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# Aggressiveness of *Phaeoisariopsis griseola* isolates and reaction of common bean varieties to the isolates under greenhouse conditions

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**Angular leaf spot caused by *Phaeoisariopsis griseola* is one of the most destructive diseases on common bean in Southern and Southwestern parts of Ethiopia. However, systematic and focus studies were lacking to this important disease. Therefore, greenhouse study was conducted to test aggressiveness of *P. griseola* isolates and evaluate resistance reactions of bean varieties to pathogenic isolates. 55 leaf samples were collected and seven isolates were recovered, but three isolates namely Dolla1, Dolla2 and Dorebafano1 were successfully multiplied and tested to evaluate their pathogenicity and aggressiveness on susceptible variety Dinknesh. Dolla1 and Dolla2 isolates were collected from Bolloso Sore district, while Dorebafano was from Hawassa Zuria. 21 days old plants were inoculated with spore concentration of $2 \times 10^4$ conidia $ml^{-1}$. The study was factorial arranged in a completely randomized design in four replications. All tested isolates were found aggressive to susceptible check with PSI value ranging from 83.34 to 93.52%. 75% of tested varieties showed susceptible reaction to isolates, but overall aggressiveness was higher on varieties Dimtu, Babile, KATB1, KATB69 and Dinknesh. PSI values ranged from 87.96 to 100% in most varieties. The apparent infection rate was faster on susceptible varieties. The disease progress curve attained sigmoid shape in most varieties. Varieties SER125, SER119 and Nasir were identified as a good source of resistant gene to develop ALS resistance. However, further studies on identification of genes and genetic diversity of the pathogen should capture research priority. Varieties evaluated under greenhouse conditions should also be tested under field conditions.**

**Key words:** Aggressiveness, angular leaf spot, common bean, incidence, reaction, severity.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely cultivated in tropical and subtropical countries. It is an important source of proteins, carbohydrates, fiber, vitamins and minerals (Voysest, 2000). Its high nutritional

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value and high rate of consumption make common bean an important food for many people in developing countries in Africa, Asia, and Latin America (Beebe, 2014). Worldwide, about 22.8 million metric tons (MT) of dry beans were produced (FAOSTAT, 2014). The crop is also widely grown in areas between 1400 and 2000 m.a.s.l. in Ethiopia. According to CSA (2017), about 290,202.43 ha of land was allocated for common bean production and a total of 483,922.66 ton was obtained with an average productivity of 1.60 t $ha^{-1}$ for white and 1.69 t $ha^{-1}$ for red common beans in 2016/2017 main cropping seasons in the country.

However, productivity of common bean is constrained by several factors including a wide range of biotic and abiotic factors that limit the genetic potential of the crop (Singh and Schwartz, 2010). Among biotic factors, diseases and pests are affecting the production and productivity of common bean in Ethiopia (Kutangi et al., 2010). Of diseases, anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromysis appendiculatus*), angular leaf spot (*Phaeoisariopsis griseola*) and common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) are the dominant ones (Yesuf, 2005). The same diseases are known to be the major diseases of common bean in tropical regions including Ethiopia (Fininsa and Tefera, 2002; Fininsa and Yuen, 2002).

Angular leaf spot (ALS), incited by *P. griseola*, is one of the most destructive diseases of common bean in tropical and subtropical regions of the world (Allorent and Savary, 2005). The disease is known to cause a yield loss of 70 to 80% depending on variety susceptibility, environmental conditions and pathogenicity of the isolates or pathotypes (Singh and Schwartz, 2010). Studies done in southern highland of Tanzania showed that ALS infection cause to some high yield losses of 61% on commonly grown bean varieties (Mongi, 2016). About 47% yield reduction of common bean due to ALS was reported so far in South and Southwestern Ethiopia (Fikre et al., 2011).

*P. griseola* is a facultative hemibiotrophic and/or necrotrophic fungal pathogen that inflicts plant health, grain yield and seed quality in common bean. The pathogen has great variability, which explains the large number of existing races and the complexity of genetic resistance (Damasceno-Silva et al., 2008). The disease is favored by intermittent dry-wet and warm-cool weather (Correa-Victoria et al., 1989). Under favorable temperature conditions of 18 to 24°C, high humidity (>70%) and in the presence of a susceptible host, the pathogen has the ability to colonize different parts of plants including leaves, pods and seeds (Stenglein et al., 2003). Leaf and stem infections by *P. griseola* result in premature defoliation, shriveled pods and shrunken seeds, thus reducing the yield potential of beans (Strenglein et al., 2003).

Angular leaf spot can be managed through fungicide sprays in Ethiopia (Shiferaw, 2017). However, majority of the common bean growers are subsistence type and/or from rural areas who could not afford fungicides due to poor access and high cost. In addition, fungicide is also known to pose long term consequences to human health and the environment. Consequently, using resistant varieties is seen as a cost effective, practical, economical and environmentally friendly approach for ALS disease management under subsistence farming systems (Mahuku et al., 2009). But, deployment of resistant varieties for the control of ALS requires identifying the possible source of resistant gene and understanding of epidemiological factors (Singh, 2007).

Durable disease resistance depends on many factors like genetic inheritance, variability in the pathogen population and the utilization strategies of resistance alleles in the host (Adugna, 2004). Thus, better sources of multiple resistances to ALS need to be identified to exploit the genetic diversity that characterizes commercially available common bean varieties. Despite the importance of the disease, limited work has been done so far in Ethiopia on identification of resistance sources and reaction of available common bean genotypes to ALS. Knowing such information is crucial to identify adapted common bean varieties and identify sources of resistance genes for breeding purposes; and subsequently, reach common bean growers to improve grain yield. Therefore, the objectives of this study were to (1) test the pathogenicity and aggressiveness of *P. griseola* isolates, and (2) evaluate the resistance reactions of selected common bean varieties to *P. griseola* isolates.

## MATERIALS AND METHODS

### Collection of angular leaf spot infected leaves

Angular leaf spot infected common bean leaves were collected from naturally infected bean leaves in south and southwestern parts of Ethiopia, including Bako Tibe, Kerisa, Bolosso Sore, Awassa Zuria, Hawassa and Jimma Agricultural Research Centers, which were selected purposively upon the survey periods as the disease frequently occurs in the stated areas (Yesuf, 2005; Fikre et al., 2011). Three peasant associations (PAs) were selected from Bolloso Sore district and 17 fields were assessed from them. Similarly, two PAs each were selected from Awassa Zuria and Bako Tibe districts, and 12 and 10 fields were assessed, respectively. On the other hand, only one PA and seven fields were assessed from Kerisa district. The rest nine fields were assessed from research centers (four from JARC and five from HARC) and a total of 55 fields were surveyed from all districts and research centers.

Fields were randomly selected at intervals of about 5 km along the accessible main and feeder roads and five to six fields per PAs were inspected for disease status and leaf sample collection. The plants were systematically selected by making an inverted ‘‘V’’ pattern. From each field, 10 plants were sampled and a sub-sample of two trifoliate leaves per plant was collected from middle canopy of main stem. Totally, 55 composite leaf samples were collected from all surveyed areas. Collected leaf samples were wrapped between envelopes to absorb available moisture and labeled with their corresponding information in a field. The leaf samples were then taken to laboratory for isolation of the pathogen. Air dried leaf samples were kept in boxes at room temperature until isolation proceeds (CIAT, 2015).

**Preparation of isolation medium**

The ideal medium for isolating and multiplying *P. griseola* is V8 juice. The juice was prepared from eight vegetables, namely parsley (15 g), beet (100 g), tomato (500 g), pepper (30.8 g), onion (150 g), garlic (20 g), lemon juice, clove (10 g) and 1 L distilled water. Then, the medium was prepared from ingredients of 15 g agar, 3 g calcium carbonate and 1 can of V8 juice at 200 ml and about 5 g dextrose was added to enhance the sugar content of the medium. The ingredients were weighed, placed in a beaker and about 800 ml of sterile distilled water was added to bring the volume to 1000 ml. Then, the resulting solution (V8 culture medium) was poured into Erlenmeyer flasks and autoclaved for 15 min at 121°C and a pressure of 20 psi for sterilization. The flasks were left until the medium is cooled enough and poured into Petri dishes (CIAT, 2015). The medium preparation as well as all other isolation process were performed in Haramaya University Plant Protection Laboratory.

**Isolation of *P. griseola***

The infected leaves were placed on stages of dissecting microscopes and focused to see the *synnemata* on the underside of the leave. Once the fungal development and *synnemata* were examined using a stereoscope, a small piece of V8 agar media with the point of a flame sterilized needle was taken and gently touched one or more sporulated *synnemata* so that conidia was adhered to the agar. Then, the conidia was transferred to V8 medium five times in different location of medium, labeled and incubated in darkness for 14 to 18 days to allow the germination of conidia and the colonies to develop following procedures of CIAT (2015). Finally, about seven isolates were successfully recovered especially from Boloso Sore and Hawass Zuria districts as the cultural behavior of the pathogen is difficult and also act as semi-obligate most of the time.

**Multiplication of *P. griseola* monosporic isolate**

The monosporic isolate to be multiplied were selected and a conidial suspension was prepared by picking a 14-day old single colony using forceps and placed into clean Petri dish containing 2 ml sterile distilled water. Then the colony was crushed to release spores and about 250 µl of conidial suspension was transferred to a Petri dish containing V8 culture medium using sterilized pipette. The suspension was spread over the medium's entire surface using a flame-sterilized glass rod. Several Petri dishes ware prepared using the same procedure and incubated at 24°C for 14 to 18 days for mass multiplication of the target isolate. Finally, there monosporic isolates were multiplied and maintained in cold condition until inoculation (CIAT, 2015).

**Pathogenicity test**

Three isolates of *P. griseola* (Dolla1, Dolla2 and Dorebafano1) were tested to evaluate their pathogenicity and aggressiveness on susceptible common bean variety (Dinknesh). Dolla1 and Dolla2 isolates were isolated from samples of Bolloso Sore district, while Dorebafano was isolated from Hawassa Zuria district. Seeds of Dinknesh (collected from Melkassa Agricultural Research Center, MARC) were surface-sterilized in 5% sodium hypochlorite for 5 min and rinsed in three changes of sterile distilled water before sowing. Six seeds were sown in pots of 15 cm diameter containing sterile soil mixture composed of sterilized loam soil, sand and manure in the ratio of 2:1:1 (Leitich et al., 2016). After full germination, seedlings were thinned to three plants per pot and 21 days old plants were inoculated by spraying on the upper and lower side of primary trifoliate leaves until runoff with finally adjusted spore concentration of $2 \times 10^4$ conidia $ml^{-1}$. The control common bean plants were inoculated with sterilized distilled water and each treatment was replicated four times under greenhouse. Then, the inoculated plants were covered with transparent plastic bags for about 48 h to enhance infection through increasing humidity (CIAT, 2015).

**Raising of common bean varieties in greenhouse**

Twelve commercial common bean varieties were used to evaluate the resistance reactions against isolates of *P. griseola*. Both the pathogenicity test and this reaction study were conducted in Haramaya University Agricultural Research Station (Rare). All seeds of each variety, except Babile, were obtained from MARC, which is responsible to develop and offer certified clean seeds of common bean. Seeds of Babile variety were obtained from Lowland Pulse Research Program of Haramaya University. Some of the important characteristics of these common bean varieties are shown in Table 1.

Seeds of each variety were surface-sterilized in 5% sodium hypochlorite for 5 min and rinsed in three changes of sterile distilled water before sowing. Six seeds were sown in pots of 15 cm diameter containing sterile soil mixture composed of sterilized loam soil, sand and manure in the ratio of 2:1:1 (Leitich et al., 2016) under greenhouse conditions. Two weeks after germination, the seedlings were thinned to three plants per pot to have proper plant population. The pots were factorial arranged in completely randomized design with four replications. Varieties Dinknesh and SER125 were used as susceptible and resistant checks to ALS, respectively (Tumsa et al., 2015).

**Inoculum preparation and inoculation**

Petri dishes showing the best growths (18 days old culture) were selected and inoculum was prepared by adding about 10 ml of sterile distilled water to each plate and by scraping the surface of the culture three times. Conidia suspensions from all cultures of the same isolate were mixed and the spore suspension obtained was filtered through a sieve to remove the mycelial mass and other residues of the medium. The filtrate containing conidia from all the Petri dishes was collected in a beaker and the spore concentration was estimated using a haemocytometer by counting the conidia observed in different grids of a hemocytometer. The final concentration was adjusted to $2 \times 10^4$ conidia $ml^{-1}$ using sterile distilled water (CIAT, 2015).

One drop of Tween 20 was added per 100 ml of inoculum and mixed thoroughly before inoculation to prevent inoculum spilling and to stick on leaf surfaces. After that, 21 days old plants were inoculated by spraying on the upper and lower side of primary trifoliate leaves until runoff using a hand sprayer. Then, the inoculated plants were covered with transparent plastic bags for about 48 h to enhance relative humidity and inoculation efficiency. Cross contamination was avoided by keeping the plants inoculated with the same isolates together. Each isolates were inoculated separately (CIAT, 2015). In addition, mats were spread on floor and misted twice per day to maintain humidity of indoor environments as close as required.

**Disease assessment**

Disease parameters such as incidence and severity were recorded to evaluate the responses of susceptible variety for each isolates in pathogenicity test. Disease parameters including severity and AUDPC were recorded to evaluate the resistance reactions of

**Table 1.** Description and characteristic features of common bean varieties used to evaluate resistance reaction against *P. griseola* isolates.

| Variety/Breeder Code | Altitude | Seed color | Productivity (t/ha) | | Year of release | Diseases resistance reaction[1] | Seed maintaining center[2] |
|---|---|---|---|---|---|---|---|
| | | | On station | On famer | | | |
| Ado (SAB 736) | 1300-1800 | Large White | 2-2.5 | 1.8-2.2 | 2014 | … | MARC |
| Tafach (SAB 632) | 1300-1800 | Speckred | 2.2-2.6 | 1.9-2.4 | 2014 | … | MARC |
| Awash-2 | 1300-1700 | White | 2.8-3.1 | 1.8-2.2 | 2013 | CBB, Rust and HB | MARC |
| SER-119 | 1450-2000 | Red | 3.3 | 2.5 | 2014 | CBB and Rust | MARC |
| SER-125 | 1450-2000 | Red | 3.5 | 2.2 | 2014 | CBB, Rust, HB and ALS | MARC |
| Dendesu (KATB69) | 1300-1650 | Red | 2.2-3.0 | 1.9-2.3 | 2013 | CBB, Rust and HB | MARC |
| Dimtu | 1200-1800 | Red | 2.14 | 2.2 | 2003 | … | MARC |
| Dinknesh | 1400-1850 | Red | 2.5-3.0 | 2.0-2.4 | 2006 | … | MARC |
| Deme | 1300-1800 | Red Speckled | 1.9-2.0 | 1.8-2.2 | 2008 | … | MARC |
| Nasir | 1200-1800 | Red | 2.03 | 2.3 | 2003 | … | MARC |
| Babile | 1600-2200 | Red | 3.6 | 3.0 | 2012 | CBB, Rust and ALS | HU |
| KATB1 | - | - | - | - | - | CBB, Rust and HB | MARC |

[1]CBB = Common bacterial blight; HB = halo blight, ALS = angular leaf spot. [2]MARC = Melkassa Agricultural Research Center, HU = Haramaya University.

common bean varieties with respective isolates. On appearance of the first typical ALS symptoms (12 days after inoculation, DAI), evaluation of the disease severity was performed from the whole inoculated plants using a 1-9 severity scoring scale (Pastor-Corrales and Jara, 1995); where 1= plants without disease symptoms; 2 = the presence of up to 3% leaf lesions; 3 = the presence of up to 5% leaf lesions, without sporulation; 4 = the presence of sporulating lesions covering 10% of the leaf area; 5 = the presence of various sporulating lesions from 2 to 3 mm in size, covering 10-15% of the leaf area; 6 = the presence of numerous sporulating lesions > 3 mm in size, covering 15-20% of the leaf area; 7 = the presence of numerous sporulating lesions > 3 mm in size, covering 20-25% of the leaf area; 8 = the presence of numerous sporulating lesions > 3 mm in size, covering 25-30% of the leaf area; and 9 = severe symptoms of the disease (>30%), resulting in early leaf drop and plant death.

Severity scoring was performed four times at an interval of three days and reaction type categories were determined from the averages of disease scores for each isolate-cultivar combination. The severity grades were converted into percent severity index (PSI) values. The AUDPC values in %-days were calculated using the midpoint rule method as used by Campbell and Madden (1990) form the PSI values.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where $t_i$ is time in days of each evaluation for the $i^{th}$ disease assessment, $y_i$ is the disease severity in percentage representing the infected foliage at each evaluation and n is the number of evaluations. Host responses were categorized as resistant (R) for AUDPC value scores ≤13.5, intermediate resistant (IR) for AUDPC = 13.5-27 and susceptible (S) for AUDPC >27%-days (Ddamulira et al., 2014a).

### Data analysis

Quantitative data such as PSI and AUDPC were subjected to ANOVA using the Genstat 16th edition computer package. Mean separation used LSD at 5% level of significance for pathogenicity test, while for resistance reaction study means were separated using DMRT according to Gomez and Gomez (1984) at 5% probability level. Logistic, ln [(Y/1-Y)], (Van der Plank, 1963) and Gompertz, -ln [-ln(Y)] (Berger, 1981) epidemiological models were compared for estimation of disease progression from each treatment. The transformed disease severity data were regressed over time (DAI) to determine the rate. The fitness of the models was tested based on the magnitude of the coefficient of determination ($R^2$) and residuals (SE) obtained using each model (Campbell and Madden, 1990). As a result, there were higher coefficient of determination ($R^2$) and lower standard error (SE) for logistic model than from Gompertz model. Therefore, rate of increase in ALS was estimated and compared using the logistic model. The slope of the regression line estimated the disease progress rate. Regression was computed using Minitab version 18.0 for windows®, 2010.

## RESULTS

### Pathogenicity and aggressiveness of *P. griseola* isolates

Inoculations of *P. griseola* isolates on the

**Table 2.** Efficiency of *P.griseola* isolates to infect common bean (Dinknesh variety) and establish disease evaluated under greenhouse conditions (preliminary test of pathogenicity).

| Isolate | Incidence (%) | Severity (%) of ALS at different days after inoculation[1] | | | |
|---|---|---|---|---|---|
| | | 11 DAI | 14 DAI | 17 DAI | 20 DAI |
| Dolla1 | 100 | 31.48[a] | 54.63[a] | 75.93[a] | 88.89[ab] |
| Dolla2 | 100 | 21.30[b] | 52.78[a] | 69.44[a] | 83.34[b] |
| Dorebafano | 100 | 25.93[ab] | 62.96[a] | 78.63[a] | 93.52[a] |
| Control | 0 | 11.11[c] | 11.11[b] | 11.11[b] | 11.11[c] |
| CV (%) | - | 27.7 | 17.9 | 14.6 | 5.7 |
| LSD (5%) | - | 9.57 | 12.49 | 13.27 | 6.11 |

[1]DAI = Days after inoculation, CV = coefficient of variation, LSD = least significant difference at 5% level of significance. The value 11.11 in control treatment obtained was due to the fact that the disease scoring scale starts from 1(no symptom). Means in a column followed by the same letter(s) are not significantly different at 5% level of significance.

susceptible variety led to the production of typical symptoms in all inoculated plants between 11 and 14 DAI and are shown in Table 2. Results indicated that all tested isolates were highly aggressive to the inoculated susceptible variety Dinknesh. Analysis of variance for PSI values showed significant (P<0.05) difference among treatments. The plants treated with sterile water (control) developed no symptom and significantly different from the rest treatments in all observations. During the initial date of disease assessment (11 DAI), disease severity in Dolla1 was about 32.33 and 17% higher than severity in Dolla2 and Dorebafano isolates, respectively. Severity recorded for isolate Dolla1 was significantly ($P < 0.05$) different from Dolla2 but not from isolate Dorebafano at 11 DAI.

There was no significant difference between aggressiveness of isolates at the second (14 DAI) and third (17 DAI) date of disease assessments (Table 2). However, during the last date of disease assessment, mean disease severity in Dolla1 was only 6.2% higher than Dolla2 and 4.9% lower than Dorebafano isolate. Dolla2 was significantly different from Dorebafano isolate at 20 DAI but not from that of Dolla1. Conversely, isolate Dolla1 was not significantly different from that of Dorebafano in all observations. The overall results indicated that all tested isolates incited more severe ALS symptoms on the tested susceptible common bean variety.

## Responses of common bean varieties to *P. griseola* isolates

Artificial inoculation of common bean varieties using three isolates of *P. griseola* resulted in typical symptoms and development of the ALS, which took only 12 to 15 DAI to develop and interaction effect of *P. griseola* isolates × common bean varieties is shown in Table 3. The analysis of variance for AUDPC indicated that there were significant (P<0.05) differences among variety-isolate interactions. AUDPC values ranged from 9.0 (SER125-Dolla1 and Dorebafano isolates) to 70.56%-days (KATB1-Dorebafano isolate combination). The AUDPC values for isolate Dolla1, Dolla2 and Dorebafano ranged from 9.0%-days (SER125) to 64.75%-days (Dimtu), 10.25%-days (SER125) to 63.62%-days (Dimtu), 9.0%-days (SER125) to 70.56%-days (KATB1), respectively. Out of the 12 common bean varieties evaluated, 75% of them were rated as susceptible (AUDPC >27.0%-days) to most *P. griseola* isolates. Varieties, namely, KATB1, Babile, Deme, Dinknesh, Dimtu, KATB69, SAB632 and SAB736 showed susceptible reaction to all tested isolates. Variety Awash2 also showed susceptible reaction to isolate Dolla1 and Dorebafano, but rated as intermediate resistance to isolate Dolla2.

On the other hand, variety SER125 was found resistant to all isolates and also showed complete resistance to isolates Dolla1 and Dorebafano, which were regarded as the most aggressive isolates to the other varieties in the study. Variety SER119 was also rated as resistant to Dolla2 and Dorebafano (AUDPC ≤ 13.5%-days) as the resistant check; but was rated as intermediate resistance to isolate Dolla1 (AUDPC value of 13.5-27.0%-days). However, there was no significant difference between variety SER119 and SER125 in all pathogen host interactions, but significantly different from other variety-isolate interactions. Moreover, variety Nasir noted as intermediate resistance to all isolates.

### *Disease severity*

Analysis of variance for interaction effects of treatments indicated that there were significant PSI differences (P≤0.001 and P<0.05) among variety-isolate interactions at all dates of assessments (Table 4). Mean PSI values ranged from 11.11% (SER125) to 41.66% (Dimtu-Dolla2) during the initial date of disease assessment (12 DAI). Only variety Dimtu was not significantly different from susceptible check for isolate Dolla1 at 12 DAI. Varieties

**Table 3.** Resistance reactions of common bean varieties to *P. griseola* isolates evaluated under greenhouse conditions.

| Variety | Responses of common bean varieties to *P. griseola* inoculations[1] | | | | | |
|---|---|---|---|---|---|---|
| | Dolla1 | | Dolla2 | | Dorebafano | |
| | AUDPC | RC | AUDPC | RC | AUDPC | RC |
| KATB1 | $48.25^{efghij}$ | S | $54^{cdefgh}$ | S | $70.56^{a}$ | S |
| Babile | $28.96^{lmn}$ | S | $35.32^{klm}$ | S | $45.65^{ghijk}$ | S |
| Nasir | $26.38^{mn}$ | I | $25.12^{mn}$ | I | $25.12^{mn}$ | I |
| Deme | $42.75^{hijk}$ | S | $45.88^{ghijk}$ | S | $40.88^{ijk}$ | S |
| Dinknesh | $57.88^{bcde}$ | S | $49.12^{efghij}$ | S | $60.87^{abcd}$ | S |
| Dimtu | $64.75^{abc}$ | S | $63.62^{abc}$ | S | $66.62^{ab}$ | S |
| KATB69 | $43.75^{hijk}$ | S | $50.5^{defghi}$ | S | $57.38^{bcdef}$ | S |
| SER125 | $9.0^{p}$ | R | $10.12^{p}$ | R | $9.0^{p}$ | R |
| SER119 | $13.88^{op}$ | I | $12.75^{p}$ | R | $10.88^{p}$ | R |
| Awash2 | $35^{klm}$ | S | $23.5^{no}$ | I | $43.38^{hijk}$ | S |
| SAB632 | $50.38^{defghi}$ | S | $44.62^{ghijk}$ | S | $55.75^{bcdefg}$ | S |
| SAB736 | $38.12^{jkl}$ | S | $43.88^{hijk}$ | S | $46.5^{fghijk}$ | S |
| CV (%) | | | | | 17.1 | |

AUDPC = Area under disease progress curve; RC = resistance reaction category; R = resistant; S = susceptible and I = intermediate resistance. CV = Coefficient of variation. Means in columns and rows followed by the same letter(s) are not significantly different at 5% level of significance, DMRT.

KATB69, Babile, Awash2, KATB1, Nasir, Deme and SER119 were not significantly different from resistant check at 12 DAI. For isolate Dolla2, again Dimtu was severely affected and also significantly different from all other varieties including the susceptible check. However, there was no significant difference between varieties KATB69, KATB1, Babile, SAB736, Dinknesh, SAB632, Nasir, Awash2, SER119 and SER125 with susceptible check at initial date of disease assessment for isolate Dolla2. On the contrary, there was significant difference between varieties Dimtu, KATB1, Nasir, SER119, SER125 and the susceptible check for isolate Dorebafano at initial date of disease assessment.

There were also highly significant (P≤0.001) difference between the interaction effects of variety and isolates during the second date of disease assessment (15 DAI) (Table 4). At 15 DAI, there was highly significant difference between the susceptible check and other varieties excluding Dimtu, SAB632 and KATB1 for isolate Dolla1. In contrast to isolate Dolla1, there was highly significant difference between variety Dimtu and the susceptible check for isolate Dolla2. The PSI value of Dimtu variety was 27.16% higher than that of susceptible check during the second date of disease assessment for isolate Dolla2. On the other hand, the difference between varieties Babile, Dimtu, KATB1, KATB69 and SAB632 and the susceptible check was not significant for isolate Dorebafano.

The interaction effects of variety and isolates were also significant (P<0.05) during the third date of assessment (18 DAI) (Table 4). Higher epidemics (PSI ranged from 81.48 to 95.37%) were recorded in many host-pathogen interactions at 18 DAI. Varieties other than Dimtu, SAB632, KATB1 and Babile had comparatively higher mean PSI value and also significantly different from the susceptible check in isolate Dolla1 at 18 DAI (Appendix Figure 1). In the case of isolate Dolla2, varieties Awash2, SAB736, Nasir, SER119 and SER125 were significantly different from susceptible check at 18 DAI (Appendix Figure 2). Five varieties namely, Dimtu, Babile, KATB1, SAB632 and KATB69 had higher mean PSI value and were non-significantly different from the susceptible check in isolate Dorebafano too (Appendix Figure 3). However, there was significant difference between epidemics of other varieties and the susceptible check at 18 DAI.

There were also highly significant (P≤0.001) difference between the interaction effects of variety and isolates during the last date of assessment (21 DAI) (Table 4). The mean percent severity index was reached at 100% in variety Dimtu for all isolates at 21 DAI. The PSI values were above 87.96% in many interactions apart from variety Dimtu and the susceptible check. The epidemics in variety Awash2 and Nasir were significantly different from both susceptible and resistant check at 21 DAI. However, there was no significant difference between variety SER119 and the resistant check even at last date of disease assessment. Generally, the epidemic was very high in varieties Babile, KATB1, KATB69 and SAB632 apart from variety Dimtu and the susceptible check in all host-pathogen interactions at 21 DAI. On the contrary, SER119 was the most outstanding variety in resisting the disease until the end of the epidemic period like that of the resistant check.

**Table 4.** Angular leaf spot severity (%) on common bean varieties in response to inoculation of *P. griseola* isolates under greenhouse conditions

| Variety | Angular leaf spot percent severity index recorded at different days after inoculation under greenhouse conditions[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 DAI | | | 15 DAI | | | 18 DAI | | | 21 DAI | | |
| | D1 | D2 | DF | D1 | D2 | DF | D1 | D2 | DF | D1 | D2 | DF |
| KATB1 | 19.45$^{f-m}$ | 27.77$^{c-f}$ | 36.11$^{abc}$ | 51.85$^{e-i}$ | 64.81$^{b-f}$ | 75$^{abc}$ | 74.07$^{c-j}$ | 82.41$^{a-g}$ | 88.89$^{abc}$ | 87.96$^{a-f}$ | 90.74$^{a-e}$ | 96.3$^{ab}$ |
| Babile | 18.52$^{g-m}$ | 25d$^{e-h}$ | 32.41$^{b-e}$ | 44.44$^{ghi}$ | 59.26$^{c-g}$ | 84.26$^{a}$ | 71.29$^{d-k}$ | 79.63$^{b-i}$ | 94.44$^{ab}$ | 89.81$^{a-f}$ | 93.52$^{abc}$ | 98.15$^{a}$ |
| Nasir | 16.67$^{h-m}$ | 15.74$^{h-m}$ | 16.66$^{h-m}$ | 24.07$^{jk}$ | 25.93$^{jk}$ | 25$^{jk}$ | 37.96m | 34.26$^{m}$ | 36.11$^{m}$ | 54.63$^{j}$ | 50$^{j}$ | 47.22$^{j}$ |
| Deme | 15.74$^{h-m}$ | 17.59$^{g-m}$ | 21.3$^{f-k}$ | 43.52$^{ghi}$ | 53.7$^{d-h}$ | 43.52$^{ghi}$ | 66.67 $^{f-l}$ | 67.51$^{f-k}$ | 60.16$^{jkl}$ | 80.56$^{d-h}$ | 79.63$^{e-h}$ | 74.08$^{ghi}$ |
| Dinknesh | 33.34$^{a-d}$ | 19.44$^{f-m}$ | 25.93$^{d-g}$ | 66.58$^{b-e}$ | 54.63$^{d-h}$ | 76.85$^{ab}$ | 83.33$^{a-e}$ | 73.15$^{d-j}$ | 86.11$^{a-d}$ | 95.37$^{abc}$ | 88.89$^{a-f}$ | 99.08$^{a}$ |
| Dimtu | 37.04$^{ab}$ | 41.66$^{a}$ | 37.96$^{ab}$ | 78.7$^{ab}$ | 75$^{abc}$ | 84.26$^{a}$ | 82.59$^{a-f}$ | 91.67$^{ab}$ | 95.37$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |
| KATB69 | 17.59$^{g-m}$ | 20.37$^{f-l}$ | 33.33$^{a-d}$ | 44.44$^{ghi}$ | 56.48$^{d-g}$ | 70.37$^{a-d}$ | 66.67$^{i-l}$ | 73.15$^{d-j}$ | 81.48$^{a-h}$ | 84.26$^{b-g}$ | 94.45$^{abc}$ | 93.52$^{abc}$ |
| SER125 | 11.11$^{lm}$ | 11.11$^{lm}$ | 11.11$^{lm}$ | 11.11$^{k}$ | 12.04$^{k}$ | 11.11$^{k}$ | 11.11$^{n}$ | 12.04$^{n}$ | 11.11$^{n}$ | 11.11$^{k}$ | 14.81$^{k}$ | 11.11$^{k}$ |
| SER119 | 11.88$^{lm}$ | 11.88$^{lm}$ | 11.88$^{lm}$ | 15.42$^{k}$ | 14.82$^{k}$ | 12.96$^{k}$ | 18.2$^{n}$ | 16.51$^{n}$ | 13.89$^{n}$ | 22.22$^{k}$ | 19.45$^{k}$ | 14.82$^{k}$ |
| Awash2 | 17.59$^{g-m}$ | 13.89$^{i-m}$ | 23.15$^{f-j}$ | 35.18$^{ij}$ | 22.22$^{jk}$ | 49.07$^{f-i}$ | 51.85$^{l}$ | 33.34$^{m}$ | 61.11jkl | 67.59$^{l}$ | 49.07$^{j}$ | 77.78$^{f-i}$ |
| SAB632 | 23.15$^{f-i}$ | 16.66$^{h-m}$ | 23.15$^{f-j}$ | 58.33$^{c-g}$ | 50$^{e-i}$ | 66.67$^{b-e}$ | 74.08$^{c-j}$ | 66.67$^{g-l}$ | 82.41$^{a-h}$ | 83.33$^{c-g}$ | 80.56$^{d-h}$ | 92.59$^{a-d}$ |
| SAB736 | 22.06$^{f-k}$ | 24.07$^{e-h}$ | 22.22$^{f-k}$ | 37.96$^{hij}$ | 46.3$^{ghi}$ | 49.99$^{e-i}$ | 57.4$^{kl}$ | 62.04$^{jkl}$ | 68.52$^{e-k}$ | 69.44$^{hi}$ | 80.56$^{d-h}$ | 83.32$^{c-g}$ |
| CV (%) | | 24.6 | | | 22 | | | 15.5 | | | 10.5 | |

[1] DAI = days after inoculation; D1 = Dolla1 isolate; D2 = Dolla2 isolate and DF = Dorebafano isolate. CV = Coefficient of variation. Means in columns and rows followed by the same letter(s) are not significantly different at P ≤ 0.05 level of significance, DMRT.

### *Disease progress rate (r)*

Disease progress rates and parameter estimates of ALS severity are shown in Table 5. The computed disease progress rates showed wide variations among different host-pathogen interactions. Angular leaf spot progressed at rate of more than 0.20 units day$^{-1}$ in all varieties, except the resistant check and SER119 for isolate Dolla1. Disease progressed faster in variety Dimtu (1.05 units day$^{-1}$), Dinknesh (0.67 units day$^{-1}$), Babile (0.43 units day$^{-1}$) and KATB1 (0.38 units day$^{-1}$) than rates obtained in other varieties. The disease progressed at a rate of one and half times faster in variety Dimtu than in Dinknesh. Comparatively, the disease was progressed slowly in variety SER119 (0.07 units day$^{-1}$), Nasir (0.21 units day$^{-1}$) and SAB736 (0.24 units day$^{-1}$) for isolate Dolla1.

Surprisingly, SER125 was infected by isolate Dolla2. However, its rate of disease development was reduced by one and half times compared to variety SER119. The rate of disease progress ranged from 0.03 in SER125 to 1.01 units day$^{-1}$ in variety Dimtu for isolate Dolla2. The disease progress rate in Dimtu was about 59.15, 43.94, 46.12 and 46.32% higher than rates calculated in varieties Dinknesh, Babile, KATB1 and KATB69, respectively. Similarly, there was about 27.13, 24.17 and 23.89% faster disease development in varieties Babile, KATB1 and KATB69, respectively, than disease progression in susceptible check for isolate Dolla2.

Angular leaf spot progressed at a rate of 1.06, 0.96, 0.928 and 0.907 units day$^{-1}$ in varieties Dimtu, Babile, KATB1 and the susceptible check, respectively, in response to isolate Dorebafano inoculation. On the other hand, no disease progression was recorded on resistant check, while there was slow disease development in SER119 (0.021 units day$^{-1}$). The rate of disease progress in variety Nasir was one and half times slower than rates computed in Deme and Awash2 for isolate Dorebafano. The overall disease progression was very rapid in varieties Dimtu, KATB1, Babile and Dinknesh for all isolates. In contrast, ALS development was relatively slow in variety Nasir, Awash2 and SER119. It seemed that the disease was progressed more rapidly in varieties inoculated with isolate Dorebafano than the rest two isolates. Hence, isolate Dorebafano is

**Table 5.** Disease progress rate (units day$^{-1}$) and parameter estimates of angular leaf spot (*P. griseola*) of common bean varieties evaluated under greenhouse conditions.

| Variety | *Phaeoisariopsis griseola* isolates used in resistance reaction evaluation study under greenhouse conditions[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dolla1 | | | | Dolla2 | | | | Dorebafano | | | |
| | Rate | SE of rate | SE of intercept | $R^2$ (%) | Rate | SE of rate | SE of intercept | $R^2$ (%) | Rate | SE of rate | SE of intercept | $R^2$ (%) |
| KATB1 | 0.38 | 0.336 | -5.823 | 94.3 | 0.542 | 1.736 | -7.593 | 55.6 | 0.928 | 2.8 | -12 | 58.6 |
| Babile | 0.433 | 0.579 | -6.766 | 87.8 | 0.564 | 1.675 | -8.079 | 59.3 | 0.96 | 2.495 | -12.23 | 65.5 |
| Nasir | 0.205 | 0.299 | -4.155 | 85.8 | 0.184 | 0.221 | -3.882 | 89.9 | 0.169 | 0.309 | -3.649 | 79.4 |
| Deme | 0.357 | 0.536 | -5.818 | 85.1 | 0.316 | 0.432 | -5.045 | 87.3 | 0.263 | 0.401 | -4.376 | 84.7 |
| Dinknesh | 0.677 | 2.055 | -9.312 | 58.3 | 0.411 | 0.642 | -6.24 | 84.1 | 0.907 | 1.899 | -12.56 | 74.6 |
| Dimtu | 1.05 | 2.139 | -13.96 | 75.7 | 1.01 | 1.553 | -13.44 | 84.3 | 1.058 | 1.695 | -13.74 | 83.4 |
| KATB69 | 0.361 | 0.384 | -5.811 | 91.9 | 0.54 | 1.134 | -7.995 | 74.5 | 0.371 | 0.761 | -4.913 | 75.3 |
| SER125 | 0.000 | 0.000 | -2.08 | - | 0.031 | 0.166 | -2.479 | 31.6 | 0.000 | 0.000 | -2.08 | .. |
| SER119 | 0.07 | 0.469 | -2.831 | 22.4 | 0.051 | 0.497 | -2.604 | 11.7 | 0.021 | 0.342 | -2.25 | 4.5 |
| Awash2 | 0.261 | 0.44 | -4.648 | 81.9 | 0.2 | 0.253 | -4.272 | 89 | 0.264 | 0.263 | -4.228 | 92.8 |
| SAB632 | 0.349 | 0.908 | -5.195 | 65.5 | 0.334 | 0.405 | -5.362 | 89.7 | 0.42 | 0.571 | -5.973 | 87.4 |
| SAB736 | 0.241 | 0.352 | -4.133 | 85.8 | 0.287 | 0.37 | -4.574 | 88.6 | 0.342 | 0.65 | -5.303 | 78.1 |

[1]Disease progress rate (units day$^{-1}$) was obtained from regression line of disease severity against time (days) after inoculation; SE = Standard error of parameter estimates, $R^2$ = coefficient of determination of the Logistic model.

more aggressive compared to the other *P. griseola* isolates.

### *Disease progress curve*

The disease was progressed differently in different host pathogen-interactions. The disease was progressed increasingly and attained closelysigmoid shape in most susceptible varieties. Disease severity progressed increasingly starting from first date of disease assessment in most of the varieties evaluated. The overall disease progression was high in varieties Dimtu, Babile, KATB69, KATB1 and susceptible check, while the development was slow in other varieties in response to the isolates (Figure 1).

The disease progress in variety SER119 and resistant check were very slow at all dates of assessments and attained a sort of straight line in response to all tested isolates. On the other hand, the progress in variety Nasir was high compared to resistant varieties, but progressed low compared to susceptible varieties. The disease was progressed slightly at initial date of assessments. However, ALS rapidly developed during the second epidemic recording period in most varieties, except the intermediate resistant and resistant ones. Then, the epidemics were increased gradually to the third and last epidemic level (Figure 1). Disease development due to isolate Dolla1 inoculation was progressed highly in variety Dimtu, followed by Dinknesh compared to the epidemics developed from the rest varieties (Figure 1A). Likewise, the disease progress was also higher in variety Dimtu, followed by KATB1 and Dinknesh in response to isolate Dolla2 than others (Figure 1B). The progresses were higher and closer between varieties Babile and Dimtu, followed KATB1 even more than the susceptible check in response to isolate Dorebafano (Figure 1C). The intersection points of the graphs indicated that there were similar epidemics among varieties at that particular time in response to specific isolate.

## DISCUSSION

Percent severity index was used to detect the difference in pathogenicity and aggressiveness of *P. griseola* isolates on susceptible common bean variety. Information on the aggressiveness of the isolates is important to understand the variability of the pathogen and also to select most aggressive isolates for further evaluation of genotypes for different geographical regions. Irregular brown

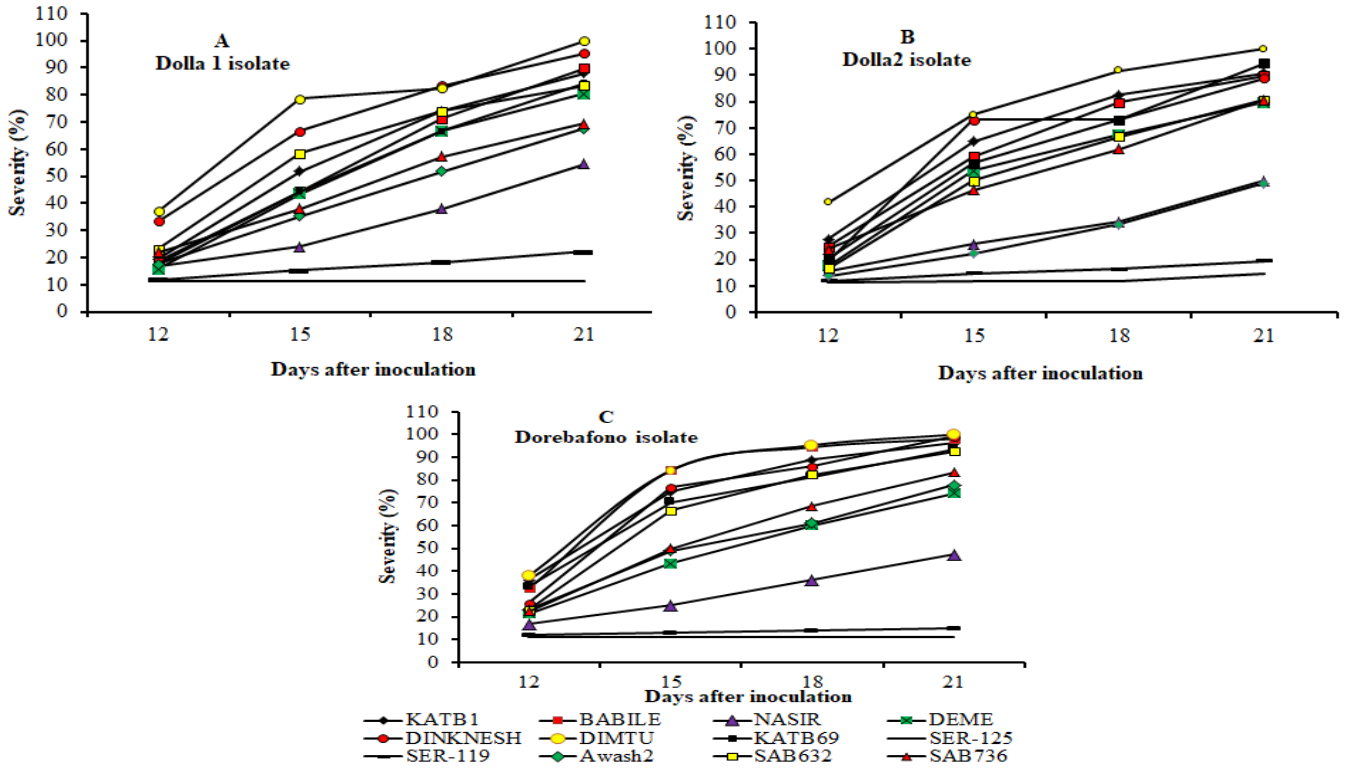


**Figure 1.** Angular leaf spot (*P. greisola*) disease progress curves on common bean varieties in response to artificial inoculation of three isolates under greenhouse conditions.

spots were evident on inoculated leaves at 11 DAI. Ng'ayu-Wanjau (2013) also observed characteristic ALS symptoms on susceptible checks CAL 96, BRB 191 and MCM 5001 between 11 and 14 days after inoculation. This could confirm that pathogenic and aggressive isolates took few days to establish infection and express symptoms. Similarly, Karwitha (2009) noted that *P. griseola* caused circular lesions which enlarged and attained larger sizes expressed on primary leaves 10 to 15 days after inoculation with three *P. griseola* races, which could imply the presence of high pathogenic variation among the isolates tested (Wagara et al., 2005a).

The presence of pathogenic and aggressiveness variability in *P. griseola* isolates are required for identifying possible sources of resistance in common bean. The present study revealed that there was a wide variation in the response of bean varieties to three aggressive *P. griseola* isolates. Among twelve commercial varieties tested, 75% of them showed closely susceptible reaction to *P. griseola* isolates. Variety Babile was previously reported as resistant to ALS, but now lost its resistance and became susceptible to all tested isolates indicating the evolvement of new aggressive strains through time. This finding is not in line with Fikre and Abush (2005) who reported about 38.36, 42.47 and 19.18% resistant, intermediate resistant and susceptible responses out of 73 bean genotypes evaluated. The loss of resistance among commercial bean varieties might be due to the breakdown of host resistance by the pathogen and/or due to inherent evolutionary variability of *P. griseola* (Pedro et al., 2006).

However, some varieties showed resistance and moderately resistance reaction to each isolate. In this regard, varieties SER125 and SER119 maintained their resistance to *P. griseola* isolates. Variety Nasir also showed moderate resistance to all isolates tested. In line with this finding, Charimbu (2009) reported that common bean lines showed incompatible interaction, and complete to moderate resistance reactions to different races of *P. griseola* isolates in Kenya. The resistant and intermittent resistant responses of varieties could also be due to the presence of different major and minor genes for resistance and could be important sources for ALS resistance breeding. Several studies have also demonstrated that ALS resistance in common bean is mediated by both major and minor genes that are either dominant or recessive with complementary or epistatic effects and acting alone or in combination (Mahuku et al., 2004; Caixeta et al., 2005).

Variations in resistance responses in bean varieties is very common in that bean cultivars might be resistant to only some *P. griseola* isolates but there are no varieties that are resistant to all the pathogen isolates (Stenglein et

al., 2003) and several other studies on *P. griseola*-bean combinations also confirmed the same in different parts of the world (Ng'ayu-Wanjau, 2013; Ddamulira, 2014b; Adikshita and Sharma, 2016; Kijana et al., 2017). On the other hand, there might be a variation among the tested isolates in terms of genetics, which make differences in infecting and damaging potentials against bean varieties. Related to this result, wide variations in virulence patterns, even isolates collected from the same bean cultivar, either at the same or different locations was obtained in Kenya (Wagara et al., 2005b).

Difference in PSI values among isolates tested might be due to difference in pathogenicity and aggressiveness and/or due to physiological specialization among isolates. Such characteristics enable isolates to infect, invade and colonize the host tissue rapidly and resulted in more leaf damages in which aggressive isolates had shorter incubation period to express symptoms, for greater spore production and damage on the host plant (Muiru et al., 2010). There was also huge variation with regard to PSI values of evaluated bean varieties. However, the overall epidemics found higher on some varieties, namely Dimtu, Babile, KATB1, KATB69 and the susceptible check for all isolates inoculated than other variety-isolate interactions. On the other hand, the epidemics were lower in variety Nasir and SER119 besides the resistant check. These variations might be associated with the difference in defense mechanisms of hosts and/or the virulence pattern of the isolates. Previous studies also demonstrated that the severity or the susceptibility of bean lines to ALS were variable among different genotypes and pathogen interactions (Ng'ayu-Wanjau, 2013; Ddamulira, 2015; Pereira et al., 2015; Mongi, 2016; Kijana et al., 2017).

The level of resistance and susceptibility of common bean varieties tested also revealed different apparent infection rate. The apparent infection rate was inversely proportional with degree of resistance. That is to mean rate was high in susceptible varieties as compared to moderately resistant and resistant varieties. Disease progression was faster per day in most susceptible varieties, namely Dimtu, Babile, KATB1, KATB69 and the susceptible check, while it was slow in variety Awash2, Nasir and the resistant ones. This could be due to the higher multiplication and growth of fungus on the susceptible varieties than resistant ones. Similarly, most common bean landraces exhibited low infection rates against least aggressive races (Mongi, 2016). On the other hand, the moderately susceptible cultivars exhibited minimum infection rate of 0.054 to 0.095 units $day^{-1}$, while highly susceptible cultivars exhibited high infection rate ranged from 0.169 to 0.195 units $day^{-1}$ (Adikshita and Sharma, 2016).

Angular leaf spot was progressed increasingly and attained closely sigmoid shape in most varieties, except some moderately resistant and resistant varieties. Such disease progress curves are characteristic to polycyclic diseases (Van der Plank, 1963). The rate of development was rapid at particular time and then became slowly increased to the final epidemic level. This could be due to the loss of nutrients (host tissue) and increase of competition among fungal populations that make rate of progress slow at final stage of developments. In this regard, factors such as availability of host tissue and initial amount of the inoculum and/or initial disease progress rate had an important effect on the final severity of the disease (Fininsa and Tefera, 2005; Madden et al., 2008).

## CONCLUSIONS AND RECOMMENDATIONS

Although the tested varieties are highly productive and preferred by growers, the majority of them are susceptible to *P. griseola* isolates considered in the study. This implies that there is an urgent need to develop commercial common bean varieties with multiple angular leaf spot resistance as disease management option in the resistance breeding programs. The variation in isolates for pathogenicity and aggressiveness also implies that use of many source of resistance is needed to manage ALS effectively. The identified intermediate resistant (Nasir) and resistant varieties (SER119 and SER125) could be a good source of resistant gene to develop durable angular leaf spot resistance. However, genetic studies on the type, number and mode of inheritance of resistant genes should be conducted. In addition, more isolates covering wide agro-ecologies shall be included in the resistance reaction evaluation studies to exploit crop resistance gene potentials. Furthermore, common bean varieties evaluated under greenhouse conditions in this study should also be tested under field conditions to confirm the consistency of results obtained.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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**Appendix Figure 1.** Responses of twelve common bean varieties to *P. griseola* isolate, Dolla1.


**Appendix Figure 2.** Responses of twelve common bean varieties to *P. griseola* isolate, Dolla2.


**Appendix Figure 3.** Responses of twelve common bean varieties to *P. griseola* isolate, Dorenafano.