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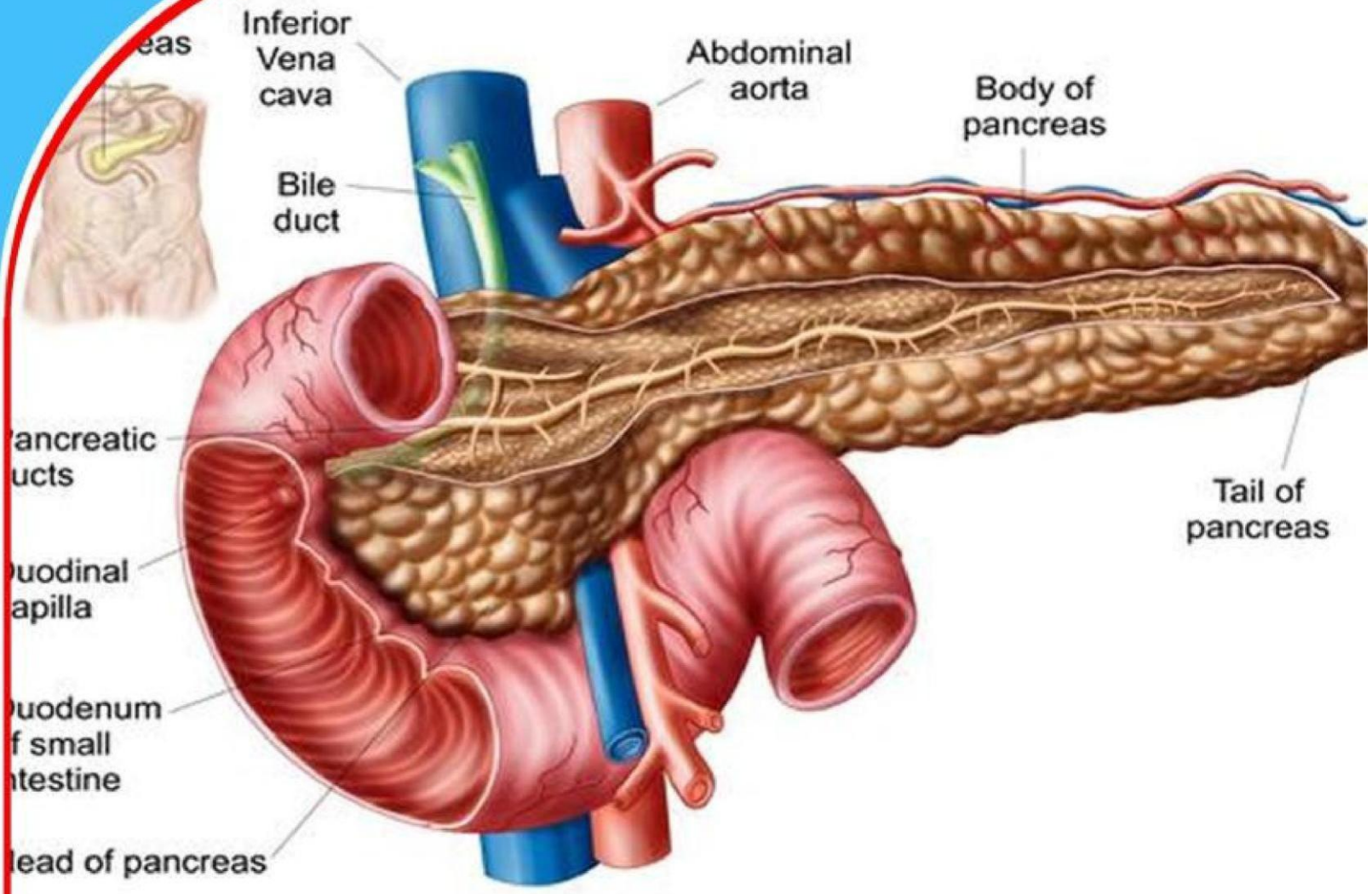

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SCREENING OF COMMON BEAN GENOTYPES FOR RESISTANCE AGAINST *Xanthomonas campestris pv. phaseoli* IN KAKAMEGA COUNTY, KENYA

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Abstract

Purpose: To screen nine available bean genotypes for resistance to common bacterial blight disease under green house and field conditions.

Methodology: Experiments were conducted in randomized complete block design with three replications in a 9×2×2 factorial factor of 9 bean genotypes, grown in sterile or non-sterile soil and inoculated or non-inoculated (control) during the greenhouse and a 9×2 field screening of 9 bean genotypes, and inoculated or non-inoculated. During growth, data on plant height number of pods per plant, length of pods and size and number of CBB spots was taken. Yield parameters were also assessed.

Findings: The findings from the experiment revealed a significant variation ($P<0.05$) on the entire traits studied among the nine bean genotypes. Data from the field and greenhouse experiments were in conformity. None of the evaluated genotype was immune to CBB. In the green house, it was observed that disease symptoms were severe in beans planted in non-sterile soil and inoculated with *Xap* compared to those planted in sterile soil and non-inoculated respectively.

Contribution to practice and policy: There was a strong positive correlation between size and number of CBB spots and growth and yield parameters. CAL77 and Cal 156A genotypes exhibited high levels of resistance to CBB which could improve the bean yields resulting to sufficient food supply, improved nutrition, health and improvement of the source of income to the local communities.

Key words: *Common Bean, Pathogenicity, Resistance, Xanthomonas Axonopodis, Yield Loss*

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is rich in nutritional content and economically potent food crop which is grown globally (Leitich *et al*., 2016 Tugce *et al*., 2018). Common bean represents 65% and 32% of total protein consumed and energy respectively and therefore, it is a vital nutrient resource to approximately 500 million people in parts of Africa and Latin America, (Blair *et al*., 2010; Tugce *et al*., 2018). Bean production in Africa has been declining despite the fact that they are the most affordable plant protein source to the many rural poor. This is due to both biotic and abiotic factors, poor agricultural practices and lack of access to certified and improved seed varieties (Katungi *et al*., 2009; Kajumula and Muhamba 2012). In Kenya, declining bean production is associated with soil infertility, pests and diseases.

Yields of beans on farmer's fields are lower (400 kg/ha) than the yields attained at research centers (1500 and 2500 kg/ha) due to a gradual fall in soil fertility and rising levels of pests and diseases (Otsyula and Wambulwa, 2010). Diseases are estimated to be the second largest constraint after low soil fertility (Leitich *et al*., 2016). Common bacterial blight disease (CBB) caused by *X. axonopodis pv. phaseoli* is the main threat to common bean production. The disease is destructive during high rainfall, humidity and temperature (25-35°C) with maximum development occurring at 28°C and results in quality and yield losses (Akhavan *et al*., 2013). The CBB pathogen is mainly disseminated through infected seed (Fourie *et al*., 2011). Using resistant beans varieties is one of the most resourceful, cost effective and ecologically compatible strategy in the control and management of diseases (Otsyula, 2010). This study evaluated the reactions of nine selected bean genotypes to Common Bacterial blight.

2.0 LITERATURE REVIEW

2.1 Bean production practices in Western Kenya

Beans are grown in Western Kenya mainly for food and as an income generating activity (Mhango *et al*., 2013). Most of the farmers plant beans twice annually within the long and short rains periods with majority of them using their own kept seeds from the previous seasons during planting (Buruchara, 2011)

2.2 Economic importance of *Xanthomonas axonopodis pv. Phaseoli*

The main threat to common bean (*Phaseolus vulgaris* L.) production all over the world is common bacterial blight disease (CBB) caused by *X. axonopodis pv. phaseoli* (Xap) (Belete and Bastas, 2017). The disease is destructive during high rainfall, humidity and temperature (25-35°C). High quality and yield losses are experienced when the temperature is at its optimum (28°C) (Akhavan *et al*., 2013). The pathogen is therefore dissemination through contaminated seeds (Belete and Bastas 2017) and the disease is spread through infected germplasm (MOARD 2014)). Most economically disadvantaged farmers store and reuse the bean seed during planting (Otsyula 2016). This coupled with poor storage facilities cause a threat to bean farming and bean production (He, 2010).

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2.3 Disease symptoms of *Xanthomonas axonopodis pv. Phaseoli*

The symptoms on the foliar parts are small, light green, angular, water-soaked, or translucent spots (Belachew *et al*., 2015). Infected pods have a central cream or yellow colored bacterial colony seen as water-soaked lesions that drip as yellow lots of bacterial, which with time, become shallow and dark reddish-brown blotches (Chen *et al*., 2012). Infected seed may rot or shrivel if contamination occurs during the period of pod and seed development (Buruchara *et al*., 2011).

3.0 MATERIALS AND METHODS

3.1 Study materials

Bean seeds used were advanced lines that have been developed by bean breeding program at KALRO-Kakamega for different disease constraints and are in the national performance trials pending release to the farmers. Characteristics of genotypes of beans used during this study are described in Table 3.1

Table 3.1. Characteristics of bean genotypes used in greenhouse evaluations at KARLO-Kakamega in 2013

Bean genotype	Colour	Size	Reaction to *Pythium* root rot	Reaction to BCMV	Reaction to BCMNV
CAL 181	White Calima	Medium	RR	Unknown	RR
CAL271A	White Calima	Medium	RR	RR	Unknown
CAL 274	Red Calima	Small	RR	RR	Unknown
KK 8 CAL 285	Red Calima	Medium	RR	RR	RR
CAL 87	Red Calima	Medium	RR	Unknown	Unknown
CAL156A	Red Calima	Small	RR	Unknown	Unknown
CAL 256	Red Calima	Medium	RR	Unknown	RR
CAL 77	Red Calima	Medium	RR	Unknown	RR
	Red Calima	Small	MR	Unknown	Unknown

RR = Resistant reaction, MR = Moderately resistant reaction, SR = Susceptible reaction, Large =
45-50 gm/100 seeds, Medium = 35- 40 gm/100seeds, Small = 15-25 gm/100 seeds according to Otsyula (2010). BCMNV (bean common mosaic necrotic virus) BCMV (bean common mosaic virus) CAL (Calima) KK (Kakamega)

3.2 Leaf Sample Collection and Bacteria Isolations

The strains of *Xap* bacteria were isolated from the leaves of common beans that were collected during surveys in the KALRO-Kakamega and from farmers around the research station. Leaves with characteristic CBB symptoms were collected and dried between paper towels. For each leaf sample, tissues (16 mm^2) were cut along the lesion border, placed in a drop of distilled water on a microscope slide and macerated. A 10-fold serial and sequential dilution (to 10_{-5})was used. 0.1

ml of each undiluted and diluted extracts were streaked on three plates of semi-selective media. 72 hours plates incubated at 25 °C were observed. All the bacterial colonies showing typical *Xanthomonas* characreristics including yellow pigmentation, convex margins with mucoid colonies were examined and counted. Two tests which included the Gram reaction and pathogenicity tests were carried out on suspected bacterial colonies.

3.3 Preparation of Growth Medium

Top soil from Kakamega forest, chicken manure and sand used to fill the pots were mixed in the ratio of 3:2:1 by volume respectively so as to ensure good drainage and ample plant food. To eradicate harmful organisms, weed seeds, and pathogens,part of the mixture was steam sterilized at 121°C for 3 hours and allowed to cool before being packed in half of the pots. The same mixture but of non-sterilized soils was used in control pots

3.4 Planting and Inoculation

In the greenhouse trial experiments, two surface sterilized bean seeds were planted in each pot at a depth of 5 cm and a spacing of 5 cm between the plants. Pots were arranged on a greenhouse bench in a randomized complete block design. A 10^8 CFU ml^1 suspension of the bacteria was sprayed onto the aerial parts of the fully expanded trifoliate leaves of the plant (fifteen days old plants), using a high-pressure hand sprayer. After inoculation, transparent nylon paper was used to cover the plants for three days to create a microclimate necessary for infection to take place and prevent drifting effect (Dursun *et al*., 2002). The control plants were sprayed with sterile water.

Field experiments comprised a complete randomized block design of 9×2 factorial design of nine bean genotypes (9 factors), with or without the pathogen. The treatments applied were; inoculation with 10^8CFU ml^1suspension of the bacteria and control (distilled water) by spraying onto the aerial parts of plants using a high-pressure hand sprayer.

3.5 Data collection and disease score

Disease incidence and severity in the form of CBB spots on the leaves and pods was scored ((Hira *et al*., 2016). In addition, growth parameters of the genotypes like the mean height at flowering was assessed (Nkalubo, 2007; Mwesigwa, 2009). Yield parameters including mean number and weight of pods per plant and mean seed weight per plant were also assessed (Nkalubo, 2007; Mwesigwa, 2009).

3.6 Data Collection and Disease Score

Plants were scored from 20–25 days after inoculation using the CIAT 1-9 scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2011). Resistance (R) was assigned to plants with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible (S).

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3.7 Data analysis

Data was analyzed using SAS portable software for scientific data analysis. For each of the greenhouse and field experiments, disease severity index was statistically analyzed using analysis of variance (ANOVA; P=0.05).

4.0 RESULTS

4.1 Pathogen characterization

The bacterial isolates recovered from leaf samples were categorized as *Xanthomonas* like, based on their yellow pigment and convex mucoid morphology (Plate 4.1A). Biochemical test results also confirmed that the colonies were rod shaped and gram negative. All the bacteria isolated and recovered from the extract of leaves made the plants to develop symptoms ten days after inoculation during the pathogenicity test on bean plant (Plate 4.1 B).

A B Plate 4.1: (A) Yellow, mucoid colonies of *Xap* (B) Necrotic spots and yellowing of bean leaves caused by common bacterial blight disease

4.2 Evaluation of selected bean genotypes for resistance to common bacterial blight in the green house experiment

The findings from the greenhouse experiment revealed a significant variation ($P<0.05$) on the entire traits studied among the nine bean genotypes. There was a significant variation between *Xap* inoculated and non-inoculated plants in disease severity rating (CBB score) indicating that all the bean genotypes were infected by CBB. The bean genotypes inoculated with *Xap* recorded a significantly ($P<0.05$) higher CBB score compared with the bean genotypes that were not inoculated but grown in non sterile soil (Table 4.1.)

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Table 4.1. Effect of the *Xap* inoculum on CBB severity and plant growth parameters on nine bean genotypes in the greenhouse

	**CBB score	Plant field weight (g)	Plant length (cm)	Yield/ Plot (g)	No. Seed / pod	No. Pod / plant	Pod length (cm)
Genotype*							
CAL 156A	3.50±0.22ab	6.35±0.64ab	5.61±0.04f	4.62±0.53b	3.00±0.13bcd	2.00±0.18c	5.35±0.36b
CAL 181	2.50±0.22c	6.76 ±1.19ab	8.73±0.25a	4.01±0.58c	4.28±0.52a	3.00±0.18ab	8.00 ±0.76a
CAL 256	3.41±0.20ab	5.72±0.84ab	6.66±0.2d	3.97± 0.39c	3.36 ±0.38b	2.50±0.28abc	7.63±0.49ab
CAL 271A	3.33±0.17ab	6.35±0.51ab	6.06±0.24e	5.22 ±0.49a	3.67±0.17ab	2.00 ±0.28c	5.01±0.47b
CAL 274	3.50±0.22ab	6.36±1.02ab	4.10±0.23g	4.12±0.46c	2.50 ±0.32cd	3.25±0.38a	7.56±0.72ab
CAL 285	3.17±0.17b	5.17 ±0.92b	8.90±0.32a	4.18±0.46c	2.42±0.2d	2.00±0.28c	7.46±0.56ab
CAL 77	3.75 ±0.35a	6.81 ±1.22a	5.40±0.09f	4.14±0.47c	3.37±0.22b	2.00 ±0.28c	5.16 ±0.45b
CAL 87	3.75±0.35a	6.54±0.86ab	8.42±0.38b	3.88±0.60c	3.25±0.38bc	2.50±0.28abc	7.88±0.82ab
KK8	3.25±0.17ab	5.91±0.82ab	7.12±0.23c	4.03±0.49c	3.45±0.36b	2.25±0.38bc	7.86±0.48ab
Treatment							
Xap Inoculated	3.70±0.15	4.39±0.09	6.76±0.32	3.13±0.09	3.00±0.14	2.33±0.17	6.76±0.34
Non-inoculated	3.00±0.0	8.06±0.28	6.80±0.33	5.35±0.09	3.51±0.19	2.44±0.14	7.01±0.36
P values							
Genotype	<.0001	0.0429	<.0001	<.0001	<.0001	0.0002	0.0012
Treatment	<.0001	<.0001	0.3180	<.0001	<.0001	0.4196	0.5454
Genotype × treatment	<.0001	0.0030	<.0001	0.0345	<.0001	<.0001	0.8875

*Values (Mean ± SE) followed by different letters in the same column are significantly different based on Tukey's HSD at 95 % probability level.

** Transformed CBB scored on a scale of 1-9 based the CIAT scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2010). Resistance (R) was assigned to plants with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible (S).

Here was a significant interaction (P<0.05) in CBB severity between the inoculated and non-inoculated bean. Except for Cal 285 and Cal 156A, the CBB score was significantly higher on *Xap* inoculated bean genotypes grown on non-sterile soils (CIN) compared with the *Xap* inoculated and non-inoculated genotypes grown on both sterile and non-sterile soils (Figure 3.1). All the bean genotypes were tolerant to *Xap* infection except Cal 181 that was slightly resistant based on the CBB score and resistance rating score on the CIAT scale

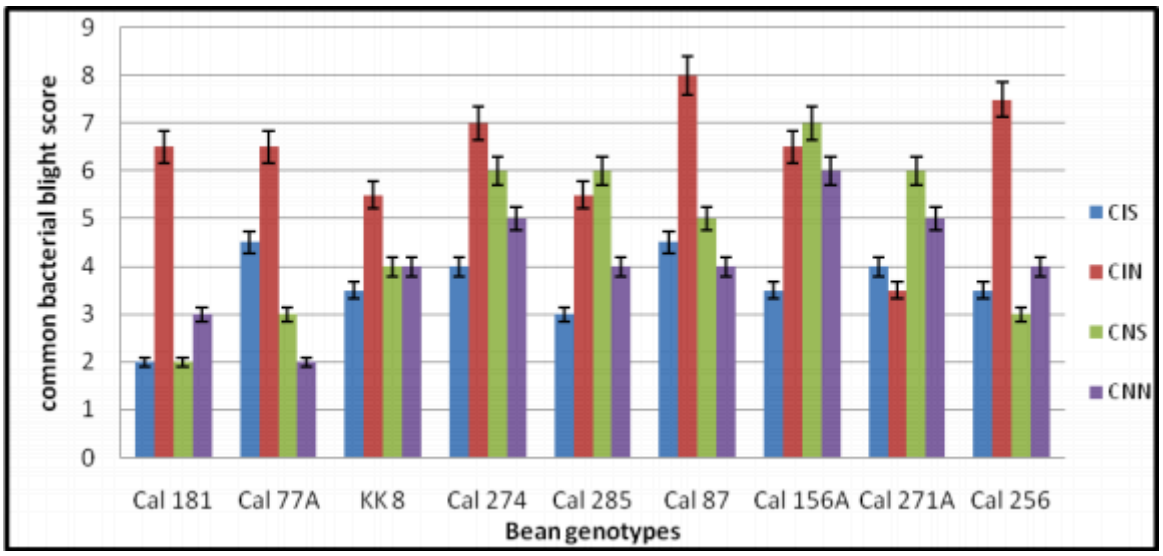

Figure 4.1: CBB score in the greenhouse for the nine bean genotypes in different soil treatments.

Cal (Calima), KK (Kakamega), CIS-CBB score in *Xap* inoculated plants in sterile soil, CINCBB score in inoculated plants in non-sterile soil, CNS-CBB score in non- inoculated plants in sterile soil, CNN-CBB score in non-inoculated plants in non-sterile soil.

CBB was scored on a scale of 1-9 based the CIAT scale (Buruchara *et al*., 2011).

Although the plant length of *Xap* inoculated and non-inoculated beans did not differ significantly among the treatments, there was a significant difference (P<0.05) established among the genotypes with a significant interaction between the treatments and genotypes being established. The yields/plot in the greenhouse differed significantly (P<0.05) among the genotypes and the treatments with *Xap* inoculated genotypes recording significantly (P<0.05) lower yields compared to the non-inoculated bean genotypes (Table 4.1).

Inoculated genotypes recorded significantly fewer seeds/pod compared to non-inoculated bean genotypes. In addition, the pods/plant and pod length were lower in the inoculated genotypes

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although there was no significant difference (P<0.05) established (Table 4.1). **4.3 Field experiment on screening of bean genotypes to Common bacterial blight**

Field experiment (Table 4.2) revealed that the mean CBB disease severity was lower in bean plants that were not inoculated compared to those that were inoculated. There was a significant (P<0.05) variation between inoculated and non-inoculated plants in disease severity rating. The bean genotypes that were inoculated with *Xap* bacteria recorded a significantly (P<0.05) higher CBB score compared with the bean genotypes that were not inoculated. The genotype response to *Xap* infection in the field conditions varied from slightly resistant to tolerant with all the genotypes being tolerant except Cal 156A, Cal 256, Cal 271A and Cal 274 that were slightly resistant based on the CIAT score index (Table 4.2)

Table 4.2. CBB severity and yield components of bean genotypes in the field long rains 2014 KALRO-Kakamega

	CBB score#	Plant field weight Yield/ Plot (g) (g)	Plant height (cm)	No.Seed / pod	No.Pod / plant	Pod length (cm)
Genotype*						
CAL 156A	4.50±0.72 a*	85.21±50.02c	26.32±6.66c	20.50±1.52b	3.33±0.42a	6.33±1.40a	8.83±0.40c
CAL 181	2.83±0.16bcd	169.51±27.63abc	60.91±13.39bc	29.50±2.55ab	3.67 ±0.42a	11.00±2.26a	8.83±0.70c
CAL 256	4.00 ±0.44ab	83.90±29.87c	32.47±10.04bc	24.50±1.92ab	3.83± 0.70a	6.00±1.41a	9.50±0.84bc
CAL 271A	3.66±0.61ab	158.56±25.89abc	64.40±10.50bc	31.00±3.60ab	4.50± 0.56a	8.50±0.99a	11.16±0.60a
CAL 274	3.50±0.56abc	129.86±35.14bc	48.35±17.66bc	35.83±4.14a	3.33±0.55a	9.66±3.09a	9.83±0.70bc
CAL 285	2.33±0.21cd	172.63±40.86abc	58.80±18.44bc	37.33±4.13a	3.50±0.34a	8.83±1.24a	10.33±0.33ab
CAL 77	2.16±0.16d	266.95±35.35ab	102.63±10.17ab	32.50±2.27ab	4.17±0.30a	9.33±1.70a	11.16±0.79a
CAL 87	2.16±0.16d	316.81±65.34a	167.25±28.72a	29.83±2.12ab	4.00±0.57a	9.50±1.40a	11.16±0.70a
KK8	2.33±0.21cd	119.88±30.50bc	49.01±16.51bc	25.83±1.72ab	4.00±0.25a	9.16±1.90a	9.00±0.25bc
Treatment						
Non inoculated	2.52±0.11 3.59 ±0.29	205.49±23.58 128.59±19.00	80.46±11.30 55.13±9.24	32.15±1.59 27.15±1.43	4.44± 0.17 3.19 ±0.19	10.82 ±0.80 6.59±0.68	10.74 ±0.30 9.22±0.29
Inoculated **P values**						

	<.0001	< 0.0015	<.0001	0.0038	0.5001	0.5326	0.0077
Genotype	<.0001	< 0.0053	0.0259	0.0125	<.0001	0.0006	0.0003
Treatment	0.0026	0.09747	0.9927	0.9876	0.7246	0.9783	0.9403
Genotype* Treatment							

*Values (Mean ± SE) followed by different letters along the column are significantly different according to Tukey's

HSD at 95 % probability level.
**Cal (Calima), KK (Kakamega)

#CBB was scored on a scale of 1-9 based the CIAT scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2011). Resistance (R) was assigned to plants with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible(s).

Inoculated bean genotypes recorded significantly ($P<0.05$) lower plant weight compared to non-inoculated (Table 4.2). In addition, the interaction between the treatments and genotypes varied significantly ($P<0.05$). The bean yields/plot in the field differed significantly among the genotypes and the treatments with inoculated genotypes recording significantly ($P<0.05$) lower yields compared to the non-inoculated bean genotypes (Table 4.2). Genotype Cal 87 had significantly higher yield compared to Cal 156A which recorded significantly lower yield. Although the plant length of inoculated and non-inoculated beans did not differ significantly, the plant length varied significantly ($P<0.05$) among the genotypes with a significant interaction between the treatments and genotypes being established (Table4.2).

There was no significant difference ($P>0.05$) among the genotypes in the number of seeds/pod and the pods/plant (Table 3.2). Inoculated genotypes recorded significantly fewer seeds/pod compared to non-inoculated bean genotypes. In addition, the pods/plant and pod length were lower in the inoculated genotypes although there was no significant difference established (Table 4.2).

5.0 DISCUSSION

Most bean genotypes exhibited CBB disease symptoms following inoculation with *Xap* both in the green house and in the field. The CBB severity and incidence varied significantly between sterilized and unsterilized soil indicating that there was CBB inoculum in the forest soil that was used in the experiment. All the nine bean genotypes grown in non-sterile soil recorded a higher CBB score. The high CBB score could be attributed to higher *Xap* population in the soil. Based on the CIAT scale, the genotype response to *Xap* infection in the field conditions varied from slightly resistant to tolerant with all the genotypes being tolerant except Cal 156A, Cal 256, Cal 271A and Cal 274 that were slightly resistant as indicated in Table 4.2. Genotypes Cal 87, Cal 181 and Cal 274 exhibited reduction in height when inoculated with *Xap* in the field. Therefore,

the five bean genotypes (Cal 271A, Cal 256, Cal 87, Cal 274, and Cal 181) may be considered susceptible to CBB disease based on reduction in plant height.

Five genotypes; KK8, Cal 256, Cal 87, Cal 181 and Cal 274 may be considered susceptible to the pathogen based on pod counts, pod weights and mean seed weight per plant. By contrast, genotypes Cal77, Cal 271A, Cal 285 and Cal 156A did not exhibit reduction in pod counts or pod weights when inoculated with *Xap*, and could be considered more tolerant based on the three yield-related parameters. Five genotypes; KK8, Cal 256, Cal 87, Cal 181 and Cal 274 may be considered susceptible to the pathogen based on pod counts, pod weights and mean seed weight per plant. By contrast, genotypes Cal77, Cal 271A, Cal 285 and Cal 156A did not exhibit reduction in pod counts or pod weights when inoculated with *Xap*, and could be considered more tolerant based on the three yield-related parameters. This is in agreement with a previous study by Belachew *et al*. (2015)

With the CIAT disease severity scale, genotypes CAL 77 and Cal 156A are the only ones among the nine genotypes that were more tolerant to CBB. Indications of their CBB resistance were still evident in most of the other plant growth and yield parameters. However, the reduction in pod length is a clue that genotype Cal 77 may be less tolerant compared to CAL 156A. Throughout the study, the beans that were inoculated with *Xap* showed the CBB symptoms on the leaves and the pods. The disease severity was more in the genotype Cal 271A and Cal 256 compared to the non-inoculated bean plants. The two bean genotypes can be seen to be susceptible to CBB. This resulted in the reduction on the other plant growth parameters including the height of the plant, length of the pods, number of pods per plant and the overall yield. The degree of yield decline caused by CBB in the present study agrees with the results obtained by Binagwa *et al*. (2018) who established that yield losses in beans caused by CBB were a product of both low seed weight and fewer seeds per plant.

6.0 CONCLUSION AND RECOMMENDATIONS 6.1 CONCLUSION

The experiments confirmed the presence of *Xap* pathogen in diverse leaf sources in the area under study with the leaves obtained from research trial site having lower levels of pathogen infection compared to leaf from farmer's field. Results on CBB disease severity and incidence indicate that CAL 77 and Cal 156A genotypes exhibited high level of tolerance to CBB. Seven genotypes namely Cal 285, Cal 256, CAL271A, Cal274, KK 8, CAL 181 and Cal 87 exhibited moderate level of tolerance reaction based on the CIAT disease severity scale. CBB disease caused both quantitative and qualitative yield losses depending on bean genotype and its resistance.

6.2 RECOMMENDATIONS

The findings recommend further evaluation is done using other isolates of *Xap* that are known to commonly occur in Western Kenya. Also, there is a need to establish the actual factors that confer high levels of tolerance to CBB in genotype CAL 77A and Cal 156A and the other seven susceptible genotypes may be tried in different locations that are less prone to *Xap.*

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