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Screening for resistance of the common bean genotypes to common bacterial blight, and bean common mosaic virus

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Abstract

Common bacterial blight (CBB), and bean common mosaic (BCMV) limit common beans (Phaseolus vulgaris L.) production worldwide. This study was carried out to perform phenotypic screening and asses the leaf reaction of a resistant line to CBB and BCMV. The experiment was conducted using a Completely Randomized design with three replications under screen-house conditions. Four improved bean genotypes for bruchid resistance were collected from bean improvement projects at the Sokoine University of Agriculture and one commonly cultivated susceptible cultivar was collected from a local market. Bean seeds were sown in a pot with sterilized soil and Xap inoculated by spraying with a bacterial suspension at 18 days after planting, while mechanical inoculation was performed for BCMV on 10 days old leaves. Disease severity of CBB was assessed three times at 14, 21, and 35 days after inoculation using a 1-9 CIAT scale, while for BCMV, symptoms were assessed at 15 days after inoculation. Results show significant differences ($p \leq 0.001$) on resistance to both diseases among the common beans genotypes tested. 13A/59-98-3x3-3A (scored 1.3 for CBB; no infected plant with BCMV), AO 29-3-3A (scored 2.0 for CBB; no infected plant with BCMV) and KT020 (scored 1.3 for CBB; only 1 plant was infected with BCMV) had resistance to both diseases while BR59-63-10 was resistant to BCMV and intermediate resistance (scored 3.5) to CBB. Kablanketi was susceptible to both diseases (scored 8 for CBB; 2 plants infected with BCMV). This study verified the resistance against CBB and BCMV in three lines obtained from SUA used for breeding multiple disease resistant cultivars.

Keywords: *Phaseolus vulgaris L., Phenotypic screening, resistance, susceptible*

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Introduction

Common bean (*Phaseolus vulguris* L.; 2n=2x=22), is the most preferred consumable legume and being distributed worldwide (Razvi *et al.,* 2018). It is an important and essential component of diets in most households of Tanzania (Letaa *et al.,* 2020). Common beans are cultivated as vegetable (Laizer *et al.*, 2019). Their grains which have high dietary protein content around 22% or even higher on a dry matter basis (Philipo *et al.,* 2020). It is the source of essential minerals, and vitamins (Mazengo *et al.*, 2019). Its proteins and carbohydrates provide calories of up to 25% of the diet (Beebe *et al.,* 2013). Their nitrogen fixing ability contributes about 50N kg per ha to soil fertility (Bänziger, 2004; Hillocks *et al.,* 2006).

Common bean is essential to smallholder farmers to meet their daily nutritional needs and for income generation (Mangeni *et al.,* 2020).

Tanzania ranks first in Africa and sixth in world top bean producers, with total production of 1.14 million metric tons and average of yield of 0.9 tons per hectare (Letaa *et al.,* 2020). However, its productivity is still low because the crop has been stressed with both abiotic and biotic factors, including diseases and insect pest (Mishili *et al.,* 2011; Mazengo *et al.,* 2019). Pests are estimated to be the second biggest constraints to bean production after low soil fertility and its annual loss caused by pests vary from 20 to 100% (Oladzad *et al.,* 2019; Dramadri *et al.,* 2019). Reduction in yield has been attributed by the effect of disease and insect pest, specifically, Common Bacterial Blight (CBB), Bean Common Mosaic Virus (BCMV) and/or Bean Common Mosaic Necrotic Virus (BCMNV) (Tryphone *et al.,* 2012; Chilagane *et al.,* 2013; Alladasi *et al.,* 2018), and secondarily from bruchid (bean weevils) damage (Kipato *et al.*, 2015; Kusolwa *et al.*, 2016).

CBB and BCMV are both seed-borne diseases in which the infected seeds play a great role as the primary source of inoculum for the diseases. In addition, BCMV can be transferred over short distances from the infected plants to healthy ones through vectors such as aphids in a non-persistent manner (Mwaipopo *et al.,* 2017). Breeding for host plant resistance is most reported to be a more effective and long-term solution to control these diseases and many. CBB and BCMV resistant lines have been developed in this regard. Resistance of CBB has been reported being governed by quantitative trait loci (QTL), while BCMV is being controlled by qualitative gene (Tryphone *et al.,* 2012; Alladasi *et al.,* 2018). Screening of the breeding material is very essential in order to be sure of the plant reactions to the disease races. It has been reported that, there is differential expression of resistance to CBB in different plant parts (Alladasi *et al.,* 2018). Infection in leaves and pods is reported as a major challenge in controlling CBB disease in common bean and therefore past studies have focused on the association between leaf and pods to *Xap*/*Xapf* (Alladasi *et al.,* 2018). Armaud-Santana *et al.,* (1994) reported lower genetic correlation between leaf and pod reactions and leaf and seed reaction to CBB disease. Similarly, Part *et al.* (1998) found low to intermediate correlation between leaf and pod reactions to CBB in common beans. Jung *et al.,* (1997) also reported different genes controlling CBB resistance in leaf, pod and seed in common beans. All findings have shown that some CBB resistant genotypes possess resistance to CBB in only one organ; thus, screening of multiple organs is important in order to obtain the resistant line with combined resistance. According to Belarmino, (2015) screening of genetic resources against the specific pathogens is significant in developing resistant cultivar. Therefore, the objective of this study was to screen and assess the plant reaction of the provided resistant lines using inoculum for Common Bacterial Blight (CBB), and Bean Common Mosaic Virus (BCMV).

Materials and Methods

Description of the Study site

The study was conducted in the screen house of Horticulture Section at Sokoine University of Agriculture (SUA). The University is located at latitude 6°5′ South and Longitude 37°39′ East and 549 meters above sea level on the foot of Uluguru Mountains.

Experimental Plant Materials

The experimental material used were seed of locally adopted bean cultivar 'Kablanketi', which is susceptible to CBB, BCMV but fetches high market price in local markets, used as a check in this study, KT020 (Improved genotypes from Bean Improvement Project). KT020 is derivate of Mexico54, Vax3 and Mshindi following four backcross to Kablanketi; an indeterminate climbing (Type IV) having medium sized seeds, grayish in color, and have resistance to CBB and BCMNV; 59-63-10 derived from crossing black seeded (APA-ICA Pijao x G40199) x Kablanketi followed three backcross to Kablanketi, indeterminate vine, but lacking climbing ability (Type IIIB) and have medium sized seeds, grayish in color, and have resistance to Bruchid damage and BCMV/ BCMNV; AO 29-3-3A which is resistant to bean Bruchid and , it is indeterminate bush (Type II) having medium sized seeds with kidney red color also having resistance to BCMV and BCMNV and was used as a check; 13A/59-93-9 x3-3A, a successful cross

of APA lines and AO 29-3-3A having large cream sized seeds, resistant to bruchid damage and BCMV/BCMNV, and it is indeterminate bush (Type II).

Each of these genotypes was planted per pot using a Completely Randomized Design (CRD) with three replicates where the pot was treated as replicates under screen house conditions and germinated seedlings were inoculated with respective pathogens when they were 18 days old for CBB and 10 days after planting (DAP) for BCMV pathogens.

Inocula Collection

In order to obtain inoculum for each pathogen, diseased leaves with typical disease symptoms were collected from naturally infected fields or farms from different area around Morogoro where beans are grown i.e the SUA-crop museum, Mgeta, and Kilosa. For CBB infected plants, leaves were detached from the plant and transferred into labeled plastic bags with name of bean variety, date, and location from where the sample was collected, and placed in the ice cool box for transportation to the laboratory. For BCMV specimens, fresh samples were placed on ice in plastic bags ready for inoculum preparations. The samples were then brought to the pathology laboratory in the TOSCI laboratory for isolation and characterization of the *Xap* pathogen.

Pathogen Isolation, Preparations of Inoculum and Inoculation

Common Bacterial Blight

Isolation of *Xap*

Differential media was prepared following the procedures described by Mortensen (2005). Infected leaves were taken to the laminar air flow chamber and a section from the margin of healthy and disease leaf tissue were sterilized by immersing the materials into 2% sodium hypochlorite (NaClO) for two minutes, then excess NaClO was rinsed three times using distilled water. The materials were macerated using sterile blade and forceps, then macerated leaf were placed into a 30ml bottle with addition of 2 ml/g of Phosphate buffer saline (PBS) and left overnight. Then serial dilutions of the homogenate were made; each serial bottle contains 4.5 ml of PBS and 500µl of the leaf homogenate were pipetted for each dilution and the final the homogenate was streaked on the petri dish contains Yeast dextrose carbonate agar (YDCA) media labeled with the specific dilution, name of the pathogen and date. Plates were incubated at room temperature (28^{o}C) for three days (72 hours). After three days, yellow mucoid colonies were observed (Figure 1.A). Cell suspensions were made using sterile distilled water and its concentration was adjusted to 10^{6} cfu ml^{-1} using haemocytometer (Figure 1.B).

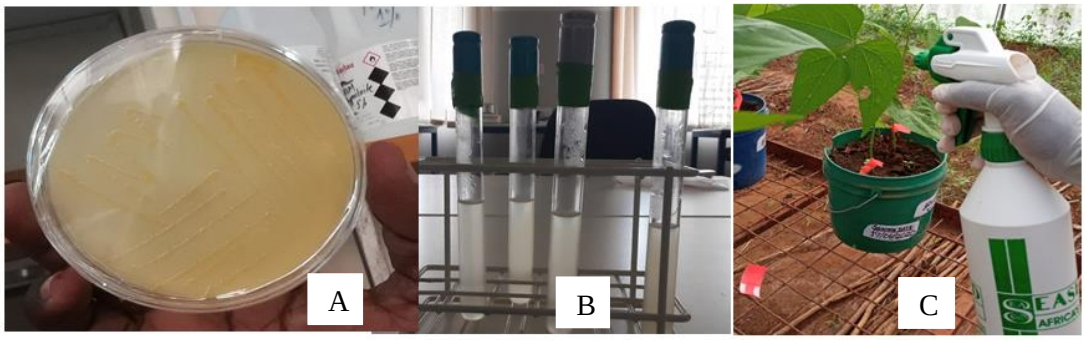

Figure 1: Isolation and preparation of common bacterial blight for inoculation of common bean genotypes. A; Xap colonies grown on the YDCA in a petri dish: B; Cell suspension after being diluted by sterile distilled water and adjusted: C; Inoculation process by spraying method

Inoculation

Plants were inoculated at 18 days after planting when they have fully expanded trifoliolate leaves by spraying the inoculum on both side of the leaves using hand pump sprayer (Figure 1.C) and covered by plastic sheets to increase relative humidity (RH) for 72 hours. After 72 hours the plastic sheets were removed and the plant pots were transferred and placed to the screen-house benches made of meshed steel, one-meter-high

for symptoms development, while the floor was kept wet for 24 hours.

Disease scoring

The disease severity was assessed on all leaves weekly from seven days after inoculation (DAI), then 14 DAI and 21DAI. The disease severity rating was estimated following CIAT 1-9 (Van-Schoonhoven and Pastor-Corrales, 1987)

Table 1

General scale used to evaluate the reaction of bean germplasm to common bacterial blight (van Schoonhoven and Pastor-Corrales, 1987**)**

Rating	category	Description	Comments
1-3	Resistant	No visible to very light symptoms resulting in little or no economic damage	Germplasm useful as parent or commercial variety
4-6	Tolerant or Intermediate	Visible and noticeable symptoms resulting only in limited economic damage	Germplasm can be used as commercial varieties or sources of resistance to certain diseases
7-9	susceptible	Severe to very severe symptoms causing useful yield losses or plant death	Not useful to be used as parent or commercial variety

Bean Common Mosaic and Necrosis Virus

Inoculum preparation and inoculation for BCMV

The fresh infected leaves with typical symptoms of disease were collected from the field, one gram (1.0gm) of infected leaf was grounded using mortar and pestle in cold 5 ml of cold 0.01 M Potassium phosphate buffer containing 0.1% Tween 20. The mixture was sieved to eliminate the plant debris, then the sieved one were used for inoculation after adding 10 g of carborundum powder (300 mesh) and sterile PBS, and the mixture were stirred.

Inoculation

Mechanical inoculation was performed; where by the index finger was dipped into the inoculum and then sap was slightly rubbed on both surfaces of the primary leaves of 10 days old plants. Control seedlings were not inoculated but simply sprayed with distilled water.

Disease severity rating

Disease was assessed at 15 days after inoculation (DAI) at which plant showing reaction or symptoms such as mosaic mottle, systemic necrosis or vein banding were counted and recorded and removed from the pots leaving the healthy plants.

Disease resistance rating

Disease was assessed at three phases which are; 14, 21 and 35 days after inoculation on trifoliate leaves. The disease scoring was done based on phenotypic observation and appearance of the leaves due to absence or presence of the typical symptoms of the CBB, using the CIAT scale of 1-9 with some modification at which the plant with score of 1-3.3 were considered as the resistant, 3.4-6.4 were considered as Intermediate resistant, and 6.5-9 were considered as susceptible genotypes as shown in Table 1 (Van Schoonhoven and Pastor-Corrales, 1987). For BCMV, assessment was done once at 15 days of inoculation where by number of plants with typical symptoms were counted, removed from the experiment and recorded its symptom.

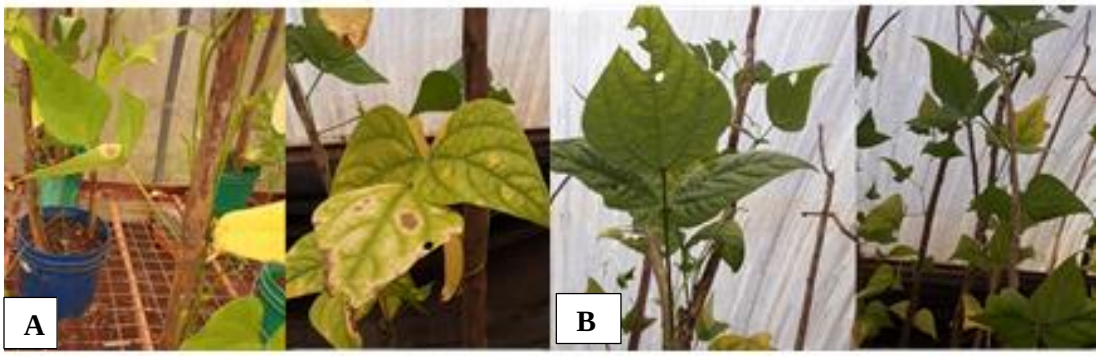

Figure 2: Showing typical leaf symptoms of the diseases after inoculation; A= CBB symptoms; B=Mosaic symptoms (BCMV)

Data collection and analysis

Data were collected on the disease severity for CBB on each genotype and for BCM/NV, counted number of plants with virus symptoms, were then subjected to the GENSTAT-16th edition (VSN INTERNATIONAL, 2013) to generate variance, standard errors and the means of disease severity on leaves were separated using Tukey's Test at probability level of 5 percent. Microsoft excel was used to construct graphs of the disease reaction.

Results

Reaction of common bean genotypes to CBB (Xap) disease

Results showed significant differences ($p \leq 0.001$) among 5 genotypes tested (Table 2). At 14 DAI all genotypes were observed to be resistant to Xap with average visual score ranging from 1.00 to 3.33 which were considered as resistance in this study (Table 2). Leaf severity scored at 21 DAI showed a significant reaction among the tested genotypes in which KT020, 13A/59-98-3X3-3A and AO 29-3-3A had visual scores of 1.00, 1.33 and 1.33 respectively (resistant). BR 59-63-10 had a score of 4.33 and Kablanketi scored 4.67 (intermediate). There were significance differences for observed reaction of the genotypes at 35 DAI to *Xap* in which KT020, 13A/59-98-3x3-3A and AO29-3-3A had visual scores of 1.33, 1.33 and 1.67, respectively and were categorized as resistant to *Xap* reaction while BR 59-63-10 was observed to have lesions on the leaves having a visual score of 4.67 (intermediate resistance) and Kablanketi had typical and large lesion on leaves with visual score of 8.00 which categorized as susceptible (Figure 1 and Table 2). Results show that there was development of the CBB symptoms over time as shown in Table 2, in which KT020 and 13A/59-98-3X3-3A did not develop any disease symptoms, while AO 29-3-3A had a few leaves with water-soaked symptoms. Kablanketi shown tremendous development of disease on leaves per time as well as BR 59-63-10.

Table 2

Leaves severity visual score rating of the tested common bean genotypes to CBB inoculum (Xap) at specified time interval

Genotype	**Leaf severity score**		
	14 DAI	**21 DAI**	**35 DAI**
BR 59-63-10	3.00 b	4.33 b	4.67 b
13A/59-98-3X3-3A	1.00 a	1.33 a	1.33 a
KT020	1.00 a	1.00 a	1.33 a
AO 29-3-3A	1.00 a	1.33 a	1.67 a
Kablanketi	3.33 b	4.67 b	8.00 c
Grand mean	**1.87**	**2.6**	**3.4**
s.e.d	**0.2981**	**0.422**	**0.558**
CV%	**17.7**	**19.9**	**20.1**

F pro.	**<.001**	**<.001**	**<.001**

*Values with same letter in the same column are not significant different (Tukey's Test, p≤0.05); DAI=Days after inoculation, CV%=coefficient of variation, s. e. d=Standard error of difference of means, F pro.=F probability at p≤0.05.

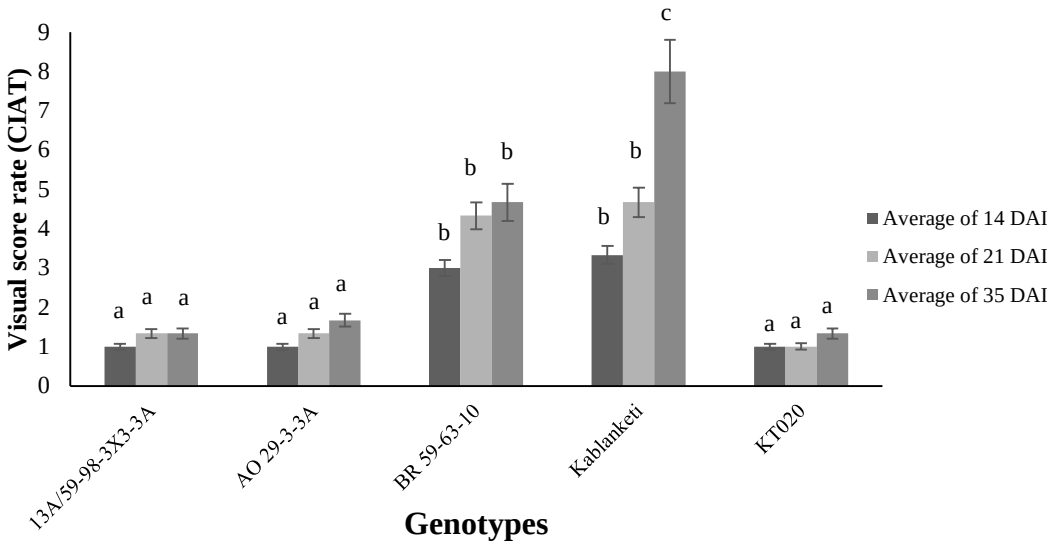

Figure 2. Average of common bean leaves severity visual score of the tested genotypes to Xap inoculum at different time intervals

Plant reaction to BCMV

There was significant difference (p≤0.05) on plant reaction to BCMV inocula whereby Kablanketi (control), observed to have an average of two plants affected and showing the typical mosaic symptoms of the BCMV while 13A/59-98-3X3-3A observed to have some mosaic symptoms with no development. In this study, A0 29-3-3A and KT020 observed with no any plant having the disease symptoms while BR 59-63-10 genotype only one plants observed to have mosaic symptoms on the leave (Table .3 and Figure .2).

Table 3

Numbers of common bean plants with typical mosaic symptoms discarded from trial after inoculated with BCMV inoculum

Genotypes tested	**Number of plant infected**
AO 29-3-3A	0.00 a
BR 59-63-10	1.00 b
13A/59-93-9X3A	0.00 a
KT020	0.00 a
Kablanketi	1.67 b
Grand mean	**0.533**
s. e. d	**0.211**
CV%	**48.4**
F prob.	**<.001**

*No significant difference to the values with same letter in the same column according to Tukey's Test at p≤0.05; s.e.d=standard error of difference of means, CV%=coefficient of variance, F prob.=F probability at p≤0.05.

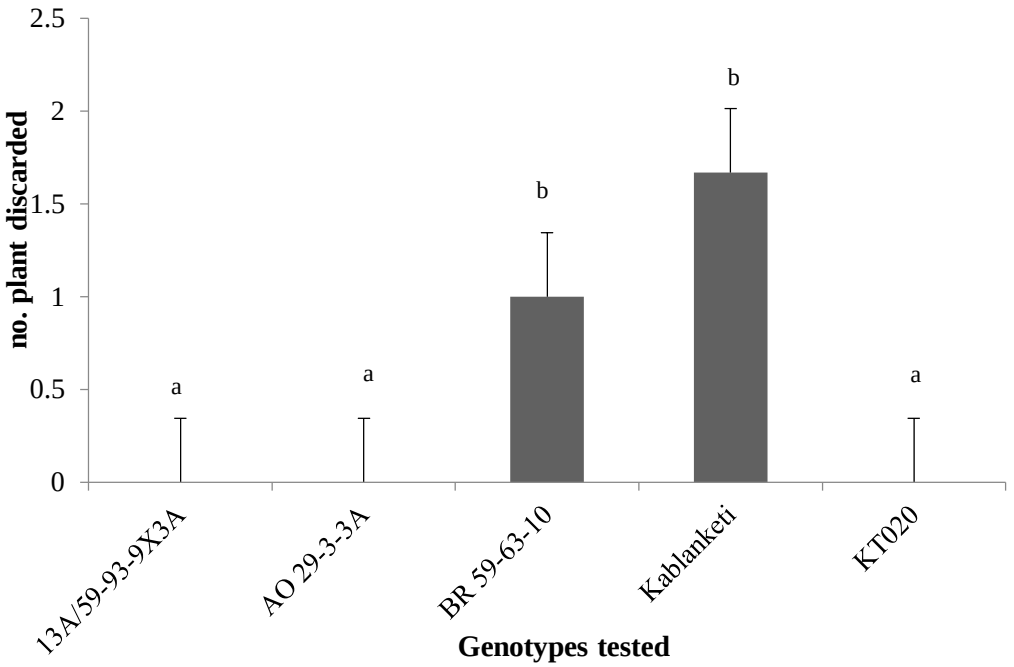

Figure 3. Number of common bean plants observed having typical mosaic symptoms after being inoculated with BCMV inoculum

Response of the genotype to both Diseases

Result showed differences among the genotypes tested to both diseases i.e CBB and BCMV (Table 4). BR 59-63-10 has intermediate resistance to CBB (4.67 visual score) and one plant showed mosaic symptoms of BCMV reaction. KT020 results on CBB severity was resistant with 1.33 visual score to *Xap* but no plant among those tested showed symptoms of the BCMV (Table 4). AO 29-3-3A showed resistance to both diseases tested (had visual score of 1.67 for CBB and no plant have mosaic symptoms for BCMV) as shown in Table 2.4 while Kablanketi showed susceptibility to both diseases in which both necrotic and typical symptoms of CBB were observed (had high visual score of 8.0); as well an average of two plants had typical BCMV mosaic symptoms (Table 4).

Table 4

Response of the common bean genotypes tested to Common Bacterial blight severity and number of plants showing symptoms of BCMV

Bean genotypes	CBB severity	BCMV reaction
BR 59-63-10	4.67 b	1.00 a
13A/59-98-3X3-3A	1.33 a	0.00 a
KT020	1.33 a	0.00 b
AO 29-3-3A	1.67 a	0.00 a

Kablanketi	8.0 c	1.67 c
Grand mean	**3.4**	**0.53**
CV%	**20.1**	**0.211**
s.e.d	**0.558**	**48.4**
F pro.	**<.001**	**<.001**

*No significant difference to the values with same letter in the same column according to Tukey's Test at p≤0.05; s.e.d=standard error of difference of means, CV%=coefficient of variance, F prob.=F probability at p≤0.05

Discussion

Phenotypic screening of the germplasm used for disease resistance incorporation is important (Alladasi *et al.,* 2018). The results in this study have revealed that, there were significant differences on visual score to *Xap* reaction observed on leaves, implying that all genotypes have different levels of resistance to *Xap*. These results agree with those obtained by, Tryphone *et al.,* (2012), Alladasi *et al.,* (2018) and Beaver *et al.* (2018) as well as in the similar study showed continues development of the disease symptoms as observed in this study particular in *Xap* reactions.

Results obtained from this study, observed three range of disease score severity on leaf reaction which suggested three categories of resistance with score of 1 to 3, intermediate with score of 4 to 6 and susceptible with score of 7 to 9 as obtained in this study. Alladasi *et al.,* (2018) and Kabeja, (2020) reported similar results which further confirmed the high genetic diversity of the common bean genotypes tested to CBB.

Based on results obtained on 35 DAI, KT020 and 13A/59-98-3X3-3A observed to have low scores indicating presence of resistance gene for CBB. Also, AO 29-3-3A showed some resistance to *Xap* reactions while BR 59-63-10 was observed to have intermediate resistance to CBB. Kablanketi observed to be susceptible to the disease which was an indication of lack of resistance gene to CBB. Kablanketi cultivar was also reported by, Tryphone *et al.* (2012), being susceptible to CBB and BCMV/BCMNV while Chilagane *et al.,* (2013) reported Kablanketi cultivar to be susceptible to ALS and BCMNV.

Results on plant reactions to BCMV showed BR 59-63-10 to possess resistance gene to the virus. AO 29-3-3A line showed resistance to BCMV pathogen used similar to results found by Kusolwa *et al.* (2016) who reported the same line having resistance to both bean bruchids and BCMV/BCMNV.

In this study, KT020 observed to have low infection reaction to both diseases followed up by 13A/59-98-3X3-3A which had few mosaic symptoms of BCMV and low scale to CBB reaction (ranged 1.0 to 1.3) considered as resistance to CBB. The genotypes showed positive response to both diseases. Tugume *et al.,* (2019), reported that gene-to-gene interaction is not involved in resistance to CBB, and our study was in agreement. There was a slight increase in CBB symptoms on BR 59-63-10 which can be considered as a negative response to CBB. Tugume *et al.,* (2019), Kiryowa *et al.* (2016) and Tryphone *et al.,* (2012) reported that, infection can be modulated by environment factors and amount or concentration of the inoculum which suggests that the BR 59-63-10 genotype might respond more negatively if the amount of *Xap* were in greater abundance. Kablanketi genotype was susceptible to both diseases in this trial.

Conclusion

Among five genotypes tested in this study, three genotypes had resistance to CBB and BCMV (AO 29-3-3A, KT020, and 13A/59-98-3X3-3A), and one was resistant to BCMV but had intermediate resistance to CBB (BR 59-63-10). This foliar disease screening trial helped to select a genotype that can be used to improve common bean without changing the market class trait especially the background color of the seed.

Based on results obtained from this study it is recommended that the germplasm tested should be screened again under greenhouse conditions.

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