

Pathotypes Characterization and Virulence Diversity of *Pseudocercospora griseola* the Causal Agent of Angular Leaf Spot Disease Collected from Major Common Bean (*Phaseolus vulgaris* L.) Growing Areas of Ethiopia

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Abstract

Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* is one of the most economically important disease affecting common bean production in Ethiopia. Until now, no information has been generated regarding the pathotype and pathogenic variability and its distribution in Ethiopia. A study was undertaken to characterize the pathotype and virulence variability among 39 *P. griseola* isolates, ALS pathogen, occurring in Ethiopia. A total of 21 pathotypes (63:63, 63:59, 63:23, 61:51, 56:36, 55:39, 49:7, 48:60, 42:59, 41:10, 34:53, 23:61, 19:33, 17:45, 8:18, 8:0, 4:16, 1:24, 1:10, 16:18. and 4:37) were determined using 12 sets of ALS common bean differentials cultivars. These results revealed the presence of high and diverse pathogenic variability of the pathogen. Among the determined pathotypes 63:59 and 19:33 were the most frequently appeared. And the occurrence of three pathotypes (63:63, 63:59 and 63:23) were confirmed as well as the previous reports in central America and Argentina. This will be the first comprehensive report of *P. griseola* pathotypes existing in the common bean growing areas of Ethiopia. Except pathotype 8:0 that were compatible with Andean common bean groups, most of the isolates were pathogenic to both Andean and Mesoamerican common bean gene pools. Based on that, the isolates were classified as Mesoamerican origin pathotypes. This specific study provided major information about the pathogenic diversity and determined the pathotype of *P. griseola* from common bean in Ethiopia.

Keywords: Pathotype; *Pseudocercospora griseola*; Pathogenic variability single spore isolates

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume next to Faba bean and which is produced all of the regions of Ethiopia with different intensity for its nutritional and economic values [1]. Among the common bean diseases, the angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous and Braun (*sin. Phaeoisariopsis griseola* (Sacc.) Ferraris) is one of the most important disease [2]. ALS cause necrotic lesions on the aerial parts of the plant, reducing the productivity and quality of common bean seed [3]. Yield loss of more than 374,800 tonnes annually have been reported [4]. The pathogen is found in nature in the form of mycelia or conidia on living tissues of the host plant (susceptible on and off-season crop, volunteer plants), undecomposed infected bean residues and infected soils. The pathogen is a seed born in most cases external contamination may occur on seed during harvesting and the pathogen has been associated with the hilum area of the seed coat [5]. This specific fungus is highly variable and has the ability to infect both Andean and Mesoamerican common bean [6] gene pools. Previous studies have revealed high levels of pathotypic variation in *P. griseola* [7-11]. In addition, many scientists reported the presence of high pathotype diversity of this pathogen. For example, Colombian 33

isolates and Brazilian 27 isolates were classified into 13 pathotypes and 21 pathotypes [8], respectively. In recent studies, high occurrence of pathogenic variability has also been reported in the neighboring Kenyan and Ugandan [9,10]. In spite of the high pathotype diversity, all *P. griseola* pathotypes have been divided into Andean and Mesoamerican pathotype groups that correspond to the two common bean gene pools [7,8,10,11]. The Andean pathotype group consists of *P. griseola* isolates recovered from large-seeded common bean genotypes of Andean origin that infect Andean genotypes only, the Mesoamerican pathotype group contains isolates that are more virulent on Mesoamerican bean and Andean genotypes [4,7,12]. To assess the degree of diversity of *P. griseola* isolates, standard methodology for *P. griseola* pathotype identification [8] which was two sets Andean and Mesoamerican with six common bean genotypes included in each set, were conducted. The common bean differential genotypes and their binary numbers includes, Don Timoteo (1), G1179 (2), Bolon Bayo (4), Montcalm (8), Amendoin (16), G5686 (32), PAN 72 (1), G2858 (2) Flor de Mayo (4), Mexico 54 (8), BAT 332 (16) and Cornell 49242 (32). The number or race designation given to an isolate is determined by the cultivars of the differential set that are infected by that isolate. For example; if an isolate infects Andean common bean genotype Amendoin and Montcalm (binary value, 16 and 8 respectively) and the Mesoamerican variety BAT332 (binary value 16) and Cornell 49242 (32) the race would be designated by adding the

values (16+8): (16+32) which is 24:48 Knowledge of pathotype variation among the isolates of *P. griseola* is important to the common bean improvement program to guide the deployment of resistance genes to ALS disease. However, there is no information on the Ethiopian *P. griseola* isolates, which puts the common bean improvement program under challenging situation to develop durable disease (ALS) resistance varieties with wider adaptation. Therefore, this specific study used the international sets of ALS differential common bean cultivars to determine and characterize the *P. griseola* pathotypes occurring in bean growing areas of Ethiopia. The objectives of the study were to determine the pathotype variability and the virulence pattern of *P. griseola* in the bean growing agro-ecologies of Ethiopia and to identify predominant pathotypes that exist in the major common bean growing areas of Ethiopia.

Materials and Methods

Sampling strategy and collection

Samples of infected leaves with symptoms of ALS were collected from the fields during survey from 2016 to 2017. They obtained from diverse agro-ecological zones in six diverse areas of Ethiopia (Areka, Dolla, Chano, Omo, Wondo and Goffa). These locations are known for its major common bean production [13] and for its high common bean disease severity especially the ALS. The sampling locations were selected based on the intensity of bean production, special ecological locations representing diverse conditions under which common beans are produced (Figure 1).



Figure 1: Different disease scoring of leaves inoculated with *Pseudocercospora griseola*, the causal agents of angular leaf spot disease, using the proposed detached-leaf inoculation method, where 1=no visible symptoms; 3=10 to 20% of plants infected and/or about 5% of the total plant area affected by the pathogen; 5=40 to 50% of plants infected and/or about 20% of plant area affected; 7=60 to 70% of plants infected and/or about 40% of plant area affected; and 9=>80%.

Random sampling technique were adopted in which infected leaves with lesion symptoms usually appear as a brown spot with a tan or silvery center that were initially confined to tissue between major

veins, that gives angular appearance were collected from 10 random farmers field from each location. Then collected leaf samples were kept in a paper bag and transported to the lab.

P. griseola isolation and inoculum preparation

Selected fresh diseased common bean leaf samples from the collections were directly used and lesions were thereafter examined under a dissecting microscope (Motic BA210) to view the synemata and assess the quality of the sporulation. Conidia of individual lesions were picked from the clean synemata by gently brushing the tips with a small piece of agar at the tip of an inoculating needle and transferred to a drop of sterile water placed on water agar (2%) as described by Mahuku et al. [14]. The inoculated petri dishes were allowed to grow the pathogen evenly. The Petri dishes were incubated at 24°C for 24 hr and then the germinated spores were picked and immediately transferred to V8 media (800 mL of distilled water, 200 mL of V8 juice, 3 g of CaCO₃ and 20 g of agar. Each Petri dishes of V8 was inoculated with single conidia avoiding mixtures during race identification and determination. Each colony that grows from a single conidium was treated as an isolate and a single spore isolates are considered pure during race determination. For the colonies to develop and multiply the Petri-dishes were allowed for about 14 days. Then each colony was treated as monosporic isolate.

Inoculation and pathotype determination

Isolation, monosporic culture and inoculation were done according to the method developed by Pastor Caralles et al. [12]. Before the inoculation, the spore concentration was adjusted in distilled water at 1.0×10^5 per ml using haemocytometer. Monosporic isolates were grown in petri dishes containing V8 medium. The resulting spores and mycelia were scrapped smoothly with a spatula and filtered through gauze and the spore concentration was adjusted to 2.0×10^4 conidia/mL. Detached leaves of the 12 common bean differential cultivars (Don Timoteo, G 11796, Bolon Bayo, Montcalm, Amendoin, G 5686, Pan 72, G 2858, Flore de mayo, Mexico 54, BAT 332, Cornell 49242), which grown in the screening house, were used for pathotype determination. Detached leaf method was conducted with minor modifications. Eighteen days after germination, the middle foliole of the first trifoliate leaves of each common bean differential plants were removed or detached when they had reached approximately two-thirds of their full development. The detached leaves were inoculated by immersion into a spore suspension and placed in petri dishes (90 mm diameter \times 15 mm height) on a cotton moistened with 3.0 mL of tap water. The experiment was replicated three times for consistent result. The petri dishes were watered regularly to maintain about 95% relative humidity to allow the growth of the pathogen. Disease severity on the inoculated plants was evaluated using 1-9 visual score scale (Schoonhoven and Pastor-Corrales, 1987, Figure 2) for 21 days at an interval of three days. Pathotypes were defined by rating scores of 1-3 to be incompatible (-) or resistant, while ratings >3 were compatible (+) or susceptible. Pathotype designation was executed by adding binary values of the differential genotypes that were compatible with the respective *P. griseola* isolates (Table 1 and Figure 1).

Code	Cultivar Id	Seed Size	Bean Race	Binary Value	R Gene Present
A	Don Timoteo	Medium	Chile	1	1 dominant
B	G 11796	Large	Peru	2	--

C	Bolon Bayo	Large	Peru	4	--
D	Montcalm	Large	Nueva Granda	8	2 recessives
E	Amendoim	Large	Nueva Granda	16	2 recessives
F	G 5686	Large	Nueva Granda	32	1 dominant
G	Pan 72	Small	Mesoamerica	1	1 dominant
H	G 2858	Medium	Durango	2	1 dominant
I	Flore De Mayo	Small	Jalisco	4	2 duplicates
J	Mexico 54	Medium	Jalisco	8	Ph-2, Ph-5, ph-6
K	BAT332	Small	Mesoamerica	16	ph-6 ²
L	Cornell 49242	Small	Mesoamerica	32	Ph-3

Source: Caixeta Source: Caixeta et al.,2002; Mahuku et al.,2004; Sartorato et al.,2002

Table 1: Common bean ALS differential cultivar and binary system for pathotype determination study.

Results

Pathotype diversity and distribution of *P. griseola* isolates from Ethiopia

We obtained 39 *P. griseola* isolates from various regions of Ethiopia and designated them as Code Pg01-Pg39 as described in Table 2. This study revealed the existence of 21 *P. griseola* pathotypes among the 39 isolates occurring in Ethiopia. Most of the isolates showed highly pathogenic on both Andean and Middle American common bean differentials, therefore they were considered as the Middle American pathotype group. This result is in agreement with the fact that Mesoamerican gene pool common bean cultivars predominantly grown in Ethiopia. The remaining few number of isolates were highly pathogenic only on the Andean differentials and these isolates infecting only the Andean differentials were considered members of the Andean pathotype group. Among 21 pathotypes, two pathotypes (63:59 and 19:33) occurred most frequently (Figure 2). Different pathotypes co-exist in certain common bean production areas. Isolates, which were obtained from similar areas or geographic location, varied in their pathogenicity. For example, two isolates coded with *Pg01* and *Pg02* from Areka district induced similar pathogenicity on some of the differentials but not on all the differential genotypes. In most cases, *P. griseola* isolates obtained from the same geographic locations varied in their pathogens confirming presence of pathotype variability in Ethiopian isolates.

Reaction of common bean differentials to *P. griseola* pathogen

All the international differential sets of common bean genotypes reacted differently for the monosporic *P. griseola* isolates obtained from the diverse common bean growing regions of Ethiopia. The 39 monosporic pure isolates *P. griseola* obtained from the infected leaves

collected from the diverse common bean growing regions of Ethiopia caused lesions of angular leaf spot symptoms in some or all of the host differential common bean genotypes. Symptoms in the leaves were brown spots that appeared on the primary detached leaves as angular brown spot limited by veins were observed. The fungal growth on the underside of the spots was observed as clusters of synemata which bared pores or conidia. Under the electron microscope, conidial were observed as obclavate cylindrical with two to four septate.

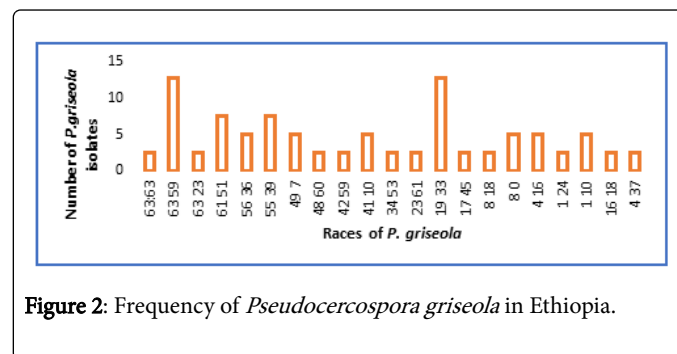


Figure 2: Frequency of *Pseudocercospora griseola* in Ethiopia.

Virulence analysis and pathotype identification

During sample collection, ALS was observed in most of the common bean fields. Variations in response of the ALS differentials cultivars were observed irrespective of the gene pool in which they were originated. *P. griseola* pathotypes are defined based on the pathogenicity reaction to a set of 12 common bean differential genotypes. In this study, the pathogenicity reaction of 39 isolates revealed the existence of pathotype variability in Ethiopian *P. griseola* isolates (Table 2). Based on the virulence reaction, 21 pathotypes were cauterized and all of the pathotypes were virulence to both the Andean and Mesoamerican common bean sets of differentials except pathotype 8:0. The result also confirmed the presence of both Andean and Mesoamerican origin pathotypes in Ethiopia (Table 2).

Code	Location	Isolate	Pathotype
Pg01	Areka	PG 37A	49:07
Pg02	Areka	R7P8	63:59
Pg03	Areka	R6P14B	61:51
Pg04	Gofa	PG44A	63:59
Pg05	Gofa	PG32C	34:53
Pg06	Gofa	CD45A	49:07
Pg07	Gofa	CD50B	55:39
Pg08	Gofa	CDPG33C	55:39
Pg09	Omo/Jinka	om418	63:23
Pg10	Chano	pg384	16:18
Pg11	Wondo	p11	23:61
Pg12	Wondo	p67	63:63
Pg13	Gofa	PG112	63:59

Pg14	Areka	R7P8	48:60
Pg15	Areka	R1P35	63:59
Pg16	Areka	R1P5C	63:59
Pg17	Areka	R1P8B	08:18
Pg18	Areka	R1PBA	17:45
Pg19	Gofa	PG 110	4:37
Pg20	Areka	R3P3A	8:0
Pg21	Areka	R4P2B	8:0
Pg22	Areka	R1P8C	4:16
Pg23	Areka	R6P11	4:16
Pg24	Wondo	B6P57	56:36
Pg25	Wondo	B3P46	41:10
Pg26	Wondo	ADP-0095	61:51
Pg27	Wondo	B6P57	56:36
Pg28	Wondo	ADP-0675	41:10
Pg29	Dolla	D 13	42:59

Pg30	Dolla	B6P14	61:51
Pg31	Chano	C D PG32	19:33
Pg32	Chano	C D PG38	19:33
Pg33	Chano	C D PG38	19:33
Pg34	Chano	C DPG 41	1:10
Pg35	Chano	C D PG 44	1:24
Pg36	Chano	C DPG 45	19:33
Pg37	Chano	C D PG46	41:10
Pg38	Chano	C D PG 50	55:39
Pg39	Chano	C D PG50	19:33

Table 2: Pathotype determination *Pseudocercospora griseola* from diverse common bean growing areas of Ethiopia.

The study identified the isolate, which is compatible to all of the 12 sets of differential common bean genotypes, as pathotype 63:63 (Table 3). Pathotype 63:63 overcomes resistance genes in 12 known sources of resistance that constitute differential of sets.

Andean						Mesoamerican						Pathotype	No of Isolates
a	b	c	d	e	f	g	h	i	j	k	l		
1	2	4	8	16	32	1	2	4	8	16	32		
+	+	+	+	+	+	+	+	+	+	+	+	63:63	1
+	+	+	+	+	+	+	+	-	+	+	+	63:59	5
+	+	+	+	+	+	+	+	+	-	+	-	63:23	1
+	-	+	+	+	+	+	+	-	-	-	+	61:51	3
-	-	-	+	+	+	-	-	+	-	-	+	56:36	2
+	+	+	-	+	+	+	-	+	-	-	+	55:39	3
+	-	-	-	+	+	+	+	+	-	-	+	49:07	2
-	-	-	-	+	+	-	-	+	+	+	+	48:60	1
-	+	-	+	-	+	+	+	-	+	+	+	42:59	1
+	-	-	+	-	+	-	+	-	+	-	-	41:10	2
-	+	-	-	-	+	+	-	+	-	+	+	34:53	1
+	+	+	-	+	-	+	-	+	+	+	+	23:61	1
+	+	-	-	+	-	+	-	+	-	-	+	19:33	5
+	-	-	-	+	-	+	-	+	+	-	+	17:45	1
-	-	-	+	-	-	-	+	-	-	+	-	08:18	1
-	-	-	+	-	-	-	-	-	-	-	-	08:00	2
-	-	+	-	-	-	-	-	-	-	+	-	04:16	2

+	-	-	-	-	-	-	-	-	+	+	-	01:24	1
+	-	-	-	-	-	-	+	-	+	-	-	01:10	2
-	-	-	-	+	-	+	-	+	-	-	-	16:18	1
-	-	+	-	-	-	+	-	+	-	-	+	04:37	1
Total													39
a=Don Timoty; b=G 11796; c=Bolon Bayo; d=Montacalm; e=Amendoin; f=G 5686; g=Pan 76; h=G2858; i=Flore de Mayo; j=Mex 54; k=BAT 332; l= Corell 49-242													

Table 3: Pathotype identification and reaction of differential common bean genotypes to the 39 isolates of *P. griseola* collected from diverse common bean growing areas of Ethiopia.

Race distribution and frequency of occurrence

The race and frequency distribution of Ethiopian *P. griseola* isolates were analysed as percentages from the total number of isolates and the results are presented in Figure 3.

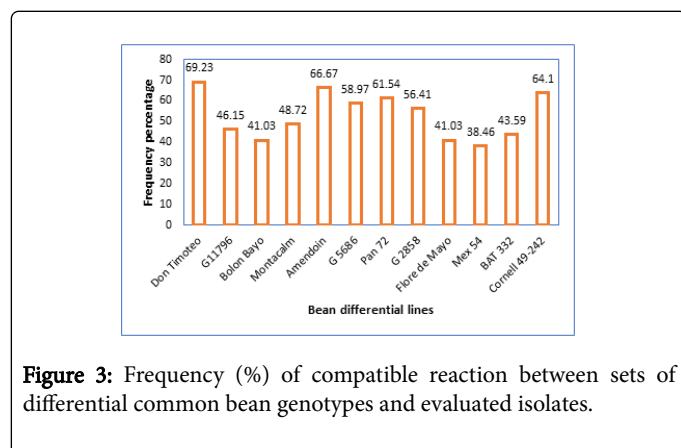


Figure 3: Frequency (%) of compatible reaction between sets of differential common bean genotypes and evaluated isolates.

A wide frequency variation revealed in which differential ‘cultivar Mexico-54’ had the lowest frequency with 38.47% while cultivars Don Timoteo (69.23%) and Amendoin (66.67%) scored the highest. The number of isolates obtained from the Middle American genotypes were greater than those from the Andean genotype. This result was in lines with the dominantly common bean production in Ethiopia is from the Middle America group and since the *P. griseola* were co-evolved with common bean gene pool.

Discussion

This study revealed that the presence of both Andean and Mesoamerican pathotypes in Ethiopia whereas the Mesoamerican pathotype was more predominant than Andean pathotype. This result supports that Mesoamerican common bean genotypes were dominantly cultivated in Ethiopia [15]. Although, no sexual reproduction was confirmed the *P. griseola* isolates from the diverse common bean growing regions showed high variation in virulence pattern. The virulence variation was high in such way that isolates collected from the same location showed differences in their virulence patterns. Several different isolates found in the same farm is not surprising. These results are in agreement with the findings of Pastor-Corrales et al., Busogoro et al. and Wagara et al. [8,9]. This result was also supported by many authors in the other countries such as Mexico, Brazil, Kenya, Uganda and Tanzania [9,10,16,17]. The high difference

in virulence patterns observed during the study indicates that the majority of the resistance genes in the host differentials common bean cultivars were effective against most of the *P. griseola* isolates from Ethiopia. This might suggest *P. griseola* isolates from Ethiopia probably have isolates with different virulence genes which might not matched with the resistance genes in the host differentials common bean cultivars. This is new report of virulence pattern of *P. griseola* isolates from the common bean growing areas of Ethiopia that includes 21 pathotypes. Among them, pathotypes 63:59 and 19:33, newly designated in this study were the most frequently observed. The majority of common bean cultivars have shown sever infections of *P. griseola*, confirming the existence of new pathotype of *P. griseola*. However, the sources of new race are currently not known and it might probably farmers practice in adjacent field. The informal seed exchange, which is dominant seed system in Ethiopian farming community, may lead to the introduction of new pathotypes and the pathotype variability. As many of the reports, the pathogen might have undergone Para sexual that facilitates exchange of genetic material within and between isolates. It might also because of chromosomal inversion, deletion and presence of transposons because all are reported to have capability to increase the variability in *P. griseola* [18-20]. Mexico-54 with low frequency percentage of pathogen infection could be used as parental lines with potential source of resistance gene in the common bean breeding program.

Conclusion

This specific study revealed the existence of virulence diversity of *P. griseola*, pathogen of common bean ALS in Ethiopia. The isolates were most predominantly from the Middle American pathotype gene pool that affects mostly both common bean gene pools of Middle American and Andean. This is in lines with the dominantly grown common bean type (the middle American gene pool) in Ethiopia. From the study, it was determined that the existence of large pathogenic and pathotype variability of *P. griseola* isolates and these pathotypes were distributed across diverse regions of Ethiopia. *P. griseola* isolates, which were obtained from the same geographic locations, showed different pathogenicity due to the district differences in response common bean differential genotypes. Hence, this would be the first report of virulence variation of Ethiopian isolates and the result from this study confirmed the presence of high pathotype in Ethiopia. The information generated from this study has a significant implication for the bean improvement program, because the *P. griseola* isolates existing in Ethiopia are with wider virulence spectrum. Hence these pathogens must be taken into consideration when developing and deploying bean cultivars with resistance to ALS. Hence the common bean breeding

program could plan at developing bean cultivars with non-pathotype specific or non-race specific resistance.

Conflict of Interest

The authors declare that they have no conflict of interest

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