R	R	R	R	R	I	I	I	S	R	R	R	R	R	I	R	R	R	R	R	I	R	I	R	R	S	S	S	R	R	I	I	R	I	R	S	R	I	S
19	R	R	R	R	I	I	R	S	S	S	R	I	I	R	R	R	R	R	R	R	I	I	S	I	R	R	R	R	R	I	R	R	R	R	R	I	R	R	R	R	R	R	R	I	I	R	R	R	R	R	R	I	S
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21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
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23	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
24	I	I	I	R	I	R	I	I	S	S	S	S	R	R	R	S	R	R	S	S	R	S	S	R	R	R	S	R	S	R	R	R	R	R	R	S	S	R	R	R	I	R	I	I	S	S	R	S	R	S	R	R	R	R
25	I	I	R	R	R	I	S	S	S	S	R	R	R	R	R	I	R	R	I	I	R	R	R	R	R	R	I	R	R	R	R	R	R	R	I	R	R	I	R	R	I	R	I	R	R	S	I	S	R	R	R	S	R	R
26	I	I	I	R	I	I	R	I	R	S	I	R	I	R	R	R	S	R	I	S	S	S	R	R	R	R	R	I	R	R	R	R	I	I	I	S	S	R	R	R	S	I	R	S	S	S	S	R	R	R	R	R	R	R
27	I	I	R	R	R	I	R	R	I	R	R	I	R	R	R	R	I	R	I	I	R	R	R	R	R	R	I	R	R	R	R	R	R	S	S	I	I	S	R	I	S	I	I	S	S	S	R	I	R	R	I	R	S
28	I	R	I	R	I	I	R	S	I	S	R	S	I	I	R	S	R	S	I	S	I	S	S	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	R	R	R	R	R	R
29	I	R	I	R	R	R	R	R	I	S	R	R	R	R	R	S	R	S	I	I	R	R	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R

R: resistance.
I: intermediate (partial resistance).
S: susceptibility.

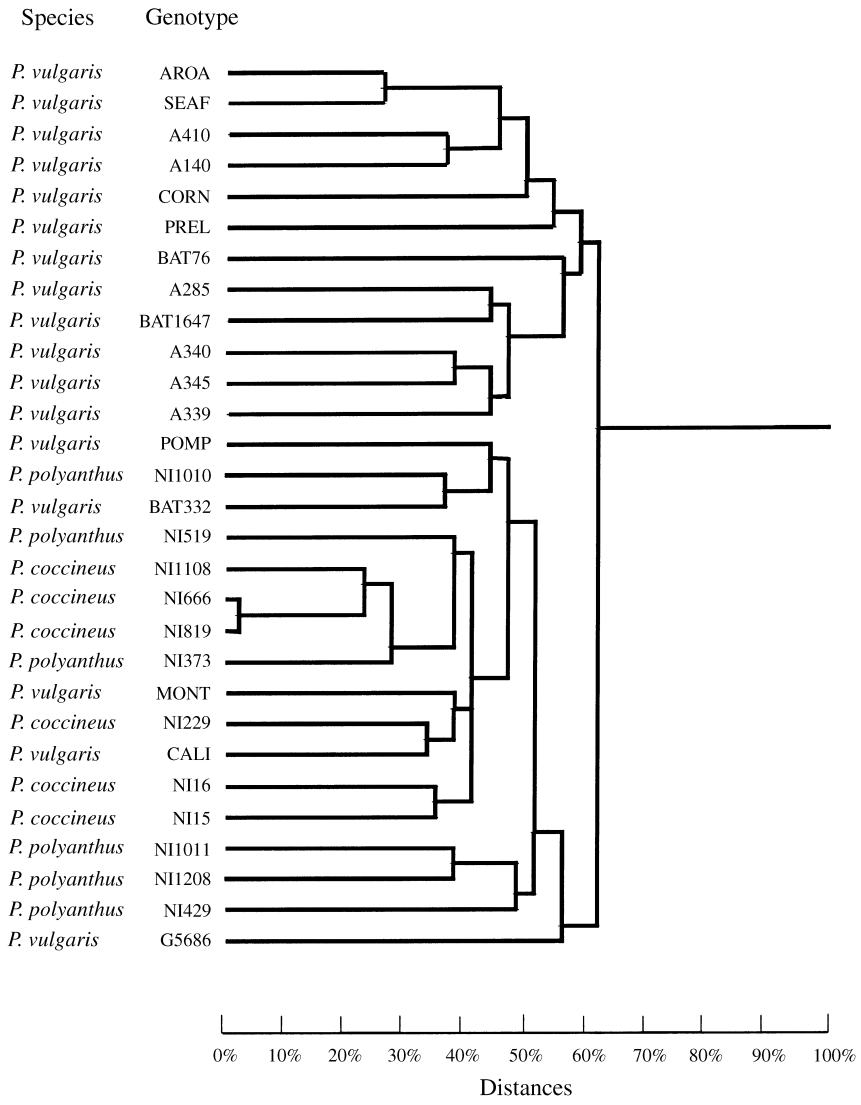

Fig. 1: Tree diagram of the 29 *Phaseolus* genotypes based on the infection types showed after inoculation by the 54 pathogen isolates. Cluster analysis is performed according to three infection types (resistance, partial resistance and susceptibility). Distances between two genotypes are calculated as the percentage of isolates for which genotypes exhibit different infection types

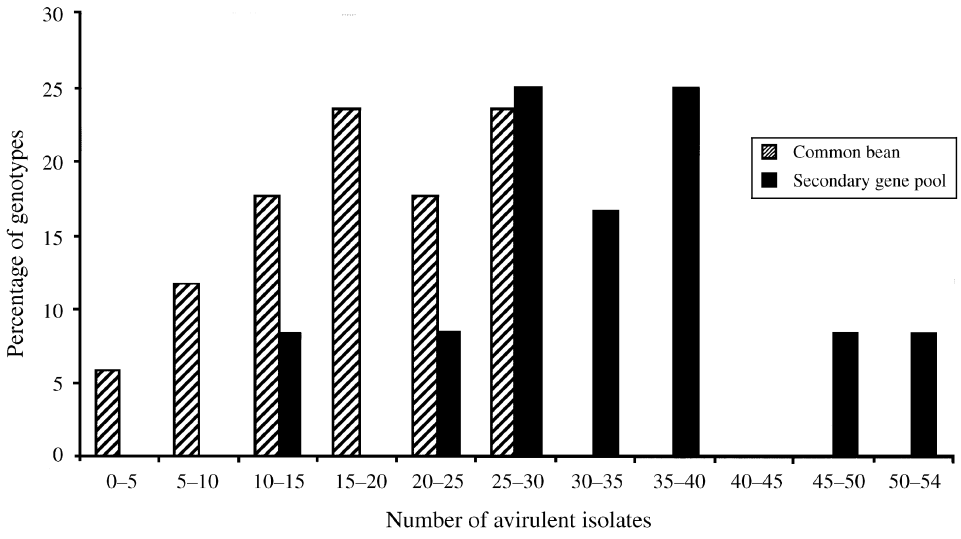

Fig. 2: Analysis of genotype resistance estimated by the number of avirulent pathogen isolates. An isolate was considered as avirulent if it caused a resistance reaction and virulent if it caused a susceptibility or a partial resistance reaction

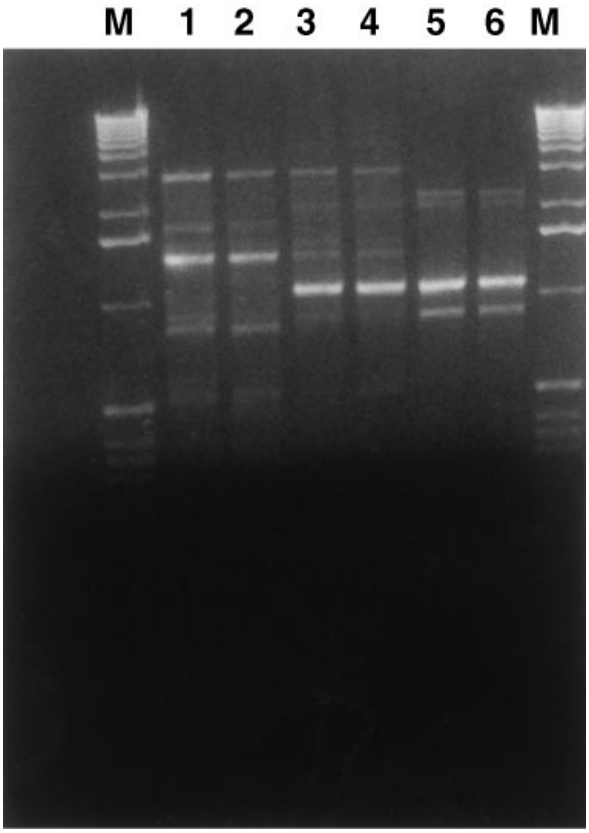

Fig. 3: Agarose-gel (1.5%) electrophoresis and staining with ethidium bromide of the RAPD amplified fragments from total DNA of the two parental genotypes (lanes 1 and 2 for 'Aroana'; lanes 5 and 6 for 'NI666') and the hybrid plant (lanes 3 and 4). M: lanes with molecular size marker (100 bp)

terns of each parental genotype exhibited specific bands which were not observed in the other parental genotype patterns, while specific bands of both parents were observed on the patterns obtained for one of the plants resulting from interspecific hybridization (Fig. 3). Inoculation tests performed with the isolate KGM6 (virulent on 'Aroana' and avirulent on 'NI666') gave rise to resistance reactions on all inoculated interspecific progenies (six), as was the case for the 'NI666' control plants and in contrast to the 'Aroana' control plants. All these hybrid plants died before flowering, suggesting a physiological deficiency for the interspecific hybridization between genotypes 'Aroana' and 'NI666'.

Discussion

The inoculation tests have shown differential interactions between plant genotypes and *P. griseola* isolates. These interactions, observed both with *P. vulgaris* and its secondary gene pool (*P. coccineus* and *P. polyanthus*), suggest the presence of vertical resistance genes (Nelson 1978). Our observations showed that such race-specific genes can be found within *P. vulgaris*, but equally within its secondary gene pool. Globally, genotypes of *P. coccineus* and *P. polyanthus* have shown resistance to more *P. griseola* isolates than genotypes of common bean. This observation should be treated with caution because genotypes of *P. vulgaris* used for the present study were mostly improved varieties and thus, not perfectly representative of the diversity of this species.

In the past, Saettler and Correa (1984) observed differential interactions between bean lines and *P. griseola* isolates. Different work, demonstrating interactions between bean genotypes and *P. griseola* isolates, as well as resistance to ALS controlled by single genes, were summarized by Beebe and Pastor-Corrales (1991). For diseases caused by variable pathogens like bean ALS (Schwartz et al. 1982, Guzmán et al. 1995, Chacón et al. 1997), it is necessary to diversify the sources of resistance (Young and Kelly 1996, Wehner and Shetty 1997). Identification of genotypes with resistance to ALS within the secondary gene pool could contribute to this management strategy.

Such resistances to common bean diseases were frequently found within secondary gene pool genotypes. For example, a resistance to common bacterial blight has been identified within a line of *P. coccineus* (Welsh and Grafton 1997). Beebe and Pastor-Corrales (1991) indicated resistance to bean golden mosaic virus within accessions of *P. coccineus* and resistance to bean anthracnose within accessions of *P. polyanthus*.

Resistance genes within secondary gene pool genotypes might be transmitted to common bean genotypes because of the feasibility of interspecific hybridization (Baudoin and Maréchal 1985). In our work, feasibility of interspecific transfer of resistance to *P. griseola* in common bean was analysed. Germination type, a property which allows one to differentiate *P. vulgaris* from *P. coccineus* species (Anderson and Ascher 1996), as well as the stem pigmentation, which was equally different between the two genotypes crossed, were considered. The intermediate morphological traits of plants resulting from the interspecific hybridization suggested that these were F_1 hybrid plants. The RAPD pattern shown by one of the hybrid plants was intermediate between the two parental genotypes.

Resistance reactions observed for the F_1 hybrid plants proved the possible transfer of resistance from the *P. coccineus* genotype ('NI666') to common bean varieties. Singh and Saini (1980) described an ALS resistance gene transferred from a *P. coccineus* genotype to a *P. vulgaris* variety. This resistance was controlled by a recessive factor because all the F_1 plants were susceptible while there was a 3 : 1 ratio of segregation at the F_2 generation. Recently, Welsh and Grafton (1997) were able to transfer resistance to common bacterial blight from *P. coccineus* to *P. vulgaris*. In our work, the resistance might be controlled by nuclear factors since the resistant parent was used as the male. More precise characterization of this resistance would come from an analysis of the segregation of the resistance in later generations. This type of analysis presents limitations for the crossing performed in our study because of the physiological deficiency in the hybrid plants. In order to overcome these limitations, reverse crossing could be tried between the two genotypes, despite the fact that having *P. coccineus* as the female parent is very difficult (Singh and Saini 1980). Another possibility would be to find common bean genotypes more compatible with the genotype 'NI666' than the cv. 'Aroana'. These genotypes could be used to transfer the resistance identified to the common bean species. Hybridization using *P. polyanthus* as a bridge species might also overcome the limitations of *P. vulgaris* × *P. coccineus* intercrossing because *P. polyanthus* is closer to *P. vulgaris* than *P. coccineus* (Schmit et al. 1993).

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