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EVALUATION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES FOR DISEASE TOLERANCE UNDER RAIN-FED CONDITIONS

SUMMARY

Breeding for disease tolerance in common bean (*Phaseolus vulgaris* L.) can improve yields for small-scale and commercial bean producers. This study evaluated promising common bean (*Phaseolus vulgaris* L.) genotypes for disease tolerance under rain-fed conditions at Harare Research Centre in Zimbabwe. A total of 25 genotypes, sourced from the International Center for Tropical Agriculture (CIAT), Malawi and Crop Breeding Institute (CBI), Harare were used in the experiment. These were replicated three times in a CRBD, and the results of disease evaluations were analysed using GenStat Discovery Edition 3 (VSN International Ltd., 2016). The germination percentages for all the genotypes ranged between 97.33 and 100% and the days to 50% flowering ranged from 35 to 42 days. The genotype CIM-NAV02-16-2 attained the highest yield of 2541 kg/ha, with genotype CIM-DWRF-CLIM01-1-1 and GCI-5Y-275-RAR-1 falling second and third with 2516 kg/ha and 2168 kg/ha respectively. There were significant ($p < 0.05$) differences in the severity scores for angular leaf spot (*Phaeoisariopsis griseola*), common bacterial blight (*Xanthomonas phaseoli*) and rust (*Uromyces appendiculatus*) among the different common bean genotypes. The genotypes GCI-5Y-275-RAR-1, CIM-DWRF-CLIM01-1-1 and DAB 524 were tolerant to angular leaf spot (*P.griseola*). All the genotypes were highly susceptible to common bacterial blight (*X. phaseoli*). The only genotype with significant levels of rust, *U. appendiculatus* was MR14215-9; the remaining 24 varieties were tolerant. Two superior breeding lines CIM-NAV02-16-2 and CIM-DWRF-CLIM01-1-1 were relatively resistant to all the diseases evaluated in the study and it is recommended that they be advanced for further assessments.

Keywords: common bean, genotypes, disease tolerance, severity scores, susceptibility.

INTRODUCTION

Beans (*Phaseolus vulgaris* L) popularly known as common or field bean is an important crop in Africa (Popovic et al., 2012). Phaseolus bean is a cheap

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source of high-quality protein, with highest consumption among the poor. It is the leading protein source in Brazil and in parts of equatorial Africa, sometimes contributing up to 30 % of protein intake and 10-15% of calories ((Broughton *et al.*, 2003). This crop belongs to the legume tribe Phaseoleae within the Papilionoideae-Leguminose family (Freytag and Debouck, 2002). Its centers of origin are the Andean and Mesoamerica regions of Latin America (Cortes *et al.*, 2013). Beans from these two centers constitute the two main biotypes; that is the Andean and Mesoamerican types. According to Bitocchi *et al.* (2012), beans of Andean origin are predominantly large seeded types whilst Mesoamerican types vary in seed size from small to medium. Common beans are warm season plants that can be either short day or day neutral and grow in a wide range of environments from sea level to more than 3000 m above sea level (Gray, 2013).

The climatic requirements for dry beans were summarized by Makini (1994). The dry bean is an annual crop which thrives under warm climatic conditions, with an optimum temperature for growth ranging from 18 to 24°C. Maximum temperatures during flowering should not exceed 30°C for *Phaseolus vulgaris* L. and 26°C for *Phaseolus coccineus*. High temperatures during the flowering stage lead to abscission of flowers and low pod set, resulting in yield loss. Day temperatures below 20 °C delay maturity and result in poor pod filling. Under rain-fed conditions dry beans require a minimum of 400 to 500 mm of rain during the growing season, but an annual total of 600 to 650 mm is ideal. According to Liebenberg (2002) dry beans should be planted in warm soils (minimum temperatures preferably above 13°C) after all danger of frost has passed. They complete their phenological growth smoothly in well drained fertile soils with a depth of at least 90 cm. Suitable soils for beans are sandy loam, sandy clay loam and clay loam with clay contents of between 15 and 35% and soil pH (water) of 5.8 to 6.5 as the crop is very sensitive to acidic soil below pH < 5.2 (Freytag and Debouck, 2002). However, with sandy soils, problems of low fertility and nematode damage may occur.

Beans have been grown in Zimbabwe for decades but yields have been declining because of factors such as low rainfall during planting time and high disease prevalence during vegetative and reproductive stages (Mupeperekwi, 2002). Low rainfall results in reduced availability of plant nutrients, resulting in depressed biomass yield, while diseases have an impact by lowering the potential yield of beans. Of the diseases caused by fungi, bacteria and viruses in beans, the most prevalent ones are the fungal diseases such as rust, anthracnose, angular leaf spot, web blight and root rots (Tadesse *et al.*, 2009). These diseases affect the duration, efficiency and size of the various photosynthesis system. The partitioning coefficient of allocating dry matter to the economic part of the plant (the pod) is reduced. *Phaseoriopsis griseola*, a leaf pathogen which causes angular leaf spot is a major problem under conditions of terminal drought (Frahm *et al.*, 2004), whereas *Xanthomonas phaseoli* and *Uromyces appendiculatus* are major pod and leaf pathogens causing common bacterial blight and leaf rust respectively in regions where intermittent drought occurs (Navarrete-Maya *et al.*,

2002). Similarly, water stressed bean crops may become prone to damage by leafhoppers (*Empoasca kraemeri*) in the tropics and subtropics (Musyimi, 2014).

Angular leaf spot (ALS), common bean blight (CBB) and rust diseases are persistent problems that growers lack tools to control. There are few practical and economic means to reduce the severity of infection, which is conditioned in part by environmental stress and decline in soil quality. In climatically variable years, including under high rainfall or drought conditions, common bean blight often suppresses bean yields by 25%, and up to 80% (Deshpande and Deshpande, 1991). Current fungicide options are too expensive and becoming highly regulated. Integrated management control options are required that are profitable and environmentally friendly. Disease effects are also exacerbated by the fact that poor disease tolerant cultivars are planted with poor sanitation. This study sought to evaluate common bean (*Phaseolus vulgaris* L.) genotypes for disease tolerance under rain-fed conditions. In the study we hypothesize that Andean Bean (DAB) lines with good adaptability to local conditions in addition to tolerance to angular leaf spot (ALS), common bacterial blight (CBB) and bean rust can be identified. We also hypothesize that genotypes with the ability to attain high yield under disease pressure can also be identified. The DAB were selected as suitable genotypes with suitable sources of disease tolerance for use in breeding programmes in Zimbabwe.

MATERIALS AND METHODS

The study was carried out at Crop Breeding Institute (CBI), Department of Research and Specialist Services, in Harare, Zimbabwe. The study site is located in Natural Region (NR) II and lies at an altitude of 1 625 m (Google earth, 2015). Natural regions in Zimbabwe are based on the relationship between physical attributes particularly the amount of rainfall, its reliability and distribution, and farming potential (Vincent *et al.*, 1960). Rainfall amounts decrease from NR I to NR V, ranging from at least 900 mm per annum in NR I to less than 450 mm per annum in NR V. Agricultural potential also decreases from NR I to NR V. The soils in the experimental area are derived from granitic alluvial.

Genotypes

A total of 25 common bean varieties sourced from International Centre for Tropical Agriculture (CIAT), Malawi and CBI, Harare, were used in the study (Table 1). Twenty two (22) were breeding lines whilst 3 were check varieties.

Experimental design and layout

The experiment was laid out in a completely randomized design (CRD) with three replications at the study site. Planting of the genotypes was done manually on 1.8 m x 3 m prepared plots, consisting of 4 rows. The inter row spacing of 0.45 m used was whilst inner row spacing was 0.05 m. Compound D (7:14:7) fertilizer was applied at sowing at a rate of 300 kg/ha then ammonium nitrate (34.5% N) fertilizer was applied at a rate of 80 kg/ha before the flowering stage as top dressing. Weeding was done whenever necessary to minimize weed infestation.

Table1. Summary of the common bean (*Phaseolus vulgaris* L.) genotypes used in the study

Genotype Number	Identity	Source	Status (2015)
1	GCI-5Y-275-RAR-1	CIAT	Breeding line
2	CIM-DWRF-CLIM01-1-1	CIAT	Breeding line
3	CIM-NAV02-16-2	CIAT	Breeding line
4	DAB 70	CIAT	Breeding line
5	DAB 194	CIAT	Breeding line
6	GLORIA	CBI	Check variety
7	DAB 84	CIAT	Breeding line
8	DAB 524	CIAT	Breeding line
9	DAB 487	CIAT	Breeding line
10	DAB 366	CIAT	Breeding line
11	DAB 311	CIAT	Breeding line
12	NUA 45	CBI	Check variety
13	DAB 539	CIAT	Breeding line
14	DAB 221	CIAT	Breeding line
15	DAB 416	CIAT	Breeding line
16	MICHIGAN PEA BEAN	CIAT	Breeding line
17	DAB 482	CIAT	Breeding line
18	MR14215-9	CIAT	Breeding line
19	DAB 82	CIAT	Breeding line
20	DAB 58	CIAT	Breeding line
21	DAB 302	CIAT	Breeding line
22	DAB 287	CIAT	Breeding line
23	PAN 148	CBI	Check variety
24	SEQ 1039	CIAT	Breeding line
25	DAB 16	CIAT	Breeding line

Introduction of diseases

The multiple needle puncture inoculation method was used to initiate angular leaf spot (*Phaseoriopsis griseola*), common bacterial blight (*Xanthomonas phaseoli*) and leaf rust (*Uromyces appendiculatus*) diseases one week after germination. Small holes were punched on the leaves of the bean plants using needles infected with the disease as described by (Andrus 2008) and by Pompeau and Crowder (2010).

Measurements

Germination Percentage

The germination percentage was calculated as the number of plants emerged per plot divided by the total number of seed sown per plot multiplied by 100.

Days to 50 % flowering

These are days when 50 % of plants in each plot had one or more first flowers and coincides with the initiation of reproductive development stage.

Disease scoring

Diseases scores were taken on three different regimes which were:

- First regime: before flowering commenced
- Second regime: at pod filling and
- Third regime: towards crop maturity

Diseases that were scored were angular leaf spot (*Phaseoriopsis griseola*), common bacterial blight (*Xanthomonas phaseoli*) and leaf rust (*Uromyces appendiculatus*). All these diseases were scored on a scale from 1-9 where 1=no symptoms of the disease and 9= severely affected by the disease.

Pod height

Pod clearance from the ground was measured in centimeters using a meter rule. This was done six weeks after germination.

Days to 95 % maturity

These are number of days from date of planting to date when 50 % of plants in each plot attained physiological maturity. Pods will be dry and they turn brown in colour.

Harvesting

Harvesting was done by hand in 4 m x 2 row plots. Grain yield data was taken from the two central rows in each plot and expressed on a hectare basis (kg/ha). Weight of harvested clean seed from the net plot was measured in grams using Nicholas scale and recorded in the field book. The weight was then converted to yield by dividing the seed mass by plot size and then converted to kg/ha.

Statistical analysis

Data for germination percentage, days to 50 %, pod clearance, disease scores, days to 95 % physiological maturity and grain yield data were subjected to analysis of variance (ANOVA) using GenStat statistical analysis software version 10.3.0.0. Error bars for bar graphs were drawn using calculated standard error of differences (s.e.d) and were used for further analysis. In most cases the data had to be transformed using square root transformation $\sqrt{(x+1)}$ to make it normally distributed before analysis.

RESULTS

The results for (1) germination percentage; (2) pod height (cm); (3) disease scores [1-10 from least to most severe] for (a) angular leaf spot (ALS), (b) common bacterial blight (CBB) and (c) rust; (4) days to 50% flowering and to (5) 95% physiological maturity and finally (6) grain yield in kg/ha are shown in Table 2. The germination percentage for all genotypes ranged between 97.33 and 100%. Seven of the genotypes were able to attain 100% germination.

Days to 50% flowering ranged from 35 to 42 days, genotype GCI-5Y-275-RAR-1 recorded the highest number of days to 50% flowering (42 days). The pod least clearance of 2.7 cm was recorded for genotype (CIM-DWRF-CLIM01-1-, while the highest was 9 cm recorded on DAB 539. Disease scores for angular leaf spot on the Cobb scale which ran from 1 to 9 recorded between 1.67 and

6.67. Common bacterial blight scores ranged from 4 to 6.67 and for rust most scores were in the resistant zone (1 to 3) except for alarming score of 6 recorded on genotype (MR14215-9). The genotype (CIM-NAV02-16-2) attained the highest yield of 2541 kg/ha with genotype CIM-DWRF-CLIM01-1-1 and GCI-5Y-275-RAR-1 falling second and third with 2516 kg/ha and 2168 kg/ha respectively. Most of the genotypes yielded between 1365 kg/ha and 2128 kg/ha. The least yielding genotype was DAB 539 with a yield of 1365 kg/ha.

Table 2. Combined means on data collected for the 25 common bean (*Phaseolus vulgaris* L.) genotypes

Genotype	Germination (%)	50 % flowering (no of days)	Pod height in cm	ALS (scale 1-10)	CBB (scale 1-10)	Rust (scale 1-10)	95% maturity (no of days)	Grain yield (kg/ha)
1	100	42	2.33	1.67	4	1	91.33	2168
2	100	39	2.33	2.67	4.67	1.333	90	2516
3	100	39.67	4	3	4.33	1	89.67	2541
4	99.67	36.33	4	3	5.33	1.333	87.67	1681
5	99.33	34.67	7	2.67	6.33	1.667	85	1743
6	99	38	7	4	5.67	2	88.67	1794
7	100	36.33	5.33	3.67	6.67	1.667	87.33	2128
8	99.33	35	6	2.67	6	2.333	85.67	1619
9	98	35	5	3	6	1.333	85	1530
10	99.33	35	5.33	2.67	6	1.333	85.67	1536
11	97.33	35	5.33	2.67	5.33	1.333	85.67	1448
12	99.67	35	5.33	6.67	5.33	1	85.67	1912
13	98	34.67	9	3.33	4.67	1.333	85.33	1365
14	98.33	34.67	3.33	2.67	6.33	1	85.33	1582
15	99.67	35	3	4	6	1.333	85	1874
16	99.67	39.67	5	3	5.67	1	90	1787
17	100	35	5	3.67	6.67	1.333	86	1911
18	100	38.33	6.67	2.33	6	6	88.67	1847
19	98.33	35	6.33	4.67	5.33	1	86.67	1375
20	99	37.33	4.33	3.33	5.33	1.333	88.33	1680
21	98.67	34.67	4.67	4.33	6.33	1.667	84.67	1590
22	99	35	5	5.33	6.33	1	85.33	1856
23	99.67	40	6	5.33	5.33	1	90	1906
24	99.67	37	2.67	2.67	5.33	1.667	91.67	1823
25	100	40	4	3	4	2.667	91.33	1976

Germination Percentage

The twenty five common bean (*Phaseolus vulgaris* L.) genotypes that were evaluated had the similar ($p < 0.05$) germination percentages as shown in Figure 1. The analysis of variance at 5 % level of significance indicated that there is no significant difference across the three replications for both released local varieties

and breeding lines ($P>0.05$). The standard error of difference (s.e.d) which was used to draw error bars was ± 0.914 .

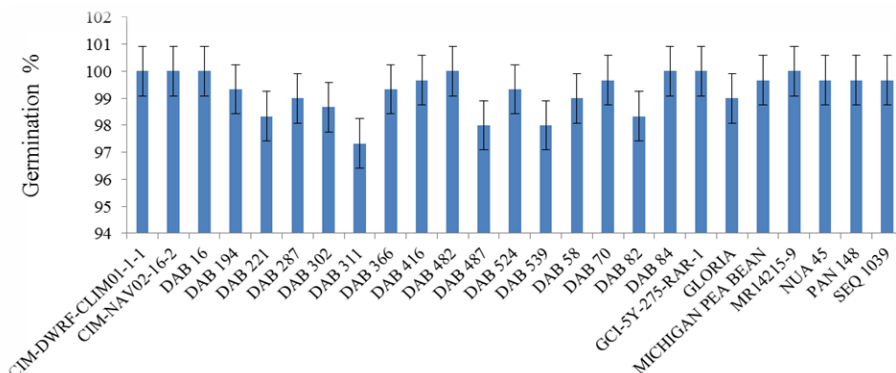


Figure 1. The germination percentage of the common bean (*Phaseolus vulgaris* L.) genotypes

Days to 50 % flowering

The analysis of variance for days to 50 % flowering at 5 % level of significance proved that there was a significant difference among the genotypes which were used as experimental units. The standard error of difference (s.e.d) which was used to draw error bars on the bar chart in Figure 2 was ± 1.147 .

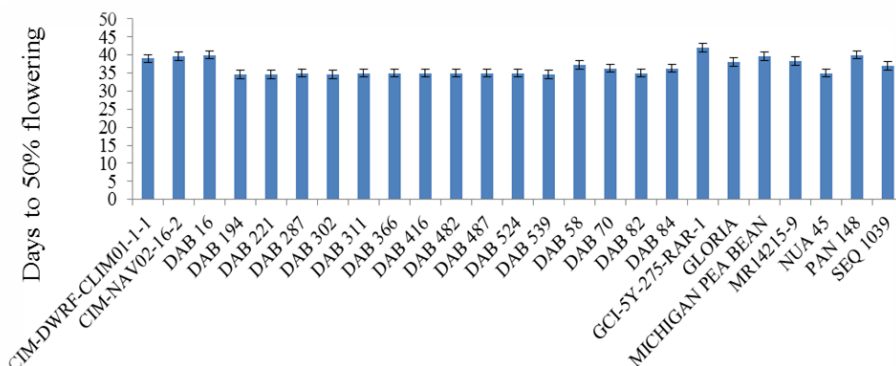


Figure 2. Bar graph on days to 50% flowering for the 25 common bean (*Phaseolus vulgaris* L.) genotypes.

Pod clearance

For pod clearance, the analysis of variance at 5 % level of significance showed that there was a significant difference between genotypes ($P<0.05$) as shown in Figure 3. The standard error of differences that used for error bars is ± 1.178 .

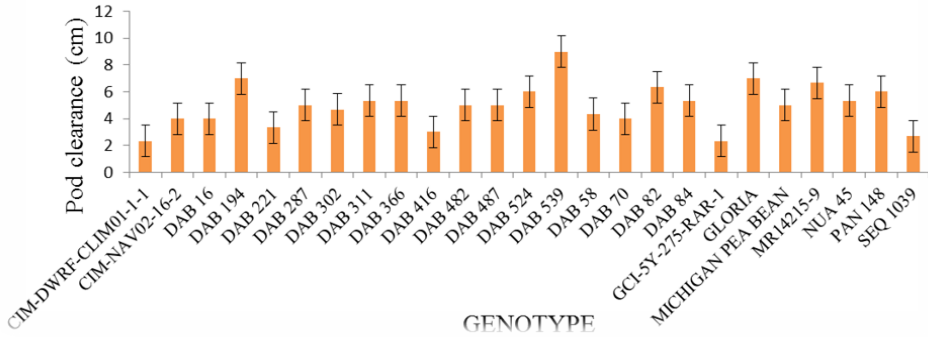


Figure 3. Bar graph showing pod clearance for the common bean (*Phaseolus vulgaris L.*) genotypes.

Angular leaf spot scores

The analysis of variance for scores recorded on angular leaf spot at 5 % level of significance proved that there were significant differences on disease scores among the genotypes as shown in Table 4.

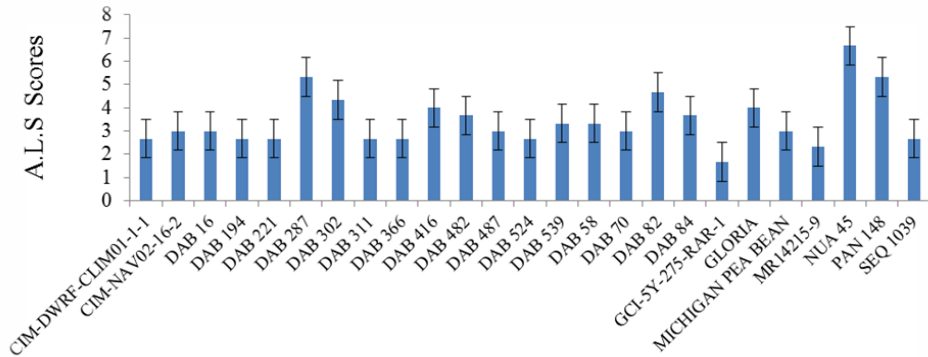


Figure 4. Bar graph on angular leaf spot scores for the 25 common bean (*Phaseolus vulgaris L.*) genotypes

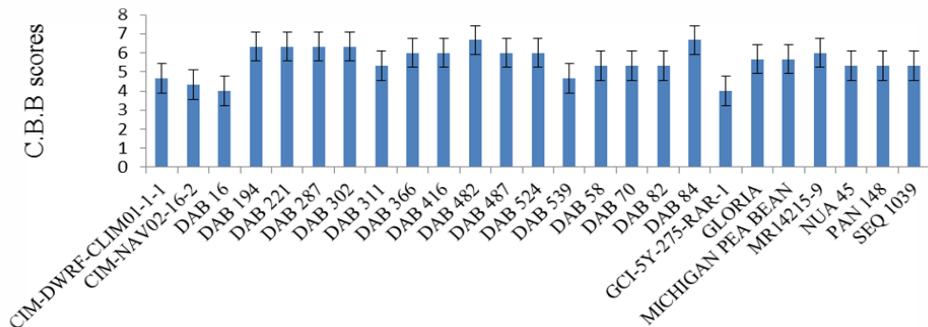


Figure 5. Common bacterial scores for the common bean (*Phaseolus vulgaris L.*) genotypes

Common bacterial blight scores

The analysis of variance for scores recorded for common bacterial blight at 5 % level of significance proved that there were significant differences on disease scores among the genotypes as shown in Figure 5. Error bars were drawn using standard error of differences value of ± 0.766 .

Rust scores

At 5 % level of significance the analysis of variance for rust severity on bean genotypes sourced from CIAT and local released varieties showed that there were significant differences ($P < 0.05$) as shown on Figure 6. The error bars were drawn using standard error of difference of ± 0.6394 .

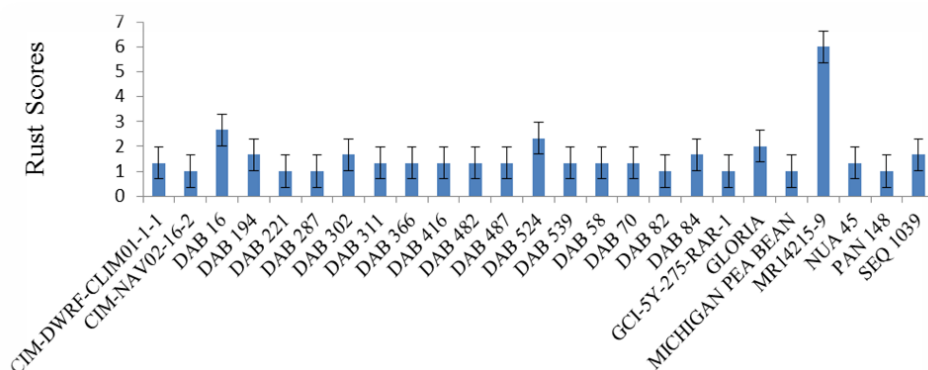


Figure 6. Rust scores for the 25 common bean (*Phaseolus vulgaris* L.) genotypes

Days to 95 % physiological maturity

The analysis of variance for days taken to attain 95 % physiological maturity by genotypes was found to be significantly different at 5% level of significance ($P < 0.05$) as shown in Figure 7. The error bars on the bar graph (figure 7) were drawn from standard error of difference (s.e.d) of ± 1.523 .

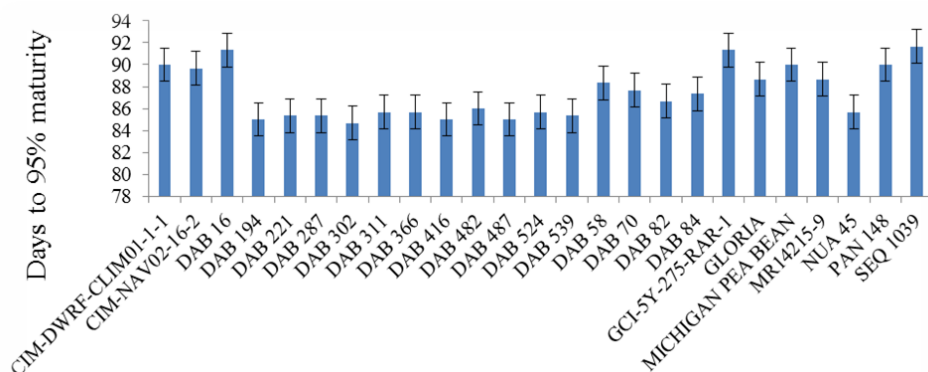


Figure 7. Days to 95% physiological maturity for the 25 common bean (*Phaseolus vulgaris* L.) genotypes

Seed yield (kg/ha)

Analysis of variance on grain yield (kg/ha) indicates that there is a significant difference in yield attained by breeding lines and local check varieties ($P < 0.05$) as shown in Figure 8. Error bars were drawn from standard error of difference value of ± 241 .

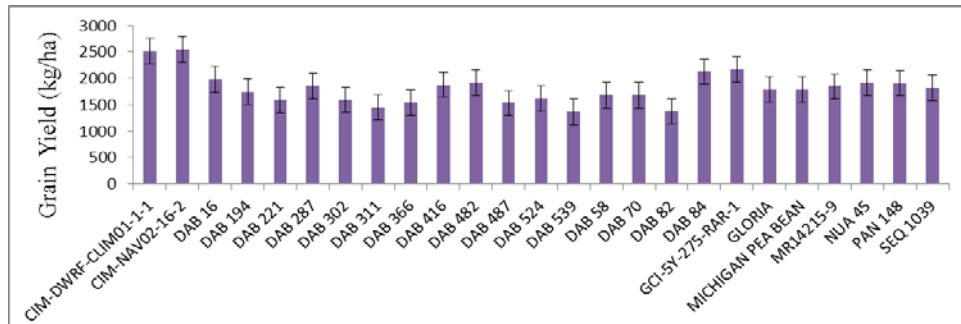


Figure 8. Grain yield for the 25 common bean (*Phaseolus vulgaris* L.) genotypes

DISCUSSION

The twenty five bean genotypes that were evaluated had the same capacity to germinate as evidenced by similar emergence counts that were obtained in this study. Results obtained suggest that these bean genotypes do not differ in terms of plant hormones that stimulate germination. Some of the germination stimulants that have been identified are electro and sorgoleone produced by cowpea and sorghum respectively (Rambakudzibga, 2000). This would suggest that bean genotypes evaluated in this study are very resistant to stem maggot infection due to their ability to produce high levels of germination percentage. Common bacterial blight and angular leaf spot infection reduced shoot biomass, pod numbers, pod weight and grain yield. Only two genotypes, CIM-DWRF-CLIM01 and CIM-NAV02-16-2 managed to produce pods and grain under infection. These results concur with findings by Mugabe (2008) and Alonge *et al.* (2001) who reported that early infection delayed the onset of flowering, reduced number of flowers and resulted in a concomitant decrease in the number of pods per plant and low grain yield.

Whilst common bacterial blight prevalence in this study suggested that all the bean genotypes were highly susceptible; the ability of the two breeding genotypes, CIM-DWRF-CLIM01 and CIM-NAV02-16-2 to produce pods and grain under *Xanthomonas phaseoli* infection suggests that these genotypes have some degree of tolerance to this bacterium pathogen. Haussman *et al.* (2000) reported that the susceptibility of the host may not be explained by the disease score on plants but probably by the host genotype. Infection of plants by *Xanthomonas phaseoli* results in an increase in sink demand in the leaves. This carbon transfer from the leaves to the photosynthetic system due to changes in sink demands largely accounts for the reduction in pod biomass and was reported to be responsible for the reduction in pod formation (Rambakudzibga, 2000). Similar findings were obtained in other host parasite associations involving tomato and tobacco infected with *Orobanche aegyptiaca* (Hibberd *et al.*, 1996) and sorghum infected with *S. hermonthica* (Cechin and Press, 1993).

For angular leaf spot, disease development started during the third week after inoculation. The more resistant genotypes to *Phaseoriopsis griseola* were GCI-5Y-275-RAR-1, CIM-DWRF-CLIM01-1-1 and DAB 524 while NUA 45 was the most susceptible genotype to *Phaseoriopsis griseola*. Angular leaf spot is not an important disease in most bean producing areas, however, under favorable environmental conditions, disease epidemics can occur. Optimal temperatures for germination of conidia of *P. griseola* range between 23 to 27°C, and infection occurs from 16 to 28°C with the optimum for infection and disease development at 24°C (Belete and Bastas, 2017) Frequent rain and high humidity are important for the initiation of disease and are considered more important than temperature. The results are similar to Pastor-Corrales *et al.* (2007) who screened 22,832 cultivated and wild common bean accessions for resistance to ALS and found most were susceptible, with only 59 showing an intermediate response and 64 showing a resistant response.

Transmission of pathogens which causes bacterial diseases in common beans is accelerated by low pod clearance. In this study, although there was a significant difference between commercial varieties and breeding lines in relation to pod height, DAB 539, Gloria and DAB 194 had the highest clearance from the ground. These genotypes have the potential to be crossed with other breeding lines with high yielding ability so as to come up with a superior variety with both high yield and pod clearance. Photoperiod (*Ppd*) controlling genes had a large effect on the height of the crop and thus on the environment, and degree of infection, that leaves were exposed to during their emergence and expansion. Disease severity at a given calendar date, for a given leaf layer was consistently greater on the more mature lines due to the longer period of exposure. It is generally assumed that, when screening for resistance, this maturity effect can be adjusted for by regressing disease severity with date of heading. However, our observations suggest that this might only be appropriate if infection conditions are consistent over time, or vary progressively due to rainfall seasonality. During the period which the project was done, rainfall was sporadic. A more appropriate method of screening would therefore be to compare leaves that emerge concurrently and therefore share similar rainfall.

CONCLUSIONS

The study concludes that the performance of advanced common bean genotypes and commercial varieties differs in relation to agronomic factors. Moreover, it can be concluded that all the common bean genotypes evaluated are susceptible to common bacterial blight infection. However, it can also be concluded that the two breeding lines CIM-NAV02-16-2 and CIM-DWRF-CLIM01-1-1 are moderately susceptible because they were able to produce highest grain yield. Therefore further selection and studies need to be done on these genotypes before release for commercial production. Genotypes GCI-5Y-275-RAR-1, DAB 311, DAB 366, MR14215-9 and DAB 524 were found to be less susceptible to angular leaf spot pathogen *Phaseoriopsis griseola*; with scores of less than 3 on the Cobb's scale. Effect of inoculating the bean genotypes with *Uromyces appendiculatus* in this experiment was not much significant, scores for most genotypes ranged between 1 and 2 except for MR14215-9 which was exceptional recording 6 on the Cobb's scale.

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