BEAN PATHOLOGY

Activity 1. Identifying common bean genotypes with resistance to angular leaf spot.

Introduction

The angular leaf spot pathogen, *Phaeoisariopsis griseola*, maintains a high level of variability, and as a result, screening for sources of resistance, identification and confirmation of resistant genotypes is a continuous activity. During 2003, we screened 87 recombinant inbred lines derived from crossing G 19833 (Andean) with DOR 364 (Mesoamerican) with different *P. griseola p*athotypes, representing the two major groups of *P. griseaola* (Andean and Mesoamerican pathotypes). The objective of this study was to identify lines that combined resistance to Andean and Mesoamerican *P. griseola* races in recombinant inbred lines (RILs) derived from a DOR364 x G 19833 cross. It was hopped that the identified lines will have the widest range of seed color and types to take advantage of the wide variability for seed type and color found in common bean. In addition, we also evaluated under greenhouse conditions advanced lines from the Mesoamerican breeding group with the most virulent pathotype (63-63) of Mesoamerican origin. It is hopped that these lines could be used as parents to improve ALS resistance in common bean.

Materials and Methods: Seven *P. griseola* races **(Table 1)** were used to inoculate plants under greenhouse conditions. These races included the most virulent and widely distributed races that we have in our collection. Inoculum production, plant inoculations and maintenance were done as described previously (CIAT 2002). Plants were evaluated starting 8 days after inoculation, using the standard procedure according to the CIAT - 9-class scale described by Schoonhoven y Pastor-Corrales (1987).

Results and Discussion: 33 recombinant inbred lines from the DOR 364 x G 19833 cross were identified that combined resistance to both Andean and Mesoamerican pathotypes of *P. griseola* **(Table 2)**. In previous evaluations, some of these lines were identified as having good levels of resistance to 6 races of *Colletotrichum lindemuthianum* **(Table 1)**. These materials combine resistance against Andean and Mesoamerican races of *P. griseola* and *C.lindemuthianum*, they therefore, from a very import potential source of multiple resistance to the two most important common bean pathogens. These materials have resistance to bean golden mosaic virus, and they should be evaluated with other races of a diverse origin to establish their suitability for managing the ALS disease of common bean.

Conclusions: The common bean lines reported here constitute very important sources of resistance for *P. griseola* and *C. lindemuthianum*. It is important that these materials are evaluated first under greenhouse conditions with isolates from different regions where ALS is an important pathogen, and other important pathogens like common bacterial blight, root rot causing pathogens etc. in order to identify lines that combine resistance to the greatest number of bean pathogens. Furthermore, the identified resistant materials should be included in an ALS nursery and distributed to our partners for evaluations under prevailing field conditions in their regions.

Table 1. Virulence phenotypes of *Colletotrichum lindemuthianum* pathotypes (races) used to evaluate recombinant inbred lines derived from a DOR 364 (Mesoamerican) x G 19833 (Andean) cross.

	Anthracnose differential cultivars*											
Pathotype	Michelite	MDRK	Perry Marrow	Cornell 49242	Widusa	Kaboon	México 222	PI207262	TO	TU4	Ab 136	G 2333
385	+							+	+			
3481	+			+	+			+	+	+	+	+
521	+			+						+		
39	+	+	+			+						

* Andean genotypes are MDRK, Perry Marrow, Widusa and Kabbon.

Table 2. Reaction of 87 recombinant inbred lines derived from a DOR 364 (Mesoamerican) x G 19833 (Andean) cross to races of *P. griseola* and *C. lindemuthianum.*

	Phaeoisariopsis griseola races							
Identification	31-19	63-59	63-63	15-55	31-47	63-0	15-0	*Anthracnose*
BT 20454- 1-1-1-1-M-M	2.3	2.0	2.8	2.1	2.3	1.0	1.9	2.4
BT 20454- 2-1-1-1-M-M	3.3	3.7	3.5	2.7	4.0	1.0	1.0	1.7
BT 20454- 9-1-1-1-M-M	3.6	5.9	3.5	2.8	2.6	1.0	1.0	4.4
BT 20454-14-1-1-1-M-M	3.0	4.0	2.3	2.6	1.1	1.0	1.0	3.8
BT 20454-18-1-1-1-M-M	1.1	2.4	1.5	1.8	1.0	1.0	2.5	1.4
BT 20454-21-1-1-1-M-M	4.3	2.2	4.8	4.8	2.0	1.0	1.0	1.2
BT 20454-22-1-1-1-M-M	4.0	3.1	4.4	3.1	2.6	1.0	1.0	2.5
BT 20454-26-1-1-1-M-M	4.9	5.4	2.4	3.8	1.8	2.3	1.0	2.7
BT 20454-30-1-1-1-M-M	2.0	1.0	2.0	1.5	2.4	1.0	2.0	1.3
BT 20454-33-1-1-1-M-M	3.2	1.0	2.7	1.9	2.4	1.0	1.0	2.3
BT 20454-39-1-1-1-M-M	4.0	4.1	4.7	3.5	3.5	1.0	2.0	2.7
BT 20454-40-1-1-1-M-M	2.0	3.9	3.2	3.0	2.4	4.5	2.0	3.3
BT 20454-42-1-1-1-M-M	4.0	4.3	2.8	4.5	2.9	1.0	1.3	3.0
BT 20454-52-1-1-1-M-M	2.0	2.2	2.3	1.7	2.2	1.0	1.0	1.5
BT 20454-53-1-1-1-M-M	2.8	2.1	2.5	2.6	2.5	1.0	1.5	1.0
BT 20454-59-1-1-1-M-M	3.7	4.7	2.3	3.5	3.0	1.1	2.6	1.5
BT 20454-63-1-1-1-M-M	2.0	3.2	2.0	1.3	2.0	1.0	1.0	1.3
BT 20454-69-1-1-1-M-M	3.6	4.8	2.7	1.8	2.2	1.0	1.0	2.1
BT 20454-70-1-1-1-M-M	4.8	3.2	3.0	2.0	3.7	1.0	1.3	2.1
BT 20454-74-1-1-1-M-M	3.0	4.4	3.5	2.4	3.8	1.6	4.7	3.0
BT 20454-75-1-1-1-M-M	3.6	3.9	2.0	1.2	1.7	1.0	1.1	2.0
BT 20454-77-1-1-1-M-M	3.0	2.4	2.0	1.5	2.0	4.7	5.1	3.6
BT 20454-78-1-1-1-M-M	4.4	3.0	2.0	1.3	1.8	1.1	1.8	2.4
BT 20454-79-1-1-1-M-M	1.5	1.8	1.3	1.0	1.0	1.1	1.2	3.2
BT 20454-81-1-1-1-M-M	2.2	3.1	3.2	2.1	2.4	1.3	1.5	2.6
BT 20454-85-1-1-1-M-M		3.9	4.0	2.6	3.0	1.0	1.1	5.0
BT 20454-88-1-1-1-M-M	3.0	2.8	2.5	1.9	1.8	1.6	1.0	4.3
BT 20454-91-1-1-1-M-M	3.2	3.1	3.7	1.5	1.7	1.0	1.0	2.7
BT 20454-93-1-1-1-M-M	1.5	3.1	1.7	1.2	1.3	1.1	3.8	1.6
BT 20454-94-1-1-1-M-M	3.1	4.2	3.2	2.3	2.2	1.0	1.0	1.1
BT 20454-95-1-1-1-M-M	3.4	4.0	3.8	1.4	1.5	1.6	1.0	2.
BT 20454-100-1-1-1-M-M	2.5	3.6	2.7	1.8	2.5	2.6	5.2	3.9
BT 20454-106-1-1-1-M-M	2.5	3.5	2.0	2.4	1.5	1.0	1.0	2.5

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Activity 2. Angular leafspot pathogen Characterized.

Random Amplified Microsatellite diversity in the common bean angular leaf spot pathogen, *Phaeoisariopsis griseola*

Introduction

With the intensification of common bean production, the incidence of the angular leaf spot disease has been on the increase, with new reports of devastation of bean production being reported every season. ALS is now wide spread in all parts of Africa, Central America, South America (Bolivia and Brazil), and the Caribbean (mainly Haiti). High genetic variability has been reported almost in all areas where the disease is a major problem, yet no sexual reproduction has been reported and preliminary analysis of the genetic population have all pointed to the fact that this pathogen is exclusively asexual. To better understand the origin and maintenance of this high genetic variation, we undertook a study to look at the molecular basis of this genetic variation, and try to elucidate its mechanism. It was hopped that this information would shed light into the evolutionary history of this pathogen and its potential to change. This information would be valuable in breeding for resistance against this pathogen and in the deployment of identified resistance genes.

Materials and Methods: A total of 808 *Phaeoisariopsis griseola* isolates from Africa, Latin America and Carribean where characterized using 5 random amplified microsatellite primers (RAMS). Population subdivion analysis was done using the POPGENE program. Genetic similarity between two isolates was calculated based on Dice's coefficient with the SimQual program of NTSYS-pc Version 1.8, and a dndrogram constructed with the help of the unweighted pair grouping by mathematical averaging (UPGMA) methods using the SAHN and TREE programs in NTSYS. Multiple correspondence analysis was used to assign isolates to groups. Haplotype diversity within and between MCA groups was calculated as described by Nei (1973). In addition, analysis of molecular variance (AMOVA) was used to partition the total genetic variation observed into that ascribed to differences between Andean and Mesoamerican *P. griseola* groups and that arising from differences of isolates within a group. Isolates had been classified into Andean and Mesoamerican based geographical origin. Nei's analysis of gene diversity in subdivided populations was used to estimate the genetic identity and genetic distances between *P. griseola* Andean and Mesoamerican groups.

Results and Discussion: Sixty (60) polymorphic fragments were generated by RAMS primer among the 808 *P. griseola* isolates. Cluster and MCA identified 395 haplotypes among 808 isolates and these were separated into two major groups **(Figures 1 and 2)**. Group 1 contained all Mesoamerican isolates, irrespective of their origin, while group 2 contained all Andean isolates. Within each group, isolates clustered according to geographical origin, with Andean isolates from Africa clustering together as did isolates from Latin America **(Figures 1 and 2)**. A similar trend was evident within the Mesoamerican group, where three groups were evident, one composed of isolates from Africa, a second of isolates from Bolivia and Brazil and a third of isolates from Central America **(Figures 1 and 2)**. Mesoamerican isolates were more diverse, compared to Andean isolates. High levels of genetic variability (H = 0.93) were observed within each group.

The total gene diversity within the total P. griseola population was estimated to be 0.2936 ± 0.0277, while it was 0.2307 ± 0.1828 in the Andean group and 0.2469 ± 0.1825 in the Mesoamerican group. The genetic identifty between Andean and Mesoamerican isolates was high (0.856) while the genetic distances between the two subgroups was 0.1555. Significant geographical differentiation (Gst = 0.402) was observed within the Andean sub-group, where the genetic identity between Andean isolates from Africa and Latin America was 0.7313, while the genetic distance between the two populations was 0.313. Although the geographical differentiation within Mesoamerican isolates from Latin America and Africa (Gst = 0.3499) was lower than that between Andean isolates, it was highly significant. The genetic identity between Mesoamerican isolates was 0.7922 while the genetic distance was 0.2330. These result reveal a significant amount of geographical differentiation within the two major sub-groups of *P. griseola*. When isolates were divided into Andean

Conclusion: These results confirm previous reports (Mahuku et al., 2002) of host and geographical specialization within *P. griseola*, revealing that host specialization is apparently the dominant factor in shapping the population structure of the ALS pathogen, *P. griseola*. The high levels of genetic variabiulity within each group reveal the mixing of pathotypes, possibly through movement of scontaminated seed or through parasexual reproduction. These results have major implications in common bean improvement for reisstance to P. griseola, by revealing that mixing Andean and Mesoamerican host resistance genes might afford lasting resistance to this pathogen.

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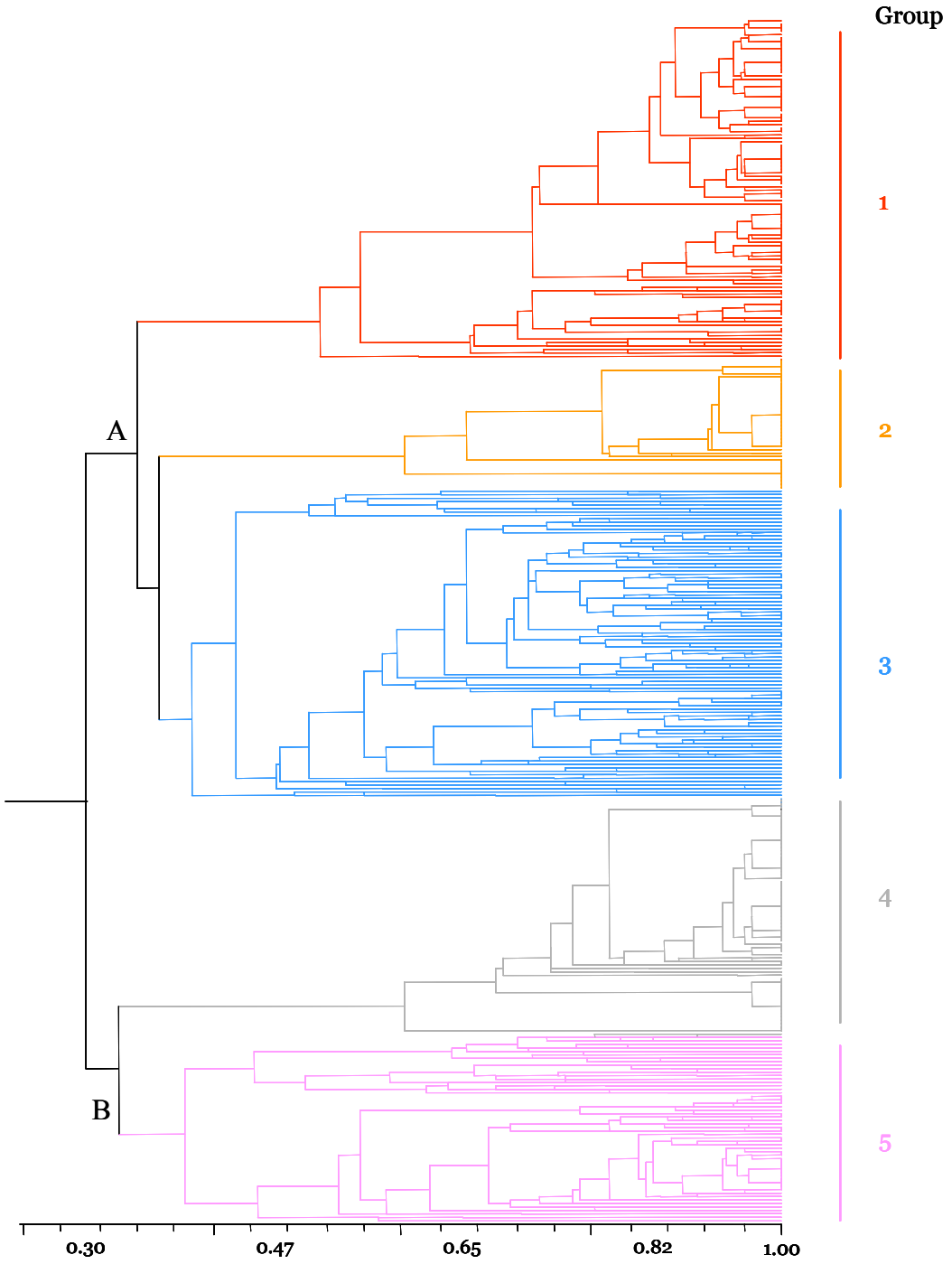
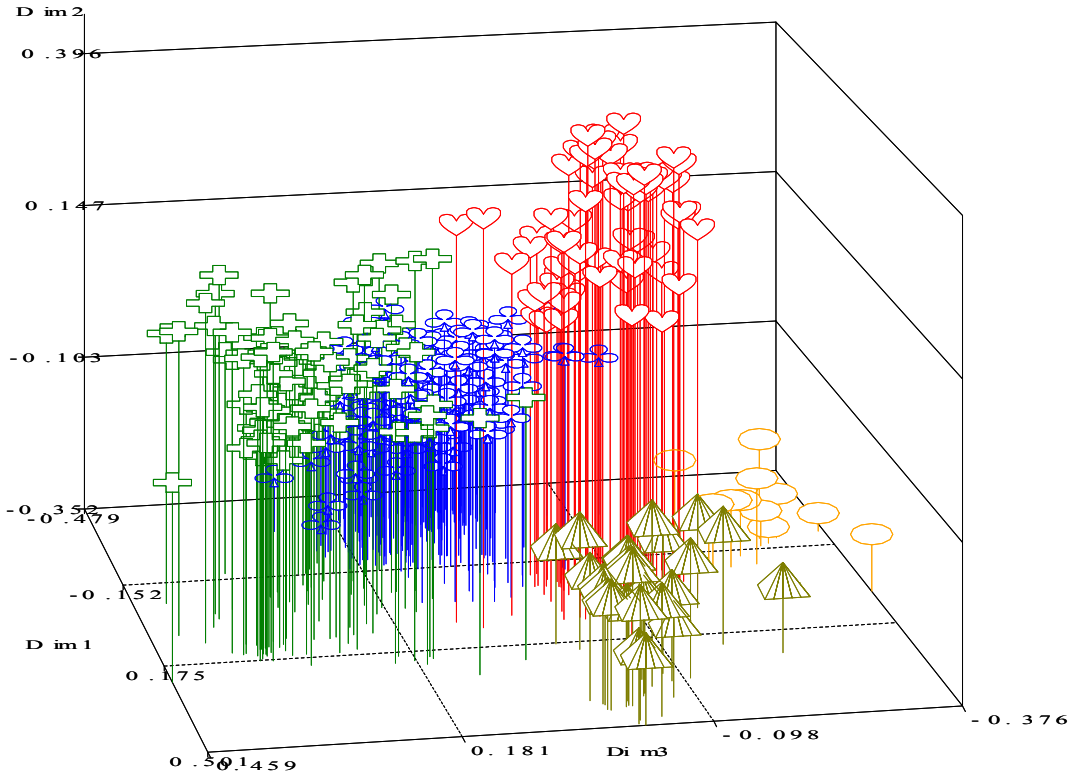


Figure 1. **Dendrogram of 808 *Phaeoisariopsis griseola* isolates plotted using data from combined RAMS data and generated using UPGMA in NTSYS. Cluster A represents Mesoamerican isolates from Brazil and Bolivia (group 1), Africa (group 2), and Central America (group 3). Cluster B represents Andean isolates from Africa (group 4) and Latin America (group 5).**



Group 1 – Mesoamerican isolates from Africa

Group 2 – Andean isolates from Africa

Group 3 – Mesoamerican isolates from Brasil and Bolivia

Group 4 – Mesoamerican isolates from Central America

Group 5 – Andean isolates from Colombia and Ecuador

Figure 2. **Three dimensional graph based on multiple corresppondence analysis of RAMS data and plotted using the spin platform of JMP program in SAS. Symbols indicate position of isolates within each cluster.**

Activity 3. Evidence that the common bean anthracnose pathogen, *Colletotrichum lindemuthiuanum* co-evolved with common bean gene pools.

Introduction

In 2002, we evaluated 40 wild and cultivated Phaseolus vulgaris genotypes of Andean and Mesoamerican genotypes using 35 *Colletotrichum lindemuthianum* isolates collected from Andean and Mesoamerican regions. Analysis of this data showed that wild beans were able to differentiate isolates into Andean and Mesoamerican subgroups, while cultivated or the established *C. lindemuthianum*, differential varieties could not clearly show this differentiation (CIAT, 2002). To further confirm the subdivion of *C. lindemuthianum* isolates into groups structured along gene pools defined for the commonbean host, we used molecular techniques (AFLP and RAMS) to characterize the 35 *C. lindemuthianum* isolates used in this study. If *C. lindemuthianum* coevolved with common bean gene pools, we would expect population subdivision analysis using molecular markers to differentiate isolates primarily into two groups that will be structured or closely so, with the two gene pools of the common bean. In addition, there should be significant levels of genetic differentiation between these groups. This information is important as it reinforces the hypothesis that pyramiding Andean and Mesoamerican anthracnose resistance genes into the same cultivar would potentially afford effective and lasting resistance.

Materials and Methods: Thirty-five *C. lindemuthianum* isolates from the bean pathology collection were used in this study **(Table 1)**. Isolates selected were either collected from Andean and Mesoamerican bean genotypes and exclusively from Andean and Mesoamerican regions. Andean isolates came from Ecuador, Colombia and Peru, and only those isolated from large seeded bean cultivars of Andean origin, while Mesoamerican isolates were selected from Costa Rica and Mexico, and were isolated from small seeded cultivars of Mesoamerican origin. Isolate recuperation, culturing, spore production, inoculum preparation and inoculations were done as described previously (Pastor-Corrales et al., 1993).

Production of mycelium and DNA extraction

Colletotrichum lindemuthianum mycelium was produced in liquid V8 juice medium as described earlier (Mahuku et al., 2002). DNA quality and concentration was determined by electrophoresis in 0.7% agarose gels, and DNA was quantified using a fluorometer (Hoefer® DyNA Quant 2000, Pharmacia Biotech, USA) and adjusted to a standard concentration of 5 ng/µl in 0.1 x TE buffer.

RAMS analysis

Seven random amplified microsatellite (RAMS) primers [(CA)n, (AG)n, (GT)n, (TG)n, (CT)n, (CGA)n, (CCA)n, (ACA)n] were used to amplify DNA from all *C. lindemuthianum* isolates. RAMS PCR reactions were carried out in 12.5 µl volumes essentially as described by Mahuku et al. (2002). The amplification product was electrophoresed in 1.2% agarose gels containing 0.17 µg ml^{-1} ethidium bromide and visualized under UV light. Gel images were captured using the Eagle Eye II gel documentation system (Strata gene) and band position was determined using the Quantity One scientific Software, Version 4 (BIO-RAD).

Table 1. Origin, year of collection and characteristic of 35 *Colletotrichum lindemuthianum* isolates used in this study.

Isolate	Origin	Host variety pool of collection	Race	Molecular group
100CRI	Puriscal Costa Rica	G 11389	1433	1
106CRI	Pejivalle, Costa Rica	Sacapobres CRS 12-1-1	1025	1
154CRI	J. Diaz Costa Rica	Criojjo CRG 4-4-1	137	1
173CRI	Turrubares, Costa Rica	Criollo CRS 52-4-1	89	1
178CRI	Mora-Tabarcia, Costa Rica	Vainica CRS 57-5-1	2001	1
41CRI	Sta. Ma. Sota, Costa Rica	Chileno	1497	1
42CRI	S. Rafael, Costa Rica	Huetar (CR 29)	3545	1
45CRI	Zapote de Z, Costa Rica	Criollo	1481	1
63CRI	Puriscal, Costa Rica	MUS 173	1435	1
28MEX	Texaco, Mexico	Amapola del camino	73	2
49MEX	Zarco; Tepatitlan Mexico	Garbancillo	5	2
13PER	Celedin-Cajamarca, Peru	Sorochuco	131	3
28ECU	Loja-Saraguro, Ecuador	Criollo	4	3
4PER	Mollepata-Cusco, Peru	Rojo mollepata	8	3
57PER	Huanbocancho, Peru	Pintado rojo	7	3
77ECU	Azuay, Valle Ecuador	Bola Amarilla	65	3
80ECU	Canton Nabon Ecuador	Bola Amarilla	6	3
82ECU	Canton-Nabon Ecuador	Bola Amarilla	129	3
84PER	Mollepata, Cusco Peru	AFR 354	7	3
89PER	Taray, Cusco Peru	Nuna Cheche local	3	3
102ECU	Chimborazo Ecuador	Silvestre-113	9	4
136 ECU	Santa Catalina Ccuador	Nuna angel poroto	133	4
224COL	Cundinamara , Colombia	Silestre	1	4
238COL	Darien, Colombia	Bolon Royo	521	4
241COL	Popayan-Cuca, Colombia	La victorie	3	4
304 COL	Rio Negro, Colombia	Cargamanto	13	4
19MEX	Zacatecas, Calera Mexico	Venezuela No. 2	1093	5
39MEX	Caeajal Mexico	A-8418-2	1088	5
59MEX	Durango Mexico	Garbancillo	129	5
70MEX	Rio Grande, Mexico	Flor de mayo	453	5
78MEX	Altos de Jalisco, Mexico	Mex 87-29-1	448	5
8MEX	FCO Madero, Mexico	G 1339	1097	5
92MEX	Calpan, Puebla Mexico	Bayo	393	5
98MEX	El Horno, Chapingo Mexico	Flor de mayo bajio	1089	5

AFLP analysis

AFLP fingerprints were generated based on the method of Voss et al. (1995), using the Life Technologies AFLP® Analysis System I. Genomic DNA (250 ng) was restricted with EcoRI and MseI (2.5 U each), and *EcoRI* and *MseI* adapters were subsequently ligated to the digested DNA. The adaptor-ligated DNA was pre-amplified with AFLP primers each having zero or one selective nucleotide, the pre-amplified DNA was diluted (1:10) and an aliquot was used for selective amplification in 12.5 µl reaction volumes with various combinations of EcoRI and MseI primers having two or three selective nucleotides at the 3' ends (E-NNN / M-NNN). The reaction products were resolved on 6% polyacrylamide gels, and the bands were detected using silver nitrate staining. Gel images were scanned and band position was determined using the Quantity One scientific Software, Version 4 (BIO-RAD).

Statistical Analysis
RAMS and AFLP markers were scored as either present (1) or absent (0) of a band. A matrix of present and absence of a band was contracted and the genetic distance between two isolates was calculated based on Dice's coefficient using SAS statistical package (SAS version 6, 1989). The similarity matrix was used to construct dendrograms with the unweighted pair grouping by mathematical averaging (UPGMA) methods using the SAHN and TREE programs in NTSYS (Rohlf, 1994). Multiple correspondence analysis was used to assign isolates to groups. Correlation between RAMS and AFLP lineages were determined using the MXCOMP option of NTSYS and Spearman's Rank Correlation Coefficients, and because RAMS and AFLP data were highly correlated, the data from the two were combined and subsequently analyzed as a single data set. In addition, analysis of molecular variance (AMOVA) was used to partition the total genetic variation observed into that ascribed to differences between Andean and Mesoamerican *C. lindemuthianum* groups and that arising from differences of isolates within a group. Isolates had been classified into Andean and Mesoamerican based on the genotype the isolates were collected from and the geographical origin. Nei's analysis of gene diversity in subdivided populations was used to estimate the genetic identity and genetic distances between Andean and Mesoamerican groups of *C. lindemuthianum*.

Results and Discussion: Of the 20 primer pair combinations tested on a set of 6 isolates from different geographical regions and classified as Andean or Mesoamerican based on virulence analysis, three (E-AGG/M-CTA; E-AAC/M-CAA y E-AAG/M-CAG) were selected for further characterization of all *C. lindemuthianum* isolates. A total of 112 polymorphic bands were generated by the three AFLP primer pair combinations, while the seven RAMS primers generated a total of 69 polymorphic fragments.

Cluster analysis separated isolates into two major groups with an average similarity index of 64% **(Figure 1)**. More variation was observed within the Mesoamerican group (0.72) compared with the Andean group(0.76) **(Figure 1)**. Multiple correspondence analysis divided isolates into five groups, groups 1, 2 and 3 constituted of isolates from the Mesoamerican region while isolates in groups 4 and 5 constituted of isolates from the Andean region **(Figure 2)**. Within the Mesoamerican group, there was a clear distinction between isolates collected from Costa Rica and those collected from Mexico, with more variation observed among isolates from Mexico. These two countries were chosen because it is in these two countries that anthracnose is a major problem. Significant genetic differentiation (Gst= 0.301), was observed among Andean and Mesoamerican *C. lindemuthianum* isolates. The average gene diversity was slightly higher for the Mesoamerican group (h = 0.2845 $\pm$ 0.1791) than in the Andean sub-group (h = 0.2029 $\pm$ 0.1964). The average gene diversity in the entire *C. lindemuthianum* population was 0.3524 $\pm$ 0.1417. Nei's unbiased measure of genetic identity between Andean and Mesoamerican *C. lindemuthianum* isolates was 0.7240, and the genetic distance between the two populations was 0.3230. AMOVA showed that 42% of the total genetic variation observed was due to differences between isolates in different groups compared to isolates within a group (58%). Although a significant amount of the total variation was ascribed to differences between isolates in a group, a larger portion was ascribed to differences between isolates in different groups, revealing that significant genetic differentiation does exist between Andean and Mesoamerican isolates of *C. lindemuthianum*. Nei's analysis of genetic diversity, AMOVA, cluster and multiple correspondence analysis all showed that the genetic structure exhibited by *C. lindemuthianum* is

congruent with the gene pools defined for its common bean host. Therefore, we can conclude that *C. lindemuthianum* coevolved with gene pools that have been defined for its common bean host.

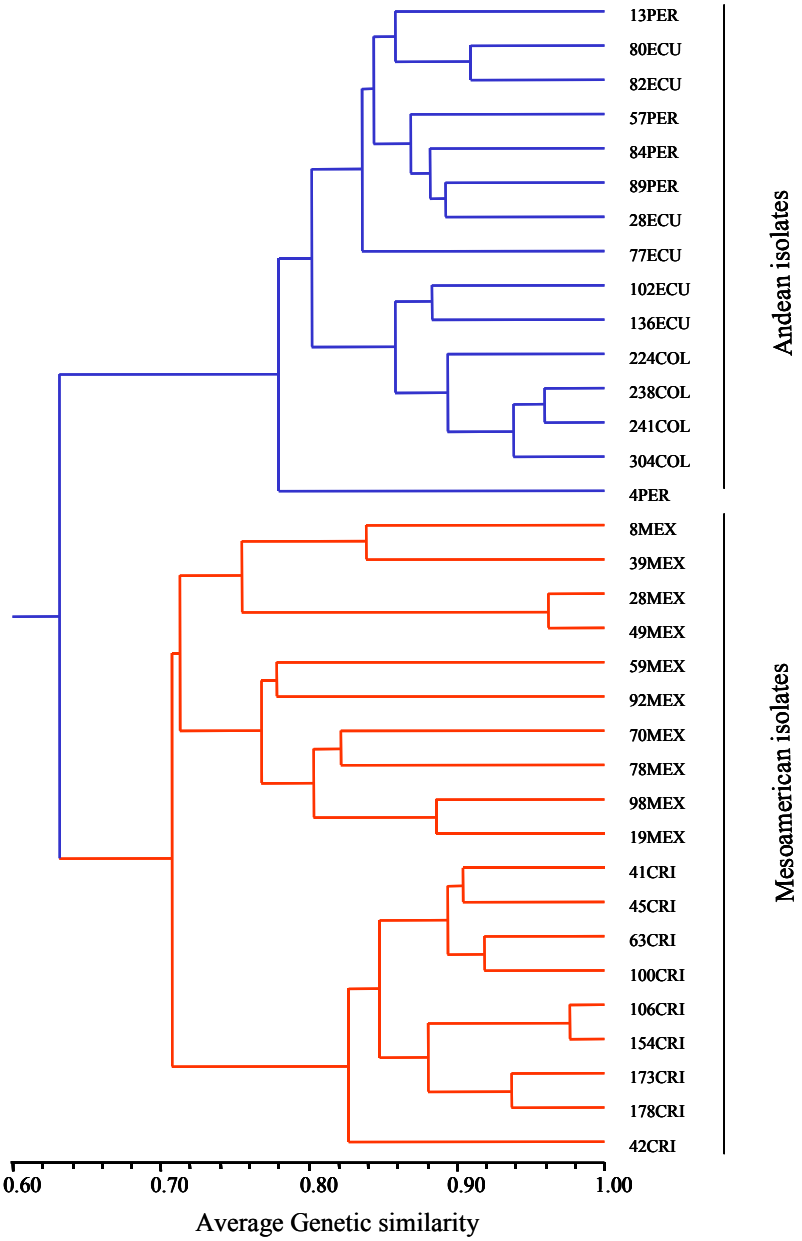


Figure 1. Dendrogram of *Colletotrichum lindemuthianum* isolates based on UPGMA methods using the SAHN and TREE option in NTSYS program with similarity coefficients calculated from combined AFLP and RAMS data. Isolates collected from Andean cultivars are shown in red and those from Mesoamerican cultivars and region are shown in blue.

Conclusion: The results reported here, combined with virulence analysis of the same isolates on 40 wild bean and land races (CIAT, 2002), clearly demonstrate that *C. lindemuthianum* exhibits a genetic structure that is congruent with gene pools defined for common bean. Therefore, *C. lindemuthianum* co-evolved with the gene pools defined for common bean.

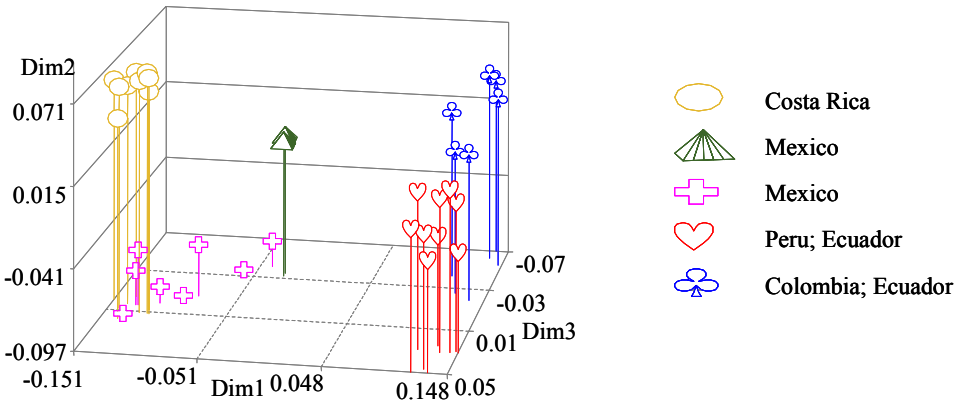


Figure 2. Three dimensional graph based on multiple corresppondence analysis of combined RAMS and AFLP data for *Colletotrichum lindemuthianum* and plotted using the spin platform of JMP program in SAS. Symbols indicate position of strains within each cluster.

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Activity 4. Characterization of *Colltotrichum lindemuthianum* isolates from Antioquia and Santander en Colombia.

Introduction

Anthracnose of common bean caused by *Colletotrichum lindemuthianum* is the most important diseases in the departments of Antioquia and Santender, Colombia. Common bean genotypes previously identified as good sources of resistance have become susceptible. In an effort to understand and elucidate the current shift in pathogenisis, a study was initiated in 2002, to look at the composition of the *C. lindemuthianum* population in these regions. This year, more isolates were collected from different production fields to increase the number of isolates tested and verify whether the change in the composition observed last year would be reflected in the new collections. The objectives was to establish whether a there was a change in the pathotype composition and structure, attempt to ascertain the sources of the new pathotypes and identify representative pathotypes that can be used to screen potential sources of resistance. This would insure that resistance genes or gene combinations that give the widest activity against the current *C. lindemuthianum* population are deployed and or used in the breeding programs.

Materials and Methods: Sixty-five *C. lindemuthianum* isolates were isolated from samples collected from farmers' fields in the departments of Antioquia and Santander, Colombia, using previously described methods (CIAT, 2002). Sixty of the isolates were characterized on a set of 12 international anthracnose differential genotypes as described previously (CIAT, 2002). In addition, 5 isolates from Darien and Popayan were also characterized.

Results and Discussion: Twenty races were identified among 42 isolates collected from Antoioquia, while 7 races were identified among the 18 isolates from Santander **(Table 1)**. Only one race (1024) characterized from Santander was not found in Antioquia. The most frequent races were 1 and 133, and race 395 was the most virulent , infecting 5 of the anthracnose differential varieties. Five new races were characterized in Santander while 10 new races wre identified in Antioquia. In addition, some races that were previously characterized were not detected. The most resistant differential varieties were Widusa, Kaboon, Tuand G 2333, which were not infected by any of the races characterized **(Table 2)**. These differential varieties have well characterized resistance genes and they can be used as sources of resistance. In addition, molecular markers for the use of some of these genes are available, and marker assisted selection can be used to speed the process of introgressing these resistant genes into susceptible but preferred varieties.

Conclusion: The activity to characterize *C. lindemuthianum* isolates from Colombia is continuing under a subproject with CORPOICA. Preliminary results have showed a shift in race structure of *C. lindemuthianum*. A total of 15 new races were characterized, while ten races that were previously identified were not detected, showing a shift in the races structure of *C. lindemuthianum* in this region. More samples have been received following the initiation of the growing season and a complete picture of the race structure will be obtained following completion of the project. Meanwhile, the new races are being used to inoculate potential sources of resistance. The differential varieties G 2333, Kaboon and Widusa have consistently

been resistant to many of the races from these two regions and these are very good sources of resistance for these two departments in Colombia.

Table 1. Common bean Anthracnose differential varieties and their respective identified resístanse genes.

Code	Differential Variety	Gene Pool[b]	Resistance(s) gene[c]	Binary Value[d]
A	Michelite	M	?	1
B	MDRK	A	Co-1	2
C	Perry Marrow	A	Co-1^3	4
D	Cornell 49242	M	Co-2	8
E	Widusa	M	Co-1^5	16
F	Kaboon[d]	A	Co-1^2	32
G	Mexico 222	M	Co-3	64
H	PI 207262	M	Co-4^3, Co-9	128
I	TO	M	Co-4	256
J	TU	M	Co-5	512
K	AB 136	M	Co-6, Co-8	1024
L	G 2333[e]	M	Co-4^2, Co-5, Co-7	2048

b = M=Mesoamerican; A= Andean
c = identified resistance genes
b=Binary value assigned for each differential variety and used for race designation
e= differential varieties that are resistant in Antioquia and Santander

Table 2. Frequency distribution of *Colletotrichum lindemuthianum* races characterized from different departments of Colombia.

Race	Altiplano North	Oriente Antioqueño	Santander	Darién	Popayán
0	1		3		
1	2	4			2
3	2	1	6		
4	3	2			
6				1	
7	1		1		
65		1			
129		2	1		
131	2	2			
133	6	6	2		
135	1	1			
136		1			
141	2		1		
387	1			2	
395	1				
1024			4		
	22	20	18	3	2

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Activity 5. Nature and inheritance of angular leaf spot resistance in ALS differential genotypes and identified resistance sources.

Introduction

Elucidating the nature and inheritance of resistance to angular leaf spot of common bean is one of the activities that has gained precedence in the Pathology activities. Over the past several years, sources of resistance to the angular leaf spot pathogen have been identified, and sufficiently characterized using different pathotypes of *P. griseola* from distinct geographical areas. A total of 19 good sources of resistance were identified following greenhouse and field characterization in different areas. To date, the genetics of resistance has been identified in only four of these sources of resistance. To effectively exploit the diversity in bean genes to combine and pyramid useful genes, sufficient characterization of the genetics of resistance is necessary. Determining and understanding the inheritance of ALS resistance in the most promising accessions, bred and differential lines would facilitate deployment of the identified resistance genes, breeding for ALS resistance, tagging the genes and identifying molecular markers for use in MAS. This is an on going study to understand the nature of inheritance of ALS resistance in common bean, with the ultimate objective of developing molecular markers that can be used to aid the transfer of resistance to well-adapted market class type bean.

Materials and Methods: Populations (F1, F2, and F1 backcrosses to resistant and susceptible parents) were made using the variety Sprite or A 36 as the susceptible parents. Populations were developed as reported previously (CIAT 2002). Green house disease evaluations, data analysis were done as described previously (CIAT 2002). Evaluations for disease severity were assessed using a CIAT 1 – 9 scale, where 1 represents no visible symptoms and 9 = severe symptoms and disease expression. Ratings of 1 to 3 were considered resistant and ratings > 4 as susceptible. Area under disease progress curves was calculated to assign genotypes to resistance and susceptibility classes. Several different genetic hypotheses were tested for each population using a Chi-squared test in the SAS program.

Results and Discussions: The observed segregation ratios from F_1, F_2, and back cross to resistant (BC1-R) and susceptible (BC1-S) parents revealed that both dominant and recessive genes, with and without epistasis conditions resistance to *P. griseola* in common bean. The resistance of Mexico 54 to race 31-55 is conditioned by 2 recessive interacting genes (epistasis). The resistance in PAN 72 and Don Timoteo to inoculations with races 15-0 and 62-0 is conditioned by a single dominant gene **(Table 1)**, while that of G 20743 to race 63-63 is due to a single recessive gene. Two dominant genes with epistasis condition the resistance of G 2858, Flor de Mayo, G 5686.

Conclusions: As previously reported for other genotypes, the results reported show the complex nature of inheritance of resistance to *P. griseola*. Major genes (whether recessive or dominant) are involved in conferring resistance to ALS and the complex segregation patterns observed in Montcalm and Amendoim reveal a possibility that minor genes might be involved as well. In addition, several of the sources of resistance and differential varieties carry more than one resistance gene that show different forms of epistatic interactions. It is essential that these genes are separated and tagged, for them to be introgressed into market class type beans.

Table 1. Nature and inheritance of angular leaf spot resistance in some differential varieties and selected resistant sources.

Source	Generation	Observed	Expected	X^2	Interpretation	Conclusion
Mex 54	F1	20:69				Partially
	F2	3929:92	4:3:9	0.95	2 rec. genes	recessive
	BC-P1	32:24	1:1	0.28	1 rec. gene	resistance due to
	BC-P2	28:86	1:3	0.91	Tendency toward rec. resistance	two genes
PAN 72	F1	137:0			Dominant	Dominant
	F2	47:15	3:1	0.88	1 dom. gene	resistance
	BC-P1					due to one gene
	BC-P2	17:16	1:1	0.86	1 dom. gene	
G2858	F1	54:37			Partially dom.	Two recessive
	F2	96:125	7:9	0.93	2 dup. rec. genes	duplicate genes
	BC-P1	27:15	3:1	0.10	2 dup. Rec genes	
	BC-P2	14:29	1:3	0.25	2 dom genes	
Flor de Mayo	F1	74:54			Partial dom. w/ het. 40% S	Two epistatic
	F2	54:33	9:7	0.32	2 dom. genes	recessive genes
	BC-P1	93:11			1 dom w/ het. 40% S	
	BC-P2	13:33			1 dom w/ het. 40% S	
G5686 x Sprite	F1	40:25			partially dom w/ 38% S	2 interacting
	F2	69:25:43	9:3:4	0.21	2 dom. genes + IA	genes
	BC-P1 (res)	18:4	3:1	0.46	Additive?	
	BC-P2 (sus)	4:64				
G5686 x A 36	F2	52:68	7:9	0.93		2 interacting genes
Amendoim	F1	0:136			Recessive	Recessive
	F2	13:194	1:15	0.96	2 rec. genes	resistance
	BC-P1	5:87	1:15	0.74	3 rec. genes	due to 2 genes
	BC-P2	0:58			Rec. resistance	
Montcalm	F1	6:49			Additive or partially rec.	2 interacting
	F2	24:214	7:57	0.63	2 rec. genes	genes (possibly
	BC-P1	5:48	1:15	0.33	3 rec. genes	complementary)
	BC-P2	3:19	1:3	0.21		
Timoteo	F1	59:3				Dominant resistance
	F2	89:40	3:1	0.12	1 dom. gene	due to 1 gene
	BC-P1	28:3			Dom, = P1	
	BC-P2	17:17	1:1	1.0	1 dom. gene	
G10431	F1	2 :38	0 :1			2 recessive genes
	F2	11:154	1:15	0.83	2 rec. genes	
	BC-P2					
G20743	F1					A single
	F2	30:61	1:3	0.08	1 rec. gene	recessive gene
	BC-P2					

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Activity 6. Inheritance of anthracnose resistance in the Andean germplasm accession G 19833.

Introduction

The genotype G 19833, a common bean germplasm accession from the highlands of Peru, was identified as a good source of anthracnose resistance. G 19833 is resistant to Andean and Mesoamerican races of *Colletotrichum lindemuthianum*, including races that overcome the resistance in the germplasm accession G 2333, which carries three anthracnose resistance genes *Co-4*^{2}, *Co-5* and *Co-7*. Previous attempts to elucidate the nature of anthracnose resistance in G 19833 were confounded by the variety used in the cross, DOR 364, which is known to have resistance against Andean races of *C. lindemuthianum*. Because of epistatic effects of the resistance genes in DOR 364 and G 19833, the nature of inheritance of anthracnose resistance could not be conclusively established. In this study, a universally susceptible variety, La Victorie, with no known anthracnose resistance gene was used as the susceptible parent.

Materials and Methods: G 19833 (anthracnose resistant) was crossed with La Victorie (anthracnose susceptible) to generate F_1, F_2 populations, and backcross populations to resistant and susceptible parents. 200 F2 plants, 100 plants derived from a backcross to resistant parent and 50 plants for backcross to a susceptible parent were independently inoculated with 4 *C. lindemuthianum* races (race 1, 7, 73 and 3481) comprising Andean and Mesoamerican races. Seeds were planted in flats and grown under greenhouse conditions for 10 days until seedlings had reached the full-expanded primary leaf stage. Seedlings of each accession were sprayed with spore suspension (1 X 10^4 spores mL^{-1}) until runoff on the stem and both surfaces of the cotyledons. After inoculation, plants were maintained in high humidity (>95%) at approximately 22C, with a 12 h light/dark cycle for 8 days. Disease was rated 10 days after inoculation using a CIAT 1 to 9 severity scale (Schoonhoven and Pastor-Corrales, 1987).

Results and Discussion: Evaluation of 200 F2 individuals with race 3481 showed a segregation ratio of 1: 3 (resistant : susceptible), and a 1:1 segregation ratio of the backcross population to the resistant parent, G 19833, a 0:1 segregation ratio to the susceptible parent La Victorie and a 1:0 reaction of the F1 individuals, suggestive of a single recessive gene conditioning resistance to this race **(Table 1)**. A segregation ratio of 7:57 was observed in 200 F2 plants inoculated with the Andean race 1, indicative of two or three complementary recessive genes conditioning resistance to this race **(Table 1)**. A segregation ratio of 7 (resistant): 9 (susceptible) was indicative of two duplicated recessive genes conditioning resistance of G 19833 to *C. lindemuthianum* race 7. When an additional 200 F2 plants were inoculated with race 73, a 9:7 segregation was observed, indicating that two dominant genes conditioned anthracnose resistance in G 19833 to race 73. This was confirmed in the segregation of the F1 (1:0) and backcross populations **(Table 1)**. The results obtained in this study reveal that more than a single gene conditions resistance of G 19833 to *C. lindemuthianum*. These genes can either be dominant or recessive, depending on the *C. lindemuthianum* race being used. In general, recessive genes condition resistance to Andean races, while both recessive and dominant genes can condition resistance to Mesoamerican races.

Conclusions: The results reported here show the complex nature of anthracnose resistance in G 19833, where different genes condition resistance to different races. At least three recessive and two dominant genes condition anthracnose resistance in this genotype. These results support the QTL analysis of RILS derived from a G 19833 x DOR 364 cross, where resistance to Andean races was localized on a different linkage group to that conditioning resistance to Mesoamerican races. G 19833 contains both major and minor genes conditioning resistance to *C. lindemuthianum.* The nature of the resistance gene depends on the classification of the pathogen race used.

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Table 1. Reaction of F1 and F2 plants derived from crossing G 19833 x La Victorie and the backcross to resistant and susceptible parents to inoculation with Andean and Mesoamerican races of *Colletotrichum lindemuthianum*.

Race	Generation	Observed Ratio	Expected Ratio	X^2	P	Comments
1	F1	2 R : 17 S				
1	F2	17 R: 179 S	7:57	1.03	0.3098	1 dominant, 2 complementary recessive genes
1	BC-R	29 R: 64 S				
1	BC-S	0 R : 50 S	0:1			
7	F1	8 R : 8 S	1:1			
7	F2	77 R: 120 S	7:9	1.741	0.187	2 recessive genes, duplicated
7	BC-R	88 R: 11 S				
7	BC-S	0 R : 50 S	0:1			
73	F1	19 R : 0 S				
73	F2	112 R : 86 S	9:7	0.008	0.928	2 dominant genes, duplicated
73	BC-R	100 R: 0 S				
73	BC-S	17 R : 32 S				
3481	F1	2 R : 17 S				
3481	F2	49 R: 145 S	1:3	0.008	0.933	1 Recessive gene
3481	BC-R	49 R: 49 S	1:1			
3481	BC-S	0 R : 50 S	0:1			

[a] M = Mesoamerican; A =Andean.
[b] Generation F2; RC = Backcross to G 19833.

Activity 7. Allelism test for anthracnose resistance genes in the germplasm accession G 19833.

Introduction

Only one anthranconse resistance gene of Andean origin (Co-1) has been characterized. Several alleles of this gene have been defined in different Andean anthracnose differential varieties; Kaboon (Co-1^2), Perry Marrow (Co-1^3), Michigan Dark Red Kidney (Co-1) and Widusa (Co-1^5) and sources of resistance AND 227 (*Co-1^4*). Given the small number of Andean genes that have been identified and characterized, there is a need to look for other Andean anthracnose resistance genes for use in breeding programs while avoiding the deployment of only a single gene, a situation that is not desirable. G 19833 has been observed to be highly resistant to isolates that infect the varieties that carry different alleles of the *Co-1* resistance loci. This study was carried out to test the independence of the resistance gene(s) in G 19833 from the *Co-1* gene and establish if the resistance gene(s) in G 19833 were the same or different from the *Co-1* alleles in the cultivars Michigan dark red kidney, Kaboon and Perry Marrow (Melotto et al, 2000).

Materials and Methods: Two hundred F2 plants derived from crossing G 19833 x Kaboon, G 19833 x Perry Marrow and G 19833 x Michigan Dark Red Kidney (MDRK) were inoculated independently using three C. lindemuthianum races (race 1, 73, and 3481) **(Table 1)**. All bean varieties are resistant to these three races. Bean seedlings with fully expanded primary leaves were sprayed with the conidia suspension ($1.2x10^6$ conidia/ml) until runoff on the stem and both surfaces of the cotyledons. Inoculated plants were incubated in a chamber at 22ºC and 90-100% relative humidity with 12 h light/dark cycle. Each isolate was evaluated separately. Plants were scored 8 days after inoculation using a 9-class scale described by Schoonhoven y Pastor-Corrales (1987). A plant with no visible symptoms or with only a few, very small lesions mostly on the primary leaf veins was scored as resistant (rating 1 to 3). A plant with numerous small or enlarged lesions, or with sunken cankers on both the lower sides of leaves and the stems was recorded as susceptible (rating 6.1 to 9). A plant with a rating score of (3.1 – 6) was considered as intermediate.

Results and Discussion: Inoculation of the G 19833 x MDRK, Perry Marrow and Kaboon with race 3481 revealed that G 19833 contains resistance genes that are different from the ones carried by MDRK, Perry Marrow and Kaboon **(Table 2)**. The resistance gene in G 19833 is different from the one in MDRK (3:1), and has two genes that are different from the ones Perry Marrow (15:1) and at least 3 genes segregating in the G 19833 x Kaboon cross (63:1).

Inoculation of the F2 populations using *C. lindemuthianum* race 73 revealed that three genes were segregating (63:1) in the G 19833 x MDRK cross and in the G 19833 x Perry Marrow cross (61:3), while in the G 19833 x Kaboon, two independent genes were segregating (15:1). Therefore, the resistance genes conditioning anthracnose resistance in G 19833 to race 73 are different from the ones in Kaboon, Perry Marrow and MDRK, and at least three genes are involved in conditioning resistance.

When these populations were inoculated with race 1, a 9:7 segregation ratio was observed for the G 19833 x MDRK cross; a 57:7 for the G 19833 x Perry Marrow cross and a 15:1 for the G 19833 x Kaboon cross, revealing that at least three independent genes are segregating.

Conclusion: The segregation ratios observed reveal that G 19833 carries resistance genes that are different from the ones in MDRK (*Co-1*), Perry Marrow (*Co-1*^{3}) and Kaboon (*Co-1*^{2}). The 3:1 segregation ratio reveal that at least one of the resistance gene is an allele to *Co-1* locus.

Table 1. Gene pool, described anthracnose resistance genes and disease reaction of bean cultivars inoculated with different races of *C. lindemuthianum*.

Bean cultivar	Gene Pool	Known Gene	Colletotrichum lindemuthianum races			
			1	7	73	3481
MDRK	A	Co-1	R	S	R	R
Perry Marrow	A	Co-1^3	R	S	R	R
Kaboon	A	Co-1^2	R	R	R	R
G 19833	A		R	R	R	R

Table 2. Test for the independence of the resistance genes in F2 populations derived from crossing G 19833 with Kaboon, MDRK and Perry Marrow

Cross	Race	Obs (R:S)	Esp	X^2	P	Coment
G19833xMDRK	1	128:70	9:7	5.62	0.02	duplicated recessive genes
G19833xMDRK	73	197:3	63:1	0.01	0.74	3 independent genes
G19833xMDRK	3481	153:47	3:1	0.240	0.62	1 dominant gene
G19833xPM	1	175:25	57 :7	0.50	0.48	3 genes, 2 are complementary
G19833xPM	73	192:8	61:3	0.21	0.65	3 genes, 2 dominant, 1 recessive
G19833xPM	3481	186:14	15:1	0.19	0.66	2 independent dominant genes
G19833xKAB	1	184:12	15:1	0.01	0.94	2 independent dominant genes
G19833xKAB	73	187:13	15:1	0.02	0.88	2 independent dominant genes
G19833xKAB	3481	196:4	63:1	0.25	0.62	3 independent dominant genes

Contributors: G. Mahuku, C. Jara, J. Fory, G. Castellanos, H. Teran, S. Beebe.

Publications

Mahuku, G.; Jara, C.; Terán, H., and Beebe, S. 2003. Inheritance of angular leaf spot resistance in selected common bean genotypes. *In*: BIC (Bean Improvement Cooperative). Annual report. East Lansing, MI, USA. v. 46, p.151 –152.

Mahuku, G.S.; Jara, C.E.; Cajiao V., C.H.; and Beebe, S. 2003. Sources of resistance to angular leaf spot (*Phaeoisariopsis griseola*) in common bean core collection, wild *Phaseolus vulgaris* and secondary gene pool. Euphytica 130: 303-313.

Mahuku, G.S.; Jara, C.E.; Cajiao V., C.H.; Beebe, S. 2002. Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Dis 86(12):1383-1387. The American Phytopathological Society.

Mahuku, G., Jara, C., Cuasquer, J.B. and Castellanos, G. (2002) Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology 51:594-604.

Submitted

Mahuku, G.S., and Riascos, J.J. (2003) Virulence and Molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. European Journal of Plant Pathology.

Mahuku, G., Montoya, C., Henríquez, M.A., Jara, C., Teran, H., and Beebe, S. 2003. Inheritance and Characterization of the Angular Leaf Spot Resistance Gene in the Common Bean Accession, G 10474 and Identification of an AFLP Marker Linked to the Resistance Gene. Crop Science.

Trips and attendance at meetings

Participate as a resource person in a Marker assisted selection workshop in Kampala, Uganda.

Training, students and courses

Three scientists (Senora Ligia Dense; Juliana Rojas, Ana Maria Serralde and Patricia Hernández) from CORPOICA in Bogotá were trained (1 week) in Técnicas para la conservación de microorganisimos a larga plazo).

A student, Monica Navia Urrutia (Universidad del Valle, Cali) has started work on infection process of Phaeoisariopsis griseola.

Maria Antonia Henríquez started her MSc thesis with the Universidad National, sede Palmira

Workshop and Conference

Jara, C., Castellanos, G., and Mahuku, G. 2003. Comparación a través del tiempo del agente causal de la antracnosis del frijol ((*Colletotrichum lindemuthianum)* en los departamentos de Antioquia y Santander. XXIV Congreso de la Asociación Colombiana de Fitopatologia y Ciencias afines (ASCOLFI) , Junio 25-27 de 2003, Armenia.

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