Genetic diversity among isolates of the angular leafspot fungus (*Phaeoisariopsis griseola*) revealed by random amplified polymorphic DNA (RAPDs) analysis

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Angular leafspot disease (ALS) of common bean, caused by the fungus *Phaeoisariopsis griseola* (PG), is found in more than 60 countries throughout the world and is a major constraint on bean production. In Brazil, ALS is now considered to be the most serious disease of common bean (L. Zambolim, pers. communication). ALS is a very important disease in East Africa, and enhanced ALS resistance is an important priority of the National Programs of Malawi, Tanzania, Rwanda, and Zambia.

Previous efforts to develop and identify ALS resistant bean genotypes have revealed that significant pathogenic variability exists among PG isolates. Correa-Victoria (1984) proposed that PG isolates from various geographic locations could be placed into races based on disease reactions on 12 bean cultivars. More recently, isozyme analysis indicated that two major patterns could be identified for PG isolates and that the patterns were correlated with geographic and bean gene pool origin (e.g. Middle American or Andean). PG isolates exhibiting pattern 1 came from Africa or Latin America and were associated with large-seeded (Andean) beans, whereas isolates exhibiting pattern 2 came from Latin America and were associated with small-seeded (Middle American) beans. These results suggested that PG isolates may have coevolved with the different bean types.

As part of an effort to improve ALS resistance in preferred bean genotypes in Malawi, a study was initiated to characterize the genetic variability of PG isolates recovered from Andean and Middle American bean genotypes in Malawi and to correlate the genetic diversity of the fungus with pathogenicity on bean genotypes of the two gene pools. The results of these studies would then be used to design crosses that would allow for incorporation of ALS resistance into susceptible Malawian materials.

RAPDs analysis of PG isolates. Bean leaf and/or pod tissues and bean seeds were collected from bean plants with ALS symptoms in the major bean-growing regions of Malawi in 1991 and 1992. A total of 44 ALS isolates were recovered and single-spore cultured for genetic analysis. The bean gene pool of the plants from which these isolates were recovered was determined as previously described by Gepts et al. (1992). To determine the genetic variability of the ALS isolates, we used the technique of random amplified polymorphic DNA (RAPDs), which uses the polymerase chain reaction and a number of short (10 nucleotide) oligonucleotide primers that will result in the random amplification of one or more DNA fragments from the genome of an organism. Genetic diversity is revealed when the primers anneal at different locations/sequences in the genomes of genetically divergent organisms, resulting in different numbers/sizes of amplified DNA bands as seen after agarose gel electrophoresis. With this method, a number of primers must be evaluated because some primers will not amplify a DNA band (particularly for organisms having small genomes), some primers will not reveal differences among isolates, but some primers may reveal genetic diversity among divergent organisms. To include PG isolates that might represent the range of genetic diversity of the species, a PG isolate from Brazil (GO), which would be predicted to have been isolated from a Middle American gene pool line, and a PG isolate (WI) from a red kidney bean (Andean gene pool) sample from Wisconsin were included in the analysis along with the 44 isolates from Malawi.

In the RAPDs analysis, some primers did not amplify a DNA band, 4 primers amplified bands but revealed no polymorphisms, and 16 primers revealed polymorphisms among isolates. Phenetic analysis of the RAPDs data indicated that the isolates could be grouped into two major

clusters that roughly correlated with the two major common bean gene pools. Thus, distinct patterns were found for isolates from Andean (pattern 1, e.g. WI isolate) versus Middle American (pattern 2, e.g. GO isolate) lines. However, it was also found that, despite our considerable efforts to collect Middle American lines in Malawi, the preponderance of materials collected were of the Andean gene pool, which represents the apparent preference for beans of this gene pool in Malawi and would be expected to have a major effect on the PG population structure in Malawi. Indeed, 41 or the 44 PG isolates from Malawi were pattern 1 isolates, whereas only 3 were pattern 2 isolates. The predominance of the pattern 1 ('Andean group') isolates is consistent with the predominance of Andean beans in Malawi. To further validate whether the RAPD groups truly reflect the bean gene pool from which the isolate was recovered will require further collections in a region where Middle American lines are predominant, e.g. Brazil.

Pathogenicity of PG isolates. To ascertain whether the genetic diversity revealed by the RAPDs analysis could be correlated with bean genotype reaction, four PG isolates, two of pattern 1 ('Andean group') and two of pattern 2 ('Middle American group') were inoculated onto selected Malawian materials representing Andean and Middle American gene pools, and known susceptible (snapbean cvs. Topcrop and Sutter Pink) and resistant (CIAT lines A240 and A286) cultivars/lines. PG isolates were grown on V8-agar for 10 days, conidia collected in sterile distilled water, and conidial suspensions adjusted to 2 X 10^4 conidia/ml. This inocula was applied to the upper and lower leaf surfaces of the first trifoliolate leaves of greenhouse-grown bean plants. Plants were covered with plastic bags for 6 days to provide adequate humidity for infection, and disease reactions were scored 14 days after inoculation using the CIAT rating scale, which is based on a 1-9 rating, with 1 representing near immunity and 9 representing extreme susceptibility.

Lines A240 and A286 were almost immune to infection by the Andean group isolates and were highly resistant to the Middle American group isolates, whereas Topcrop and Sutter Pink were highly susceptible to all isolates (Table 1). For the Malawian materials, the Andean group isolates were more pathogenic on Andean gene pool materials, whereas the Middle American group isolates were more pathogenic on Middle American materials (Table 1). Some Malawian lines were highly susceptible to isolates of both groups.

Table 1. Reaction of bean lines to *Phaeoisariopsis griseola* isolates representing the two major RAPDs patterns.

Line/cultivar	PG Group/Isolate			
	Pattern 1/Andean		Pattern 2/Middle American	
	Mal 30	Mal 38	Mal 4	Mal 7
Topcrop	8	8	8	8
A 240	2	2	3	3
Middle American (Malawian lines)				
Namajengo	1	2	8	8
14-5	2	2	8	7
13-3	7	7	8	8
Andean (Malawian lines)				
8-7	6	4	2	3
22-2	6	8	5	2
Nasaka	7	7	7	6

These results suggest that coevolution, as demonstrated in this study for this host/pathogen interaction, has resulted in the selection of more pathogenic isolates on materials of each of the gene pools. It is also evident that by conducting separate inoculations with PG isolates representing the two PG groups it is possible to identify resistance that might have been obscured in an inoculation with isolates of both groups. Finally, the A240 and A286 lines, which showed high levels of resistance to PG isolates of both groups, may be excellent donors of ALS resistance for Malawian materials.