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Pathogenic Variability of Angular Leaf Spot Disease of Common Bean in Western Kenya

Robert Kiptabut Leitich[1, *], D. O. Omayio[1], B. Mukoye[1], B. C. Mangeni[1], D. W. Wosula[1], W. Arinaitwe[2], R. M. Otsyula[3], H. K. Were[1], M. M. Abang[4]

[1]Biological Sciences Department, Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya

[2]International Centre for Tropical Agriculture (CIAT), Kawanda, Uganda

[3]Kenya Agricultural and Livestock Research Organisation (KALRO), Kakamega, Kenya

[4]Food and Agriculture Organisation (FAO) of the United Nations, Addis Ababa, Ethiopia

Email address:
kipta2012@gmail.com (R. K. Leitich)
*Corresponding author

Abstract: Common bean (*Phaseolus vulgaris* L.) is the most important legume and is second only to maize as a food crop in Kenya. Despite its importance, bean productivity is declining in western Kenya due to several biotic and abiotic constraints including several fungal diseases. Among these diseases, angular leaf spot (*Phaeoisariopsis griseola* (Sacc.) is one of the most damaging and widely distributed diseases of common bean, causing yield losses as high as 80%. Furthermore, the problem is compounded by limited information on pathogen distribution and variability in western Kenya hindering breeding for angular leaf spot (ALS) resistance. Therefore, this study was carried out to characterise the ALS pathogen (*Phaeoisariopsis griseola*) (Sacc.) into different pathotypes. Forty-two isolates of *P. griseola* were collected from different bean growing areas of western Kenya and characterized into six pathotypes (63:11, 30:26, 33:23, 63:7, 31:10 and 63:63) by use of 12 differential cultivars. Advanced lines and commercial varieties obtained from KALRO-Kakamega were separately inoculated with six pathotypes of *P. griseola* and evaluated for disease reaction in the screenhouse. A screening trial of Mesoamerican and Andean bean genotypes showed that two varieties were tolerant (disease scores 1 to 3), fourteen varieties were moderately resistant (scores 4 to 6) and four varieties were susceptible (7 to 9). The tolerant varieties were small-seeded, while the susceptible varieties were mostly large-seeded.

Keywords: Beans, Pathotypes, *Phaeoisariopsis griseola*, Severity

1. Introduction

Common bean *(Phaseolus vulgaris)* is an important crop in the daily diet of more than 300 million people worldwide [1]. Half of the world use it for direct consumption this including Eastern and Southern Africa where it's estimated to be cultivated in over four million ha of land [2]. It is the most widely grown pulse, second only to maize as a food crop and a major source of food security in East Africa, including Kenya [3], [4], [5]. Per capita consumption of beans may vary from country to country and region to region, within a country, however generally there is often a higher

consumption of bean among low income families both in rural and urban areas [6]. In Kenya, per capita consumption is estimated at 14 kg per year, but can be as high as 66 kg per year in western Kenya [7], [8].

Bean yields in Kenya have remained low on-farm with an average yield of 585 kg/ha compared to Ethiopia and Rwanda with yields of 1588 kg/ha and 913 kg/ha respectively [9]. The low bean yield in the region is attributed to a number of factors, among them diseases and use of low yielding disease susceptible varieties [10], [11]. Among the diseases, ALS is a major biotic constraint of bean production in western Kenya.

Several techniques have been used to study the distribution

and variability of fungi; some of them include; disease surveys, differential cultivars and molecular techniques [12], [13]. Characterisation based on differential cultivars and amplified fragment length polymorphism (AFLP) have revealed high levels of pathogenic and genetic variation in *P. griseola* [14], [15], [16], [17]. In Kenya, forty-four physiological races of *P. griseola* have so far been identified [17]. *P. griseola* virulence is assessed based on reaction of isolates on a standard differential set of 12 common bean cultivars proposed by CIAT [18], and divided into two sets (Mesoamerican and Andean), with six cultivars each. In this technique, a binary system based on the position of each cultivar within the series is used to define the virulence level of isolates under study [19]. Use of differential cultivars generates a true picture of virulence structure and reveals the pathogen properties related to host selection effect on the pathogen population [13].

Though, ALS has been identified to occur in Kenya, the pathotype variability of the fungus in western Kenya remains unknown and probably as a consequence of this, there is hardly any commercial cultivar either tolerant or resistance which has been developed. In addition, farmers in western Kenya usually tend to use their own seeds from the previous season or supplement their seed requirements with purchases from informal markets, coupled with continuous cropping [20], [21], [22]. This serves as primary inoculum foci for the development and spread of ALS disease. Therefore, a study to understand the variability of the pathogen and test existing bean lines for resistance to isolates form western Kenya is an important intervention in developing bean varieties which are resistant or tolerant against ALS in western Kenya.

2. Materials and Methods

2.1. Collection of Phaeoisariopsis griseola (Sacc.) Isolates

Isolates of *Phaeoisariopsis griseola* (Sacc.) were obtained from naturally infected bean leaves collected from the different AEZs surveyed. The samples were collected from diseased bean leaves of different varieties in order to capture a wider representation of pathogen diversity. The samples

were stored in paper envelopes and labelled with the host variety name and the zone where the isolate was collected. The collected leaf samples were arranged in between absorbent newspapers and pressed carefully to absorb the moisture and distribute pressure evenly across the samples to keep them intact to avoid any breakage. The samples were then, taken to the laboratory for isolation of the pathogen.

2.2. Isolation of Phaeoisariopsis griseola (Sacc.) and Inoculum Preparation

Infected leaves were incubated to induce sporulation in a sterile moist chamber. Using a dissecting microscope, well-sporulating lesions were selected and conidia were picked using a tiny piece of agar placed at the tip of a sterile mounted needle. The conidia were transferred to a drop of sterile distilled water on a microscope slide and the suspension was stirred and streaked onto water agar plates using a sterile wire loop. The plates were incubated at 24°C in a non-illuminated incubator until conidia germinated. The germinating conidia were monitored using a dissecting microscope. An agar block with a single germinating conidium was removed and transferred onto Potato dextrose agar (PDA) to obtain monosporic cultures for each isolate. Inoculated plates were incubated at 24°C for 14 days. Inoculum was prepared by adding 10 ml of sterile distilled water to each plate and scraping the surface of the culture. The spore suspension obtained was filtered through a sieve to remove the mycelial mass. Spore concentration in the inoculums was estimated using a haemocytometer and adjusted to a final concentration of 2×10^4 conidia ml-1 using sterile distilled water.

2.3. Inoculation and Pathotype Determination

In order to determine the pathotype of each isolate a set of 12 differential cultivars consisting of six Andean (Don Timoteo, G 11796, Bolon Bayo, Montcalm, Amendoin, G 5686) and six Middle American (Pan 72, G 2858, Flor de Mayo, Mexico 54, BAT 332, Cornell 49–242) were used (Table 1).

Table 1. Characteristics of the common bean differential cultivars used to characterise Phaeoisariopsis griseola (Sacc.) and the binary values were used to assign isolates to pathotypes.

Differential cultivar	Notation[a]	Seed size[b]	Gene pool	Binary values
Don Timoteo	A	L	Andean	1
G 11796	B	L	Andean	2
Bolon Bayo	C	L	Andean	4
Montcalm	D	L	Andean	8
Amendoin	E	L	Andean	16
G 5686	F	L	Andean	32
PAN 72	G	S	Middle American	1
G 2858	H	M	Middle American	2
Flor de Mayo	I	S	Middle American	4
Mexico 54	J	M	Middle American	8
BAT 332	K	S	Middle American	16
Cornell 49242	L	S	Middle American	32

Notation[a]: Differential cultivars
Seed size[b]: L = Large, M= medium, S= Small

The seeds were potted in sterile soil mixture composed of loam soil, sand and manure in the ratio of 2:1:1. Three seeds of each cultivar were sown per polythene pot and thinned to one plant two weeks after germination to attain proper plant population. Three-week old seedlings were inoculated with *Phaeoisariopsis griseola* isolates at a concentration of 2 x 10^4 conidia/ml by spraying on the upper and lower side of the leaf until runoff using a hand sprayer. Inoculated plants were maintained in high humidity through frequent mist sprays. Disease scoring was based on the presence (+) or absence (−) of disease symptoms. The pathotypes so far identified were mapped on AEZs to determine the areas where each pathotype occurred.

2.4. Common Bean Varieties Used

Twenty common bean varieties (13 advanced lines and 7 commercial varieties) obtained from KALRO- Kakamega were used in this experiment (Table 2). The seeds were obtained from KALRO-Kakamega because they offer certified clean seeds which was necessary for the experiment to avoid any seed source inoculum which could have altered results as the disease has been reported by [23] to be seed borne.

Some of the important characteristics of these bean varieties are presented in Table 2 below.

Table 2. Characteristics of bean varieties evaluated against Phaeoisariopsis griseola (Sacc.).

Variety	Origin	Seed size	Seed colour	Growth Habit
KKR IL05/RED 13	Advanced line	M	Red	II
KKR IL05/RED 16	,,	M	Red	II
KKR IL05/RED 20	,,	L	Red	II
KKR IL05/CAL 33	,,	L	Red mottle	II
KKR IL05/RED 40	,,	S	Red	II
KKR IL05/CAL139A	,,	L	Purplish	II
KKR IL05/CAL194	,,	M	Red mottled	II
KK 06/110	,,	M	Purplish	II
KK 071	,,	L	Red kidney	I
KK 072	,,	L	Red kidney	I
KK 15	,,	M	Black	III
KK 8	,,	M	Red mottled	I
KK 20	,,	M	Red	II
KK 22	Commercial variety	S	Dark Red	II
GLP-2	Commercial variety	L	Red mottled	I
GLP-585	Commercial variety	S	Red	III
GLP X-92	Commercial variety	M	Cream striped	II
GLP-24	Commercial variety	L	Red	I
GLP-1127	Commercial variety	L	Grey	II
Mwezi mbili	Commercial variety	L	Cream striped	II

Seed size: L = large; M = medium; S = small
Growth habit: I determinate bush, II indeterminate bush, III indeterminate prostrate

2.5. Isolates Used

Six isolates (63:11, 30:26, 33:23, 63:7, 31:10 and 63:63) were used in this study to evaluate the resistance of the common bean to *Phaeoisariopsis griseola* (Sacc.) pathogen. The isolates were obtained after characterisation by use of differential cultivars as shown in Table 3.

2.6. Planting and Scoring of Disease Severity

Two seeds of each variety were sown in polythene pots and thinned to one plant two weeks after germination to have proper plant population. Each variety was inoculated separately with each of the six *Phaeoisariopsis griseola* (Sacc.) pathotypes as described in section 3.2.3, when the seedlings were three-weeks-old [24] and monitored for disease reaction. The experiment was a factorial testing two factors viz: variety and severity. Twenty varieties under inoculation and uninoculation treatments repeated three times making it a total of one hundred and twenty treatments laid in a complete randomized design.

Disease symptoms on the inoculated plants were evaluated using a CIAT 1-9 visual scale [25] for 21 days at an interval of three days, as follows: 1, with no visible disease symptoms; 3, presence of a few small non-sporulating lesions that cover approximately 2% of the leaf surface; 5, with several small lesions with limited sporulation and covering approximately 5% of leaf surface; 7, with abundant and generally large sporulating lesions covering approximately 10% of leaf surface and associated with chlorosis and necrosis; 9, as 25% or more of leaf surface with large sporulating and often coalescing lesions, resulting in severe and premature defoliation.

2.7. Statistical Analysis

Data was subjected to analysis of variance using SPSS version 22, at (P <0.05) and means separated using least significant difference (LSD) at 5% probability level. The mean scores were used to partition disease severity of different ALS isolates on bean genotypes into resistant, moderately resistant and susceptible.

3. Results

3.1. Pathotype Determination

The isolates of *Phaeoisariopsis griseola* had different patterns of virulence when inoculated on 12 differential bean cultivars. Most of the isolates were pathogenic to both Andean and Middle American differentials. The isolates were characterised into six pathotypes; 63:11, 30:26, 33:23, 63:7, 31:10 and 63:63 (Table 3).

***Table 3.** Response of 12 differentials to P.griseola isolates.*

Andean						Mesoamerican							Pathotype	No. of isolates
AEZ	A	B	C	D	E	F	G	H	I	J	K	L		
	1	2	4	8	16	32	1	2	4	8	16	32		
LH1	-	+	+	+	+	-	-	+	-	+	+	-	30:26	4
	-	+	+	+	+	-	+	+	-	-	+	-	30:23	2
	+	+	+	+	+	+	+	+	+	+	+	+	63:63	1
LM1	+	+	+	+	+	+	+	+	-	+	-	-	63:11	4
	-	+	+	+	+	-	-	+	-	+	+	-	30:26	1
	+	+	+	+	+	+	+	+	+	-	-	-	63:7	2
LM2	+	+	+	+	+	-	-	+	-	+	-	-	31:10	4
	+	+	+	+	+	+	+	+	-	+	-	-	63:11	1
	+	+	+	+	+	+	+	+	+	+	+	+	63:63	1
	-	+	+	+	+	-	-	+	-	+	+	-	30:26	1
LM3	-	+	+	+	+	-	-	+	-	+	+	-	30:26	1
	+	+	+	+	+	+	+	+	+	-	-	-	63:7	2
	+	+	+	+	+	+	+	+	+	+	+		63:63	1
	+	+	+	+	+		-	+	-	+	-	-	31:10	2
	+	+	+	+	+	+	+	+	+	-	-	-	63:11	1
LM4	-	+	+	+	+	-	+	+	+	-	+	-	30:23	3
	-	+	+	+	+	-	-	+	-	+	+	-	30:26	3
	+	+	+	+	+	-	-	+	-	+	-	-	31:10	1
UM1	+	+	+	+	+	+	+	+	-	+	-	-	63:11	3
	+	+	+	+	+	+	+	+	+	+	+	+	63:63	4
Total														42

Andean cultivars: (a) Don Timoteo; (b) G 11796; (c) Bloom Bayo; (d) Montcalm; (e) Amendoin; (f) G 5686. Middle American cultivars: (g) Pan 72; (h) G 2858; (i) Flor de Mayo; (j) Mexico 54; (k) BAT 332; (l) Cornell 49–242. Pathotype designation is based on the sum (binary values) of bean cultivars (+), Compatible reaction; (-), Incompatible reaction (as per Pastor-Corrales and Jara, (1995)

3.2. Mapping of Identified Isolates Over AEZs

GLP-2 and GLP-585 were found to be infected by all the by pathotypes in all the AEZs surveyed as presented in Table 4 below. The most prevalent pathotypes across AEZs were 63:63 and 30:26. KK15 was to be infected by pathotype 63:63 in UM1, LM1 and LM2. Pathotype 63:7 infected GLP-585 and KK8 only in LM1, while pathotypes 63:11 and 31:10 infected GLP-2, GLP-585, Zaire, KAT X56 and Punda in UM1, LM1 and LM3. Pathotype 30:26 infected GLP-2, Zaire and Punda in LM4 and LH1, while pathotype 30:23 infected GLP-585 only in LM4 and LH1 (Table 4).

***Table 4.** Isolates of Phaeoisariopsis griseola (Sacc.).*

AEZs	VARIETIES							No. of Isolates
	GLP-2	GLP-585	KK8	KK15	Zaire	KAT X56	Punda	
LH1	30:26(2)	30:23(2)	63:63				30:26(2)	7
UM1	63:11	63:11(2)	63:63	63:63(2)			63:63	7
LM1	30:26	63:7	63:7	63:63	63:11	63:11	63:11	7
LM2	31:10	31:10	63:8	63:63	31:10	31:10	31:10	7
LM3	30:26	63:7	63:7		63:11	31:10(2)	63:63	7
LM4	30:26(2)	30:23(3)			30:26	31:10		7
Total								42

3.3. Symptoms of ALS Observed in the Screen House

For all the six pathotypes, the symptoms developed 10-15 days after inoculation. The first symptoms developed on the primary leaves as circular lesions which enlarged and attained larger sizes and in some lines lesions coalesced, while in some bean varieties there was extensive chlorosis on the leaves e.g. GLP-1127 inoculated with race 63-63 (Fig. 1).

***Figure 1.** Symptoms of ALS on GLP-1127 leaves; A- Intensive chlorosis, B- Large coalescing lesions of ALS.*

3.4. Reaction of Common Bean Varieties to Phaeoisariopsis Griseola Pathotypes

The reaction of bean varieties to different pathotypes of *P. griseola* is presented in Table 5 below. Pathotype 63:63 was more virulent with a mean severity of 7, followed by 63:11 with a mean severity of 6, whereas 30:26, 33:23, 63:7 and 31:10 were less virulent with a mean severity of 5.

Table 5. *Mean reaction of bean varieties to Phaeoisariopsis griseola pathotypes.*

Genotypes	33:23	31:10	30:26	63:7	63:11	63:63
KKRIL05/Red 40	3	3	2	4	3	6
KKRIL05/Red 13	4	5	3	4	4	6
KKRIL05/Red 16	4	4	6	3	6	7
KKRIL05/Red 20	6	6	6	7	5	8
Mwezi mbili	5	5	7	6	7	8
GLP-585	4	5	3	5	5	4
KK22	5	5	7	5	6	8
KKRIL05/Cal 194	4	6	4	4	4	7
KK06/110	4	3	5	5	5	7
KKRIL05/Cal 33	6	6	6	6	6	7
KKRIL05/Cal 139A	6	6	7	6	8	7
KK8	6	4	4	5	6	9
GLP-2	4	7	6	5	6	7
KK071	5	7	5	6	7	8
KK20	7	6	6	7	5	8
KK072	6	5	7	7	5	8
GLP-24	3	4	4	4	4	4
GLP-1127	6	7	7	5	8	8
GLPX-92	6	7	8	8	8	8
KK15	7	7	7	7	8	8
Mean	4.70	5.13	5.20	5.18	5.53	7.05
SE	0.24	0.27	0.29	0.25	0.26	0.23

For the varieties, KKR IL05/Red 40 was tolerant in most of the pathotypes with a mean reaction of 3 except in pathotype 63:63 where it had a mean score of 6. GLP-24, KKR IL05/Cal 194, KKR IL05/Cal 33, GLP-2, GLP-585, KK 071, KK06/110, KK20, KK 072, KK 22, KK 8, KKR IL05/ Red 13, KKR IL05/Red 16, KKR IL05/Red 20 were moderately resistant with a mean reaction of 4-6; whereas Cal 139, GLP2, KK071, KK20, KK072, GLP24, GLP-1127, GLP-X92 and KK 15 were susceptible with mean reaction of 7-9.

4. Discussion

The reaction of 42 *Phaeoisariopsis griseola* isolates on standard bean differential cultivars showed the existence of pathogenic variability of ALS pathogen. The isolates of *Phaeoisariopsis griseola* were characterised into six pathotypes. Similar findings have been reported by [15] and [26] who observed, on average, the occurrence of one pathotype for each two and seven isolates respectively. Studies by [15] and [27] found larger variability than those conducted by [26] probably due to the great diversity of the sampled places and to the hosts from both gene pools (Mesoamerican and Andean). This was clearly exhibited in the findings as six AEZs within western Kenya were covered. This explains the variability of the *Phaeoisariopsis griseola* due to diversity of the sampled places within the region. Likewise, [17] verified the existence of high pathogenic variation of *Phaeoisariopsis griseola* isolates in Kenya. She reported existence of forty-four physiological races of *Phaeoisariopsis griseola*. The wide distribution of the variability of *Phaeoisariopsis griseola* was confirmed to occur worldwide. [28] verified the occurrence of 120 pathotypes in 22 countries and, among the pathotypes identified, 71 were discovered specifically in Brazil.

This large pathogenic variation could have been associated with mutation, recombination and migration [29]. Also, specialisation in host–pathogen interactions, control measures or more general environmental constraints may singly or interact in combination to give raise to new pathotypes leading to high levels of diversity in the pathogen [15].

The study also identified pathotype 63:63 as the highly virulent compared to the other pathotypes. Though reported by [17] in Embu, it was reported for the first time in western Kenya in this study. [30] reported the pathotype as the most virulent in other countries such as Honduras and Nicaragua and Brazil.

Furthermore, in western Kenya, small and large-seeded bean cultivars are widely grown in adjacent fields and farmers have been reported to grow up to eight different cultivars on the same farm [21], [22], [17]. This farming practice may have contributed to the development of new pathotypes as isolates evolve to infect the different bean varieties. *Phaeoisariopsis griseola* is known to be seed-borne [30] and the high movement of bean germplasm from one region to another, even across national borders, may also lead to the introduction of new pathotypes through contaminated seed that led to high pathogenic variation observed in western Kenya. Furthermore, due to high cost of certified seed, farmers source seed from fellow farmers or informal markets whose supplies comes from different parts of the

country and even from neighbouring countries. These findings are in agreement with [31] who reported that most farmers in Africa practicing bean production mainly obtain their seeds from informal channels such as farm saved seeds to avoid overspending. This adversely implies farm saved seeds play a major role in harbouring more infected seeds leading to spread of seed borne diseases contributing to reduction in yield [32]. Such a seed system is liable to transmission of different *Phaeoisariopsis griseola* races across bean production areas. However, [33] demonstrated that farmers in Uganda produce and save own pure seeds for the next cropping season through proper seed production system consequently reducing the spread of seed borne diseases.

The study also found out that, some common bean varieties like GLP-2, GLP-585, KAT X56 and Punda were infected by more than one pathotype. This could be attributed to multiple leaf infections by different pathotypes which can lead to parasexual recombination [29], hence giving rise to more than one pathotypes present on a single plant.

The lesions induced by *Phaeoisariopsis griseola* pathotypes were characteristically circular at early stages but with time they attained larger sizes of angular shape. This conformed with [34] who found that on the primary leaves, the lesions tend to be circular but on the trifoliates, the spots were delimited by veins and vein lets, giving them a characteristic angular shape.

All the fourteen moderately resistant cultivars to *Phaeoisariopsis griseola* were small seeded suggesting that small seeded size derived mainly from a cross of GLP585 and KK22 that are Meso-American types. This reaction suggests that most of the pathotypes may be of Andean type as reported in Africa [24] and therefore less severe on genotypes derived from Meso-American gene pools. These results are also consistent with the report by [19] who observed that of the 19 bean accessions that were intermediate or resistant to ALS, 15 were small or medium-sized while only four were large-seeded. These results are also in support of earlier observations from [35] that the resistant bean accessions, often without any visible ALS symptoms, are the small-seeded varieties of Middle American origin. [34] also reported that most of the known sources of ALS resistance are evidently small-seeded bean varieties and that very little resistance exists among the popular large-seeded varieties in Southern Africa.

5. Conclusion

The use of standard bean differential cultivars has revealed the existence of pathogenic variability of ALS pathogen in western Kenya with pathotype 63:63 being the most virulent and frequent across the AEZs sampled. Also, most moderately resistant cultivars to *Phaeoisariopsis griseola* are small seeded suggesting that small seeded size were derived mainly from a cross of GLP585 and KK22 which are Meso-American types. Therefore, durable resistance will be required to manage the disease due to the variation exhibited by this pathogen.

Abbreviations

ALS	Angular Leaf Spot
ANOVA	Analysis of variance
AEZs	Agro Ecological Zones
BRR	Bean Root Rot
CIAT	Centre International Agricultural Tropical
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agricultural Organization Statistics
GOK	Government of Kenya
KALRO	Kenya Agricultural and Livestock Research Organisation
LH	Lower Highlands
LM	Lower Midlands
MOA	Ministry of Agriculture
UM	Upper Midlands

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