



Resistance of popular grown Kenyan Cassava cultivars to Cassava bacterial blight

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ABSTRACT

Cassava bacterial blight threatens cassava cultivation as majority of the cultivars currently grown are susceptible to the disease. The disease is associated with two causal agents *Xanthomonas phaseoli* pv. *manihotis* and *Xanthomonas axonopodis* pv. *cassavae*. Resistance has been proposed as the best management option against the disease where used in an integrated disease management system. Therefore, this study was done to identify cassava bacterial blight resistant cultivars among the popularly grown cassava varieties in Kenya. A greenhouse experiment was conducted twice to evaluate seven cassava varieties for cassava bacterial blight resistance in a factorial treatment structure within a randomized complete block design. Each variety was inoculated on the stem and leaf with 1×10^6 CFU/ml of the two causal agents either individually or in combination. A control inoculated with sterile distilled water was also included. Disease development was recorded at an interval of six days' post inoculation using a severity scale of 1-5 for six weeks. We observed that *Xanthomonas phaseoli* pv. *manihotis* was more severe on the varieties as it had the highest severity scores and area under disease progress curve values of more than 70% compared to those infected with *Xanthomonas axonopodis* pv. *cassavae* and a combination of both pathogens. The most affected varieties were mm 96/2480, Naro 56, and mm 96/1871. However, none of the varieties evaluated was resistant to the disease making it necessary to test more varieties for resistance.

Keywords: Cassava cultivars, Host resistance, Kenya, *Manihot esculenta*, *Xanthomonas axonopodis* p.v. *cassavae*, *Xanthomonas phaseoli* p.v. *manihotis*

RÉSUMÉ

La bactériose du manioc menace la culture du manioc car la majorité des cultivars actuellement cultivés sont sensibles à la maladie. La maladie est associée à deux agents causaux : *Xanthomonas phaseoli* pv. *manihotis* et *Xanthomonas axonopodis* pv. *cassavae*. La résistance a été proposée comme la meilleure option de gestion contre la maladie lorsqu'elle est utilisée dans un système de gestion intégrée des maladies. Par conséquent, cette étude a été réalisée pour identifier les cultivars de manioc résistants à la bactériose parmi les variétés de manioc les plus couramment cultivées au Kenya. Une expérience en serre a été menée deux fois pour évaluer la résistance de sept variétés de manioc à la bactériose dans une structure de traitement factoriel au sein d'un dispositif en blocs complets randomisés. Chaque variété a été inoculée sur la tige et les feuilles avec 1×10^6 UFC/ml des deux agents causaux, soit individuellement, soit en combinaison. Un contrôle inoculé avec de l'eau distillée stérile a également été inclus. Le développement de la maladie a été enregistré à intervalles de six jours après l'inoculation.

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en utilisant une échelle de gravité de 1 à 5 pendant six semaines. Nous avons observé que *Xanthomonas phaseoli* pv. *manihotis* était plus sévère sur les variétés, avec les scores de gravité et les valeurs de la courbe de progression de la maladie les plus élevés, supérieurs à 70%, par rapport à celles infectées par *Xanthomonas axonopodis* pv. *cassavae* et une combinaison des deux pathogènes. Les variétés les plus affectées étaient mm 96/2480, Naro 56, et mm 96/1871. Cependant, aucune des variétés évaluées n'était résistante à la maladie, ce qui rend nécessaire de tester davantage de variétés pour la résistance.

Mots clés : Cultivars de manioc, résistance de l'hôte, Kenya, *Manihot esculenta*, *Xanthomonas axonopodis* pv. *cassavae*, *Xanthomonas phaseoli* pv. *manihotis*

Introduction

Cassava is central to the socioeconomic life of many people residing in rural Africa. This is because the crop can flourish in difficult environmental conditions much as in low nutrient soils (Sangbamrung *et al.*, 2020; Devi *et al.*, 2022). However, cassava bacterial blight continues to cause havoc in cassava production imposing losses of over 70% and in some instance completely destroying susceptible varieties (Elliott *et al.*, 2022). The disease is often linked to two gram negative bacteria *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae* (Livoi *et al.*, 2021; Zárate-Chaves *et al.*, 2021). Of the two *Xanthomonas phaseoli* p.v. *manihotis* is the more destructive but *Xanthomonas axonopodis* p.v. *cassavae* has been reported to induce severe disease in certain environmental conditions (Verdier *et al.*, 1993; Pereira *et al.*, 1999; Zárate-Chaves *et al.*, 2021). Control measures have been developed against the disease however farmers have not proactively adopted them resulting in increased cassava bacterial blight prevalence (Lopez and Bernal, 2012; Njenga *et al.*, 2017). Therefore, resistance has been fronted as the most suitable option for farmers for use in an integrated disease management regime. Recent studies by Mbaringong *et al.* (2017) revealed that resistance to the disease is available among cultivars in Kenya as four of the varieties assessed showed some degree of resistance. However, the resistance was insufficient necessitating the identification of other resistant varieties (Bart and Taylor., 2017; Odongo *et al.*, 2019).

Furthermore, majority of the varieties have been assessed against *Xanthomonas phaseoli* p.v. *manihotis* alone yet varieties have been shown to vary in their susceptibility to *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae* (Pereira *et al.*, 2000). Therefore, this study was carried out to assess the reaction of popular Kenyan cassava varieties to bacteria blight when inoculated with *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae*.

Materials and Methods

Isolation and identification of cassava bacterial blight pathogens. The *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae* used in the study were isolated from symptomatic leaves collected from Busia County Western Kenya. The isolation was done by streaking macerate made from symptomatic leaves on YPGA (Yeast 7g, Peptone 7g, Glucose 7g, agar 15g, pH7 in 1000ml) as described by Azorji *et al.* (2016). The plates were incubated for 24 hours at 26°C. Identification was done through colony traits and pathogenicity tests as described by Livoi and Zárate-Chaves *et al.* (2021). The bacteria were then stored in NGY media (0.8g nutrient broth, 15ml glycerol, 0.2g yeast extract, 0.5g glucose in 100ml distilled water) at - 20 °C (Goszczyńska, 2000).

Experimental materials. The following cassava varieties Fumbachai, mm 96/0067, mm 96/1871, mm96/2480, mm96/3567, Naro56, and Serere assessed

in the study were acquired through Seed Enterprise Management Institute at the University of Nairobi. They were initially collected from farmer’s fields, cleaned and indexed for any viral contamination. These varieties were incorporated into the study because there possess farmer preferred attributes such as good cooking qualities, fast maturity and high yielding. They were planted in pots measuring 6 by 9 containing sterilized potting media composed of forest soil, sand and manure in the ratio of 3:2:1, respectively (Livoi *et al.*, 2021).

Experimental design. The experiment was carried out in a greenhouse at Upper Kabete Campus, University of Nairobi in Kenya. The site lies within upper mid-land zone three (UM3), on latitude 1° 15’South and longitude 36° 44’ East at an altitude of about 1800 m above sea level (Jaetzold, 2006). Seven cassava varieties were assessed for cassava bacterial blight resistance in a factorial treatment structure within a randomized complete block design. Each variety was inoculated on the stem and leaf with 1 x 10⁶ CFU/ml of the two causal agents either individually or in combination (Pereira *et al.*, 2000). A control inoculated with sterile distilled water was also included. The data collected included number of plants showing symptoms, the plant height, and the fresh biomass. The experiment was repeated for a second time within the greenhouse. The experiment comprised of the treatments (shown in Table 1).

Inoculum preparation and inoculation. Virulent isolates of *Xanthomonas phaseoli* p.v. *manihotis* xpm and *Xanthomonas axonopodis* p.v. *cassavae* (XAC) were cultured on yeast peptone glucose agar and incubated for 24 hours at 26°C (Mbaringong *et al.*, 2017).

Sterile distilled water was then poured onto the cultures after which sterile glass slides were used to scrap the bacterial colonies into a conical flask to make the stock mixture. Serial dilution was then used to make a concentration of 1 X 10⁶CFU/ml (Pereira *et al.*, 1999; Pereira *et al.*, 2000). The inoculum was then transferred into spraying cans for application. Leaves of two-month-old plants were gently rubbed using carborundum powder after which the inoculum was sprayed. Then needles were used to prick the stems and the inoculum introduced into the stem using syringe. The inoculation was done with each individual pathogen alone, and for other plants with the two combined, non-inoculated plants were used as control (Odongo *et al.*, 2019).

Evaluation of the reaction of cassava varieties to bacterial blight. Reaction of cassava varieties to bacterial blight was assessed by recording incidence and severity data after infection with the causal agents. The incidence was determined as all plant exhibiting CBB symptoms over all plants assessed multiplied by 100% to obtain percentage. Disease severity was assessed using a scale of 1 – 5 (Wydra *et al.*, (2007), where 1 = no symptoms, 2 = angular leaf spotting only, 3 = wilting, angular leaf spotting leaf blight, defoliation, gum exudates on stems or petioles, 4 = wilting, blighting, defoliation, gum exudation

shoot tip die back, 5 = wilting and blighting, defoliation and gum exudation, abortive lateral shoot formation, stunting, complete die back. The severity was evaluated at an interval of six days’ post inoculation for six weeks

Table 1. Treatment combination of cassava varieties and cassava bacterial blight causal agents *Xanthomonas phaseoli* p.v. *manihotis* (XPM) and *Xanthomonas axonopodis* p.v. *cassava* (XAC)

Varieties	Causal agents		Combined inoculation
Fumbachai	XPM	XAC	XPM + XAC
mm 96/0067	XPM	XAC	XPM + XAC
mm 96/1871	XPM	XAC	XPM + XAC
mm96/2480	XPM	XAC	XPM + XAC
mm96/3567	XPM	XAC	XPM + XAC
Naro56	XPM	XAC	XPM + XAC
Serere	XPM	XAC	XPM + XAC

Other data collected included plant height and weight. The area under disease progress was calculated on a single plant basis using the trapezoidal integration for the whole evaluation period by applying the following formula (Odongo *et al.*, 2019)

$$\sum \frac{i[(DSI + DSI - 1) \times (t_i - t_{i-1} - 1)]}{2}$$

Where “i” = (6, 12, 18, 24, 30, 36) periods of assessment, “DS” represents value on severity scale for disease and “t” stands for days after inoculation. The disease reaction was determined using a scale described by Banito *et al.*, (2010) (Table 2).

Table 2. Disease reaction scale

% AUDPC Category	Disease Reaction
0 - 33.2	Resistant (R)
33.3 - 49.9	Moderately Resistant (MR)
50 - 100	Susceptible (S)

Data analysis. The Disease intensity values were calculated for each genotype and analysis of variance done in Genstat 15th edition and means separated using Fishers Protected LSD (Odongo *et al.*, 2019).

Results

Cassava bacterial blight intensity for different cassava varieties. There was significance

($P < 0.005$) difference in cassava bacterial blight incidence only in varieties infected with *Xanthomonas phaseoli* p.v. *manihotis* (XPM) which had higher incidences in contrast to those exposed to *Xanthomonas axonopodis* p.v. *cassavae* (XAC) and both bacteria. Majority of XPM infected varieties showed incidences of up to 70%, only mm96/2480 had lower incidence. A difference of 8% between the highest and lowest incidences for XPM infected varieties was recorded. No significance difference ($p < 0.005$) in incidence levels was established among varieties exposed to XAC however they had incidences ranging between 50 and 65%. For the varieties infected with a combination of both bacteria only two had incidences below 50% while most of the remaining had more than 50% incidence. There was a significant difference in the area under disease progress curve (AUDPC) for all the varieties assessed. Varieties exposed to *Xanthomonas phaseoli* p.v. *manihotis* (XPM) had higher AUDPC values in contrast to those in us curated with *Xanthomonas axonopodis* p.v. *cassavae* (XAC) and a combination of both pathogens. Three varieties namely, mm96/1871, mm96/2480 and Naro 56 showed higher AUDPC values relative to the other varieties evaluated. Varieties infected with XPM had the highest AUDPC values compared to those inoculated with XAC as most had values of over 70%. For XAC infected varieties Fumbachai and mm 96/3567 had AUDPC values slightly less than 50% (Table 3).

Table 3. Percent disease intensity over time on cassava varieties inoculated with cassava bacterial blight pathogens *Xanthomonas phaseoli* p.v. *manihotis* (XPM) and *Xanthomonas axonopodis* p.v. *cassavae* (XAC)

Varieties	Incidence			Area under disease Progress Curve		
	XPM	XAC	XPM+XAC	XPM	XAC	XPM+XAC
Fumbachai	74bcd	64c	49a	69a	44a	63.3bc
mm96/0067	77d	48a	54ab	71.2ab	56bc	59.5ab
mm96/1871	76cd	61bc	55ab	78.8c	66d	68.8de
mm96/2480	69a	62bc	66b	82.62c	76e	72.3e
mm96/3567	73bc	51ab	59ab	77.8ab	48ab	53.1a
Naro56	77d	61bc	56ab	84.4c	67d	68.3cde
Serere	71ab	51ab	45a	71.4ab	57c	61.4bc
Mean	74	57	55	76.5	59.2	63.8
P < 0.005	0.001	0.034	0.231	0.001	0.001	0.001
LSD (D=0.05)	4.1	12.1	15.8	6.9	8.3	7.3
CV (%)	8.3	32.1	44	15.7	24.7	20.2

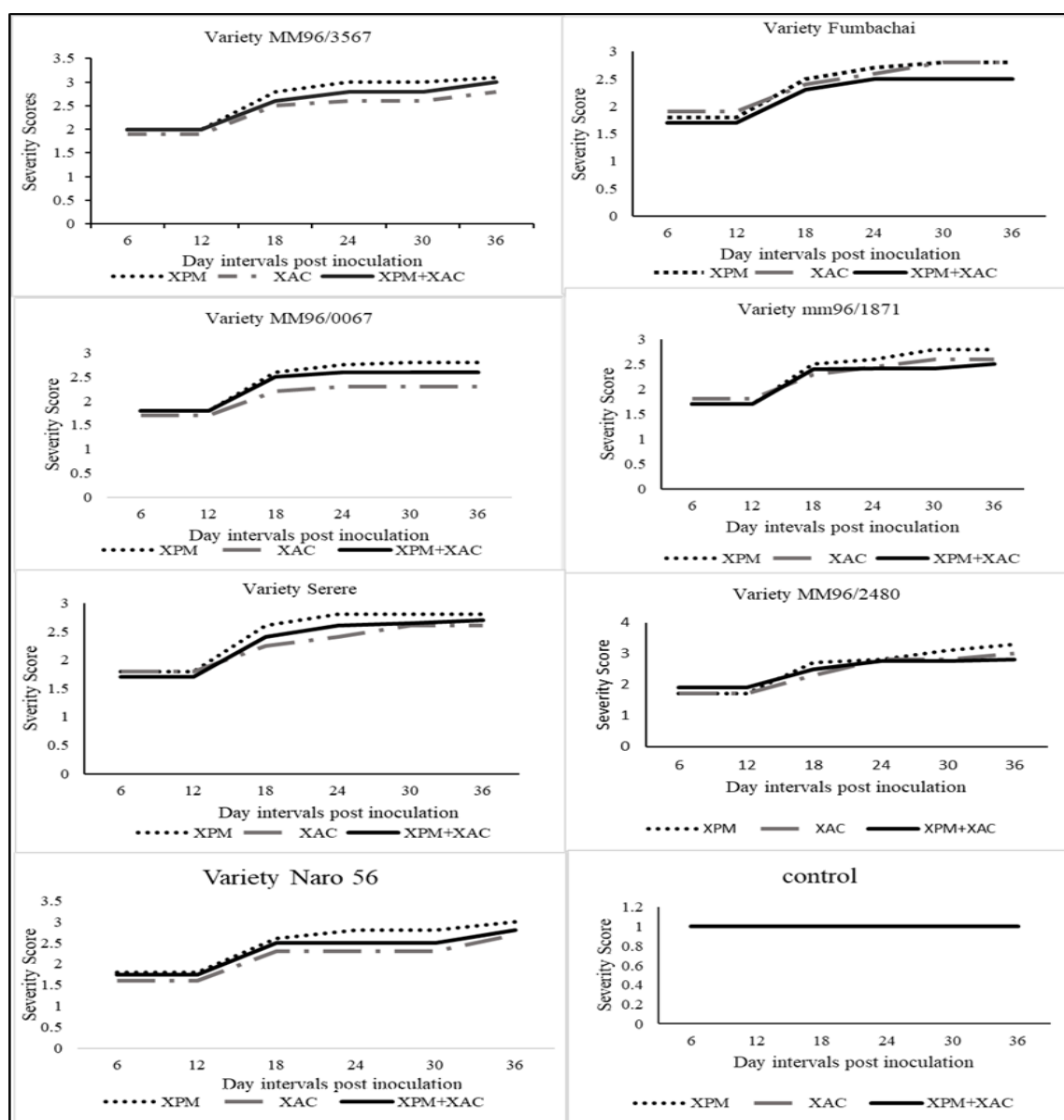


Figure 1. Disease Severity scores over time for cassava bacterial blight casual agents *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae* for each of the evaluated varieties

Severity scores of cassava bacterial blight disease in different varieties. *Xanthomonas phaseoli* p.v. *manihotis* infected varieties exhibited severe disease in contrast to varieties infected with the *Xanthomonas axonopodis* p.v. *cassavae* and a combination of both bacterial pathogens. By the end of the experiment most of the varieties infected with *Xanthomonas phaseoli* p.v. *manihotis* had a severity score of over 2.7. However, varieties mm96/3567 and Naro 56 had severity scores of above 3. The highest severity score for *Xanthomonas phaseoli* p.v. *manihotis* infected varieties was observed in mm 96/2480. For varieties infected

with *Xanthomonas axonopodis* p.v. *cassavae* most had a severity score less than 3. Only variety mm 96/2480 had a severity score of 3 while the least score was observed in variety mm96/0067. All varieties inoculated with both pathogens had scores ranging between 2.6 and 2.8 by the end of the experiment. Of all the varieties assessed mm96/2480 was severely affected by both pathogens as it had the highest score by the end of the experiment for each of the individual pathogens 3.3 for *Xanthomonas phaseoli* p.v. *manihotis* and 3 for *Xanthomonas axonopodis* p.v. *cassavae* (Fig 1).

Agronomic parameters of the different varieties inoculated with cassava bacterial blight causal agents. There was significant difference p (<0.005) in the fresh biomass at the end of evaluation. All varieties inoculated with *Xanthomonas phaseoli* p.v. *manihotis* (XPM) recorded the lowest wet weight in contrast to those exposed to *Xanthomonas axonopodis* p.v. *cassavae* (XAC) and both pathogens. Naro 56 had the lowest fresh biomass in contrast to the others varieties across all bacterial treatments while Fumbachai had the highest fresh biomass. Most of the varieties subjected to XAC had a fresh biomass of above 50 g while most of the varieties exposed to both pathogens had a fresh biomass of less than 50g.

However, there was no significant difference ($P>0.005$) in the height of cassava varieties at the end of evaluation period. The heights ranged between 28-30 cm (Table 4).

Disease reaction across different varieties inoculated with cassava bacterial blight causal agents. All the varieties were susceptible to the cassava bacterial blight pathogens at the end of the evaluation period. Only Fumbachai inoculated with XAC had an AUDPC value slightly lower than 50% making it moderately resistant, while the rest had AUDPC values of more than 50% rendering them susceptible (Table 5).

Table 4. Agronomic parameters of cassava varieties inoculated with cassava bacterial blight causal agents *Xanthomonas phaseoli* p.v. *manihotis* (XPM) and *Xanthomonas axonopodis* p.v. *cassavae* (XAC)

Varieties	Fresh Biomass (g)			Height (cm)		
	XPM	XAC	XPM+XAC	XPM	XAC	XPM+XAC
Fumbachai	53.2c	45.4a	68.4d	29.7a	29.6a	30.1a
mm96/0067	47.3b	56.8b	45.4b	29.9a	29.9a	29.9a
mm96/1871	56.4c	62.2c	55.0c	30.1a	29.9a	29.9a
mm96/2480	41.8a	45.56a	42.3a	29.9a	29.9a	29.9a
mm96/3567	47.3b	56.8b	45.4b	29.9a	29.9a	29.9a
Naro56	37.5a	41.7a	43.5a	29.9a	29.9a	29.9a
Serere	39.2a	58.1b	56.7c	29.9a	29.9a	29.9a
Mean	46.1	52.4	51	29.9	29.9	29.9
P < 0.005	0.001	0.001	0.001	0.989	0.990	0.999
LSD (p=0.05)	4.3	4.1	2.5	0.9	0.939	0.91
CV(%)	16.4	13.6	8.6	13.4	13.6	13.1

Table 5. Disease reaction of cassava varieties inoculated with cassava bacterial blight causal agents *Xanthomonas phaseoli* p.v. *manihotis*(XPM) and *Xanthomonas axonopodis* p.v. *cassava*(XAC)

Varieties	XPM	XAC	XPM+XAC
Fumbachai	S	MR	S
mm96/0067	S	S	S
mm96/1871	S	S	S
mm96/2480	S	S	S
mm96/3567	S	S	S
Naro56	S	S	S
Serere	S	S	S

Discussion

The study revealed that there was no significant difference in overall disease incidence across most treatments by the end of the experiment. Only varieties infected with *Xanthomonas phaseoli* p.v. *manihotis* showed significant difference in incidence. This was in contrast to varieties exposed to *Xanthomonas axonopodis* p.v. *cassavae*, and a combination of both bacteria which had higher disease incidence. The distinction might be because *Xanthomonas phaseoli* p.v. *manihotis* has been shown to be more lethal compared to *Xanthomonas axonopodis* p.v. *cassavae* thus invading vulnerable plants faster (Pereira *et al.*, 1999). Moreover, it has been indicated that *Xanthomonas phaseoli* p.v. *manihotis* can paralyze plant defense through production of molecules that facilitate rapid plant colonization (Zárate-Chaves *et al.*, 2021). Infection by *Xanthomonas phaseoli* p.v. *manihotis* manifests within a short time compared to *Xanthomonas axonopodis* p.v. *cassavae* (Pereira *et al.*, 1999). This was also seen in this study as by the sixth day of exposure varieties that had been infected with *Xanthomonas phaseoli* p.v. *manihotis* were showing symptoms compared to those subjected to *Xanthomonas axonopodis* p.v. *cassavae*. Although varieties infected with both pathogens showed high incidence compared to those inoculated with *Xanthomonas phaseoli* p.v. *cassavae* inoculated varieties, infection showed up much later compared to those inoculated with *Xanthomonas phaseoli* p.v. *manihotis*. This might have been caused by *Xanthomonas axonopodis* p.v. *cassavae* which has been reported to temporarily limit *Xanthomonas phaseoli* p.v. *manihotis* which the latter eventually overcomes leading to disease (Kwena, 1992; Verdier *et al.*, 1994).

Significant differences in severity scores across all the different treatments was also established. Varieties exposed to *Xanthomonas phaseoli* p.v. *manihotis* had elevated severity scores compared to those infected with *Xanthomonas axonopodis* p.v. *cassavae* or a combination of both pathogens. The severity scores were mainly moderate throughout the experiment. Which is in agreement with what has been

reported by Simiyu *et al.* (2022). Variety mm 96/2480 had the highest severity score for both causal agents compared to the rest of the varieties. Verdier *et al.* (1994) also observed varieties exposed to *Xanthomonas phaseoli* p.v. *manihotis* suffered more compared to those inoculated with *Xanthomonas axonopodis* p.v. *cassavae* and the immune response in vulnerable varieties was slower compared to formidable varieties this aligns with observations by Onyango and Mukunya (1980) and Pereira *et al.* (1999). Simiyu *et al.* (2022) also showed that defenseless varieties record high severity scores when assessed to cassava bacterial blight pathogen *Xanthomonas phaseoli* p.v. *manihotis* and variety mm 96/2480 was extremely vulnerable to the pathogen. In co-inoculated varieties disease progression was much slower at the beginning but increased towards the end of the study. This might be because of *Xanthomonas phaseoli* p.v. *manihotis* capability of overcoming the limitation imposed by *Xanthomonas axonopodis* p.v. *cassavae* as observed by Kwena *et al.* (1992) and Verdier *et al.* (1994). Susceptible plants have been shown to respond slower to CBB infection compared to resistant plants (Kpémoua *et al.* 1996; Zinsou *et al.*, 2000; Wydra *et al.*, 2004; Lopez *et al.*, 2007), this has been linked to the existence or absence of molecules that results in susceptibility or resistance when plants are exposed to CBB causal agents (Zeng *et al.*, 2018). For example, *Xanthomonas phaseoli* p.v. *manihotis* has been reported to activate the *MeSweet10a* gene which lowers plant defense against cassava bacterial blight (Cohn *et al.*, 2014). In other studies, Wei *et al.* (2017), Li *et al.* (2017), and Liu *et al.* (2018) observed that *MeRAV1* and *MeRAV2*- melatonin biosynthesis genes, *MebZIP3* and *MebZIP5*, and *MeWRKY75–MeWHY3* are critical to cassava bacterial blight resistance as plants which had this gene tampered with succumbed to the disease. The varied severity between varieties infected with *Xanthomonas phaseoli* p.v. *manihotis* and *Xanthomonas axonopodis* p.v. *cassavae* was also recorded in an experiment by

Pereira *et al.* (1999) where varieties exposed to *Xanthomonas phaseoli* p.v. *manihotis* commenced defoliation by the second week of exposure in contrast to those subjected to *Xanthomonas axonopodis* p.v. *cassavae* whose leaves persisted on the plants up to four weeks post infection. The severe disease witnessed on vulnerable plants might also have been caused by deficiency in lignin production and other phenolic compounds, absence of suberin and tyloses, lack of production of latex with high content or PR-proteins, lack of leaf level resistance such cell walls pectin which are important factors in curbing disease development in cassava (Kpémoua *et al.*, 1996; Pereira *et al.*, 2000; Cooper *et al.*, 2001; Wydra *et al.*, 2004; Mabringong *et al.*, 2017). Furthermore, area under disease progress curve values varied significantly by the end of the experiments. Varieties across the different treatments yielded AUDPC values of above 50%. All of the varieties infected with *Xanthomonas phaseoli* p.v. *manihotis* yielded area under disease progress curve values of over 70%. The extremely affected varieties included mm96/2480, Naro 56 and mm96/1871 which yielded values of over 75%. Of the varieties subjected *Xanthomonas axonopodis* p.v. *cassavae* only varieties Fumbachai and mm 96/3567 had area under disease progress curve values slightly below 50%. Finally, varieties with combined infection of *Xanthomonas axonopodis* p.v. *cassavae* and *Xanthomonas phaseoli* p.v. *manihotis* had more than 50% AUDPC values. Same observations were made by Pereira *et al.* (1999) where exposure to the different causal agents led to different area under disease progress curve values with varieties inoculated by *Xanthomonas phaseoli* p.v. *manihotis* having the highest values. A similar observation was also made by Odongo *et al.* (2019) as most of the evaluated varieties yielded AUDPC values of more than 50% deeming them susceptible to cassava bacterial blight. This suggested that most of the varieties grown in Kenya are defenseless against cassava bacterial blight which is in agreement with what we found in the study and observations from other studies (Bart and Taylor., 2017; Mbaringong *et al.*, 2017; Teixeira *et al.*, 2021). Furthermore, the

results show that *Xanthomonas phaseoli* p.v. *manihotis* is the more severe of the two causal agents. This is consistent with findings by Onyango and Mukunya (1980).

In conclusion, the findings from the study show that of the two bacterial pathogens associated with cassava bacterial blight *Xanthomonas phaseoli* p.v. *manihotis* is the more severe. Also all the varieties assessed in this study were susceptible to cassava bacterial blight as all had AUDPC values of more than 50%. The most affected varieties were mm 96/2480, Naro 56, and mm 96/1871. Therefore, attention needs to be directed towards identifying and breeding varieties that can curb cassava bacterial blight.

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Statement of No-Conflict of Interest

The authors declare no conflict of interest in the paper.

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