Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.8, No.15, 2018

Variability of Common Bean Pathogens Angular Leaf Spot Caused by Phaeoisariopsis Gresiola (Sacc.) and Anthracnose Caused by Colletotrichum Lindemuthianum Isolates in Southern Ethiopia

Shiferaw Mekonen
Southern Agricultural Research Institute (SARI), Awassa Agricultural Research Center,
P.O. Box 06, Awassa, Ethiopia

Abstract
Angular leaf spot caused by Phaeoisariopsis gresiola (Sacc.), and Anthracnose caused by Colletotrichum lindemuthianum are important fungal pathogens which limits the production and productivity of common bean in the world as well as in East Africa. Common bean breeding for resistant cultivars against these diseases are more difficult because of the existence of variability and emergence of new pathogen races of these pathogens and consequences into continuous breakdown of resistance gene of the crop. Thus, recognizing races of each region pathotype is essential before staring common bean breeding resistant program. Field experiment was carried out at Hawassa, Dilla and Halaba under field condition and in the green house during 2014 -2015 cropping season. Twelve CIAT common bean differentials cultivars were used for each pathogen to discover the race variability and its distribution in the region. Of the tested cultivars Bolon Bayq, BAT 332, and Conell 49242) were showed immune reaction to Southern Ethiopia ALS isolates. Differential Trepador and Mexico 154 showed only at Hawasa and Dilla location, and showed resistant disease reaction with mean disease score (2). The remaining differentials were showed from moderately resistant to susceptible disease reaction. Similarly for Anthracnose isolates, out of the tested differentials 66.7%, were not infected by the pathogen in both years. This preliminary result indicated the presence of variable isolates for two pathogens and resistant differential cultivar against Southern Ethiopian isolate. Therefore breeders should focus in developing common bean varieties that could resist the recorded races that attacking tested differentials in the region. The differentials those were not attacked by Southern Ethiopia isolate can be used as source of resistance to be used in the breeding program. In addition further study is needed through collecting pathogens from different common bean production area in the region and so that using molecular supported job for race identification.
Keywords: ALS, Anthracnose, common bean, differential, isolate, resistant

1. Introduction

Common bean (*Phaseolus Vulgaris L*) is one of the major pulses grown in Ethiopia (EPPA, 2004). It is an important food for people of all income categories and especially for the poor as a source of dietary protein. Southern Nation, Nationalities and People's Regional State (SNNPRS) is one of the major common bean producing regions in Ethiopia. Even though the crop is very important, the national average yield of common bean in Ethiopia is very low at 1.3- 1.4 tones/ ha (CSA, 2015), compared to the potential of the crop and the yield obtained from experimental plots, which is 2.9-3.5 t ha^{-1}.

In Southern Ethiopia common bean production is constrained by abiotic and biotic factors. Disease and insect pest are the major biotic factors which limit common bean production. Diseases such as common bacterial blight (CBB), Angular Leaf Spot (ALS), Anthracnose, rust and halo blight are the major diseases which affects common bean production in Southern Ethiopia (Habtu, 1996; Anonymous, 2012). Of the major common bean fungal diseases ALS caused by *Phaeoisariopsis gresiola (Sacc.),* and Anthracnose caused by *Colletotrichum lindemuthianum* are important fungal pathogens restricting common bean production in many countries. In Southern and South Western part of the country yield loss also has been reported to be 47 % (Fikire *et al.,* 2011). Similarly common bean Anthracnose disease can cause losses of up to 100% particularly on susceptible varieties under favorable conditions (Pastor – Corrales, 1998).

Rotate crops with non-host crops for at least two years, deep plough infested crop residue, seed treatment and the application of fungicides to the aerial parts of plants and using resistant varieties are some of the known management options which significantly reduces survival of the fungal pathogen (Vieira, 1988).

Of all the disease management options use of resistant cultivars is known as the most efficient practice to manage these diseases. Durable resistance is the most important way of controlling *Colletotrichum lindemuthianum and* leaf spot caused by *Phaeoisariopsis gresiola (Sacc.),* (Schwartz et al., 1982). However, common bean breeding of resistant cultivars against these diseases are difficult because of the existence of variability and emergence of new pathogen races and continuous breakdown of resistance (Bigirimana and Hofte, 2001).

Previous studies conducted in central America have revealed high levels of pathotypic variation in *P.*

griseola (Pastor Corrales *et al*., 1998). However in Ethiopia there was no any information about the extent of pathotype variability for *P. griseola and Colletotrichum lindemuthianum pathogens particularly* in the main common bean producing region like Southern Nation Nationalities People Regional State (SNNPRS).

Thus, determination of pathotypes is essential to support common bean breeding resistant cultivars program. Therefore the proposal was initiated to recognize the diversity of ALS and Anthracnose pathotypes in different common bean growing regions of SNNPRS.

2. Material and methods

Field experiment was carried out at Hawassa, Dilla and Halaba Research site in Southern Nation Nationalities People Regional State (SNNPRS) under field condition during 2014 -2015. The pot experiment was conducted at Hawassa Agricultural Research Center crop protection green house.

Table 1. Description of the experimental areas. asl = above sea level,

Location	Altitude (M asl)	Coordinates	Annual rainfall (mm)	Mean annual temperature (°C)	
				Minimum	Maximum
Hawassa	1 700	07°03′54″ N, 38°28′59″ E	1 046.3	13.3	27.6
Hallaba	1 772	07°18′38″ N, 38°05′38″ E	928.8	14.6	28.6
Dilla	1 519	06°22′49″ N, 38°18′25″ E	1 354.6	12.9	28.1

2.1. Experimental design and management

2.1.1. Planting materials

Twelve known common bean differential cultivars were obtained from The International Center for Tropical Agriculture (CIAT) program for each pathogen. The differentials have been known to be used for pathogen race identification using the host response.

2.1.2. Green house experiment

The obtained 12 differentials cultivars for each pathogen planted in the green house using plastic pots. Each differential were planted on five pots and made to grow in the green house for inoculation. In addition the released common bean varieties SARI one (susceptible to antracnose) and Brown speckeled (susceptible to ALS) were planted in the green house.

2.1.3. Inoculation of seedlings with ALS and Anthracnose pathogen in the green house

Diseased leaf of each pathogen were collected from each site and allowed to dry under the shelter in dark place. Using mortar and pestle the leaves were crushed. Inoculums suspension was prepared using sterile distilled water. Spore concentrations were determined with Hemacytometer and adjusted to 2 x 10^4 conidia per ml. When the seedlings attained at trifoliate leaf stage (V3 stage) each plant was wounded on their upper and lower part of the leaf and the inoculums suspension has sprayed using a hand atomize fully expanded leaves. Each inoculated plant was covered with plastic bags for 6 days after inoculation and kept in a greenhouse. Finally bags were removed and plants kept in the greenhouse through creating conducive environment to the pathogen.

2.1.4. Disease evaluation: Evaluation of the disease was made for four consecutive weeks by using severity score (1-9) scale resistant scores (1 to 3) and moderately resistant (4) whereas plants with numerous enlarged lesions were recorded as susceptible (scores 5 to 9).

2.2. Field experiment

The obtained 12 differentials seed for each pathogen were planted in hot spot area at, Hawasa, Dilla and Halaba locations. Each treatment contained a plot size of 2 m by 0.8 m and spacing 0.4 m and 0.1 m between rows and plants respectively. One known susceptible common bean variety in Ethiopia was included for each pathogen to verify the occurrence of the disease in the growing season. For Anthracnose variety SARI one was included, whereas for ALS Brown speckled were used as susceptible check. All recommended agronomic practices were applied during the experimental period in each location.

2.3. Data collection

The occurrence of the disease was assessed six days after inoculation and 20 days after planting in the green house and in the experimental field respectively. The disease score for each pathogen were recorded in each plot. Disease severity was scored at weekly intervals for a total of four ratings, using a CIAT 1-9 visual scale. Resistant scores (1 to 3) and moderately resistant (4) whereas plants with numerous enlarged lesions were recorded as susceptible (scores 5 to 9). Finally the mean disease score of the two season data for each differential work out. The pathogen group collected from each location and tested at green house and result summarized in the table.

3. Result and Discussion

In the study period ALS and Anthracnose disease were occurred in all location, despite the inoculums pressure of the pathogen is inconsistent. This might be the direct effect of the environment on the host, and lack of uniform conducive environment across locations. The susceptible varieties Brawn spectacled for ALS and SARI-1 for Anthracnose were infected by the corresponding disease across all tested locations.

3.1. Reaction of common bean differential cultivars for the *Phaeoisariopsis gresiola (Sacc.)*

Differentials Bolon Bayq, BAT 332, Conell 49242) were showed immune reaction, Differential Trepador and Mexico 154 detected only at Hawasa and Dilla and showed resistant disease reaction with mean disease score (2) in (Table 1 and 2). In remaining seven cultivars (58 %) disease score were recorded 3.3- 6 and showed from moderately resistant to susceptible disease reaction. Despite the degree of severity varied in some differentials cultivars in the green house and in the field experiment, there were no differentials that infected only in the green house and free at field condition or inverse, all have showed similar infection symptom (Table 3). Some isolates were not alike in the green house and in the field experiment for some of tested differentials. For example cultivar Amendom attacking isolate was aggressive in the green house (Score = 5) and less (Score = 3.3) in the field, whereas cultivar PAN72 infected isolate was not aggressive in the green house, instead it was severe in the field experiment. In general the reaction of differential for different isolate indicated that the occurrence of different races in Ethiopia

Table 2. Reaction of ALS differential for Ethiopian *Phaeoisariopsis gresiola (Sacc.),*isolates in the green house and field condition

Identification	Reaction of varieties for ALS	
	Green house	field experiment (over all mean)
Dontimoted	3	6
Poroto	2	4
Bolon Bayq	1	1
Montcalm 023	3	3
Amendom	5	3.3
Trepador	3	2
PAN72	3	5
Zacaticano	3.2	6
Fordemayo	5	5
Mexico 154	1	2
BAT 332	1	1
Conell 49242	1	1
Brown speckled	5	6

Table 3. The reaction common bean differentials for *Phaeoisariopsis griseolia* isolates in SNNPRS (Hawasa , Halaba and Dilla) Ethiopia, 2014-2015

Identification	Location and diseases severity(1-9 scale)						
	2014			2015			Mean
	Hawassa	Dilla	Halaba	Hawassa	Dilla	Halaba	
Dontimote	7	5	5	5	5	7	6
Poroto	7	3	3	3	3	7	4
BoloN Bayq	1	1	1	1	1	1	1
Montcalm 023	5	3	1	3	1	5	3
Amendom	5	5	1	3	5	1	3.3
Trepador	3	2	1	3	2	1	2
PAN72	5	5	5	5	5	5	5
Zacaticano	7	7	5	7	5	7	6
Fordemayo	5	5	5	5	5	5	5
Mexico 154	2	2	1	2	2	1	2
BAT 332	1	1	1	1	1	1	1
Conell 49242	1	1	1	1	1	1	1
Brown speckled	7	5	5	7	5	7	6

3.2. Reaction of Anthracnose differential for *Colletotrichum lindemuthianum* races in the green house

Out of the tested differentials 66.7%, namely Cornell 49242, Windusa, Kaboon, G1320 (PI207282), TO, TU, AB138, G233 (Coloraodo Detopsica) were not infected by the pathogen in both years and across the tested

location (Table 3). This indicates that, one; there are no *Colletotrichum lindemuthianum isolates* that can attack the mentioned common bean differential in the area. Second, these varieties might have resistance gene source for those isolates present in the tested locations (Hawassa, Dilla and Hallaba). The previous works have indicated that Michelite differential cultivar showed different resistance mechanisms towards distinct physiological races of *C. lindemuthianum* (Rava *et al.*, 1994) but in our work the tested differential showed moderately resistant reaction for those specific isolate collected from locality. The remaining anthracnose differentials MDRK, Ferry Marrow and Mexico222 commonly showed from moderately susceptible to susceptible disease reaction. This also reveals the presence of races that attack this differential in the tested locality. Even though the green house and field experiment overall mean of the anthracnose disease severity less than 2 in differential Cornell 49242, it was observed that in 2015 cropping season 5 and 2 diseases score was recorded at Dilla and Halaba field condition respectively (Table 4). This might be happen due to the favorable environmental condition created in the year and allowed multiplication for some isolates of the pathogen

Table 4. Reaction of Anthracnose differential for SNNPRS *Colletotrichum lindemuthianum* isolates in the green house and field condition

Cultivar	Severity score (1-9 sale)	
	In green house	Mean severity at field condition
MICHELITE	3	3
MDRK	6	4.5
Perry Marrow	3	4.00
CORNELL 49-242	1	1.83
Widusa	1	1
Kaboon	1	1
MEXICO 222	5	5
PI207282	1	1
TO	1	1
TU	1	1
AB138	1	1
G2333 (Colorado Dodeteopsica)	1	1
SARI - 1	7	5.67

Table 5. The reaction Anthracnose common bean differentials for SNNPRS *Colletotrichum lindemuthianum* isolates

Identifications	Diseases severity (1-9 scale)						
	2014			2015			
	Awassa	Dilla	Halaba	Awassa	Dila	Halaba	Mean
MICHELITE	3	2	2	3	3	3	3
MDRK	6	3	3	6	7	6	4.5
FERRY MARROW	3	3	3	3	7	5	4
CORNELL 49242	1	1	1	1	5	2	1.83
WINDUSA	1	1	1	1	1	1	1
KABOON	1	1	1	1	1	1	1
MEXICO 222	5	5	5	5	5	5	5
PI207282	1	1	1	1	1	1	1
TO	1	1	1	1	1	1	1
TU	1	1	1	1	1	1	1
AB138	1	1	1	1	1	1	1
G2333(ColoraodoDeteopsica)	1	1	1	1	1	1	1
SARI - 1	7	5	5	7	5	5	5.67

Conclusion and recommendation:

Host differential lines of common bean with known resistance genes have been very successful in monitoring and identifying the virulence or races of the pathogen in the tested area. Even though variation was observed in degree of severity across locations for each pathogen, the infestation of common bean differentials by both diseases was stable for most of tested differentials in both cropping seasons and locations. The experiment result indicated that the occurrence of variable phatotype in Southern Ethiopia. So, that the breeders should focus

to develop common bean varieties that could resist the mentioned pathotypes using the standard differentials. In addition the result indicated the presence of resistant differential that can be used as source of resistance to develop ALS resistant variety. The experiment was only conducted at three location and diseased samples were collected from these area. Therefore, the result points forward the needs to collect more representative samples from different common bean production area of the region for further recommendation and validation.

ACKNOWLEDGEMENT
The author expresses his appreciation to Southern Agricultural Institute and the International Center for Tropical Agriculture (CIAT) for funding the research work and for providing bean differential seeds, respectively. He also acknowledges the technical assistant and laboratory technicians at Hawassa Agricultural Research Center of Ethiopia for collection of samples and data during the study period.

References

Anonymous, 2012. Awassa Agricultural research Center crop protection progress report

Bigirimana and Hofte, 2001. Bean Anthracnose:Inoculation Methods and Influence of Plant Stage on Resistance of Phaseolus vulgaris Cultivars. Journalo of phytopathology 149(7‐8):403 - 408

CSA. 2015. Agricultural sample survey 2014/2015. Report on area and production of major crops. Central Statistical Agency of Ethiopia, Addis Ababa, Ethiopia,

Fikre Lemessa, Waktole Sori and Mulatu Wakjira. 2011. Association Between Angular Leaf Spot *(Phaeoisariopsis griseola*(Sacc.) Ferraris) and Common Bean *(Phaseolus vulgaris*

Habtu, A., I. Scache and J.C. Zadoks, 1996. Survey of cropping practices and foliar diseases of common bean in Ethiopia. Crop Protect, 15: pp 179-186

Pastor-Corrales, M.A., C. Jara and S.P. Singh, 1998. Pathogenic variation in sources of and breeding for resistance to *Phaeoisariopsisgriseola* causing angular leaf spot in common bean. Euphytica, 103: pp 161-171.

Rava C, Purchio A and Sartorato A.1994 Caracterização de patótipos de *Colletotrichum lindemuthianum* que ocorrem em algumas regiões produtoras de feijoeiro comum. Fitopatol Bras 19:167-172.

Schwartz, H. F., M. A. Pastor-Corrales, and S. P. Singh, 1982: Newsources of resistance to anthracnose and angular leaf spot of beans (Phaseolus vulgaris L.). Euphytica 31, 741—754.

Vieira C .1988. Perspectivas da cultura do feijão e de outras leguminosas de grão no país e no mundo. In: Zimmermann MJO, Rocha M and Yamada T (eds) Cultura do Feijoeiro. Editora ABPPF, Piracicaba, pp 3-20.