Evaluation of Selected Cassava (*Manihot esculenta* Crantz) Cultivars Grown in Kenya for Resistance to Bacterial Blight Disease

Gladys Ada Mbaringong[1,*], Evans N. Nyaboga[2], Virginia Wang'ondu[1], Esther Kanduma[2]

[1]School of Biological Sciences, University of Nairobi, P.O Box 30197 – 00100, Nairobi, Kenya
[2]Department of Biochemistry, University of Nairobi, PO Box 30197 – 00100, Nairobi, Kenya
*Corresponding author: gladysada2001@yahoo.com

Abstract Bacterial blight of cassava is one of the most important diseases of cassava worldwide due to its growing concern, widespread and destructive nature. Even though the use of resistant cultivars is the most effective management strategy for the disease, such cultivars are not well identified. Therefore, the objective of this study was to screen 21 cassava cultivars collected from major growing regions of Kenya, for reaction against bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). The cultivars were inoculated with *Xam* by leaf clipping and stem puncturing inoculation methods, for *in vitro* and potted plants, respectively. The cassava cultivars varied in their reaction to the pathogen, including incubation period, wilt incidence and area under the disease progress curve (AUDPC) values. Four groups of cultivars with differential reactions to *Xam* isolate were identified. Four cultivars (TME419, 30572, 98/0505 and Kibaha) were resistant, 4 cultivars (Albert, Ebwanatereka, Karibuni and 92/0326) moderately resistance, 11 cultivars (Serere, Muzege, TME7, 98/0581, Tajirika, Namikonga, Kibandameno, Mzalauka, AR40-6, Shibe and 01/1371) susceptible and the other 2 cultivars (Kiroba and Numbari) were highly susceptible. The resistant cultivars should be multiplied and made available as clean planting materials to cassava producing farmers and integrated as one of the options in disease management measures. These genotypes could also form vital germplasm of cassava bacterial blight disease resistance breeding programs. The cassava cultivars that showed a resistant reaction to the bacterial blight pathogen should be further evaluated against a large number of *Xam* isolates.

***Keywords:** cassava bacteria blight, Xanthomonas axonopodis pv. manihotis, cultivars, resistance*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a major staple food crop and source of income for more than 800 million people in tropical and sub-tropical countries of Africa, Asia and Latin America [1]. The tuberous storage roots of cassava are high in starch content (approximately 30 – 60% dry matter) and can be cooked or processed for human food, animal feed and supplies raw material for production of bio-ethanol and other desirable industrial products [2,3]. In addition to the tuberous roots, cassava leaves are consumed as a vegetable in at least 60% of the countries in sub-Saharan Africa, providing an important source of proteins, vitamins and micronutrients [4]. Cassava can be cultivated under adverse environmental and nutrient-limited conditions, hence, it is projected that cassava may become an important replacement for crops that are expected to be more vulnerable to climate change, especially in eastern Africa [5,6]. This is in part as a result of the fact that rising CO_2 concentration will have a more positive effect on cassava as a C_3 crop than it will on the major C_4 crops such as maize, sorghum and millets [7,8]. However, cassava is susceptible to several pests and diseases, including attacks from whitefly, mealybug, the widely-spread green mite, cassava bacterial blight (CBB), cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), which significantly reduce cassava yields and pose a constraint to poor farmers with little or no response capacity [9].

Of all the biotic constraints, bacterial blight disease caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is the most important disease affecting yields [10,11]. The disease is characterized by symptoms comprising water-soaked angular leaf spots, blighting and wilting of the leaves. The stems and petioles produce exudates and undergo vascular necrosis, while the plant loses leaves and might dieback [12]. Without effective control strategies, root yield losses can reach up to 100% in infected cassava plants [13]. Integrated approaches of using fungicides, bio-control agents as well as cultural practices such as the use of healthy, disease-free cuttings for planting, destruction and controlled movement of diseased plants,

cleaning of equipment that has come in contact with diseased plant material and rotation of crops have been recommended in the management of cassava bacterial blight (CBB) [14,15]. However, these control measures currently being recommended are labour intensive and not easily adopted by farmers, making the cultivation of resistant cultivars the best method of CBB control [14].

Use of resistant cultivars is a simple, effective, save and economical strategy for integrated management of major diseases in numerous crops. This strategy could be used to control CBB if resistant cultivars could be identified or developed. Attempts to develop disease resistant varieties through conventional breeding require resistant donor parents but germplasm exhibiting resistance against *Xam* has not been identified in Kenya. Continuous and intense evaluation of cassava genotypes for disease resistance is one of the basic requirements for effective and sustained implementation of integrated disease management programs. The presence of resistance/tolerance in cassava cultivars to *Xam* has been reported in West Africa though no cultivar was found to be completely resistant [16]. There is need to consider the vast wealth of cassava genetic resources in different cassava-growing regions such as East Africa. There is a high genetic diversity in cultivated cassava populations in Kenya [17] though they have not been characterized for their reaction to *Xam* pathogen, in order to find cultivars with resistance that could be recommended to farmers as part of an integrated system for CBB control. Therefore, the objective of this study was to evaluate the level of resistance of selected cassava cultivars grown in Kenya against bacterial blight disease.

2. Materials and Methods

2.1. Plant Materials

A total of 23 cassava cultivars namely TMS60444, TME14, Serere, Ebwanatereka, Kibandameno, Numbari, Albert, AR40-6, Kiroba, TME7, Kibaha, Karibuni, Kiroba, Tajirika, Namikonga Shibe, Mzalauka, 30572, TME419, 92/0326, 98/0505, 98/0581 and 01/1371 were obtained as cuttings from Kenya Agricultural and Livestock Research Organization (KALRO) field. TMS60444, TME14 are known to be highly susceptibe to CBB. The cultivars had originally been obtained from farmer's fields in major cassava growing regions of Kenya. The collected cuttings were established in plastic pots containing sterile soil in the greenhouse at the School of Biological Sciences, University of Nairobi. To establish *in vitro* cultures, shoots 10 cm in length were excised from greenhouse-grown plants and were cleaned under running tap water for 20 minutes, and then the stems cut into small pieces containing 2 nodal sections each. The nodal sections were sterilized by immersing in 70% (v/v) ethanol for 20 seconds, rinsed with double-distilled water followed by immersion in 5% sodium hypochlorite solution for 5 minutes and then rinsed three times with double-distilled water. The surface-sterilized nodal cuttings were blotted dry on sterile paper towels. All of the nodal cuttings were established on cassava basic medium (CBM; MS salts with vitamins, 2 μM CuSO4, 2% sucrose and 0.3% gelrite

at pH 5.8) at 28°C under a 16/8 h photoperiod. The *in vitro* plantlets were subcultured every 3–4 weeks. The rooted plants with 3 – 4 leaves were used for *in vitro* screening experiments.

For *in vivo* screening, plants were established in pots containing sterile soil in the greenhouse using stem cuttings. Pots containing plants were completely covered with polythene bags for 3 weeks. After 2 weeks the bags were cut at the two corners with a pair of scissors. The polythene bag was removed in the third week and watering was done on a weekly basis. The plants were maintained in the greenhouse for 8 weeks before inoculation with *Xam*.

2.2. Preparation of Bacteria Suspension Culture for Plant Inoculation

X. axonopodis pv. *manihotis* was isolated from infected plants on semi-selective medium yeast peptone glucose agar (YPGA-CC) medium (5 g of peptone, 5 g of yeast, 10 g of glucose, 1.5% agar, 50 mg/l cephalexin and 150 mg/l cyclohexamide [18]. The cultures were maintained on YPGA medium (5 g of peptone, 5 g of yeast, 10 g of glucose, 1.5% agar) at 4 °C.

A single colony of *Xam* isolate was inoculated into 25 ml of yeast peptone glucose (YPG) medium containing 50 mg/l cephalexin and 150 mg/l cyclohexamide and incubated at 28 °C with shaking at 150 rpm for 48 hours. About 5 μl of the broth was spread on semi-selective medium YPGA-CC and incubated at 28 °C for 48 hours. Fresh colonies were used for all the experiments in order to have high virulent potential of the pathogen.

2.3. Optimization of Inoculation Method

Two cultivars namely TMS60444 and TME14 known to be highly susceptible to cassava bacterial blight in the fields were used for optimization of *Xam* inoculation methods using *in vitro* and potted plants. The *in vitro* plants of cultivars TMS60444 and TME14 with well developed roots and 3 - 4 fully expanded leaves were used. Three inoculation methods were evaluated: (1) Leaf clipping method - The apical region of the central lobe of leaves were cut below their tip using a pair of scissors. A sterile toothpick was used to apply one bacterium colony directly from the Petri plate to the apex of the cut leaf lobe. Leaves of control plants were cut by scissors and water applied to the cut surface. (2) Leaf puncturing - An incision on the main veins of the central leaf lobe of cassava was made using a 27 gauge needle. A sterile toothpick was used to apply one bacterium colony directly from the Petri plate to the punctured section of the leaves. Water was applied on the incisions made on leaves of control plants. (3) Stem puncturing - A sterile blade fitted on a sterile scalpel was used to make incisions on the stem of plants. A sterile toothpick was used to apply one bacterium colony directly from the Petri plate to the wounded plantlet. Sterile water was applied on stems of control plants.

For each of the three inoculation methods tested, 10 plants were inoculated with *Xam* and 6 control *in vitro* plants inoculated with sterile water. The experiments were repeated twice. The plants were maintained in the growth

chamber at 26 ± 2 °C with a 16/8 hour photoperiod. The inoculated and control plants were assessed daily for 21 days for appearance of bacterial blight symptoms. The disease severity was done following a severity scale of 1 - 5, where 1 = dark spots or necrosis of area around the inoculation point; 2 = gum exudates on the stem; 3 = wilting of one or two leaves and exudates; 4 = more than two leaves wilted; and 5 = complete wilting and dieback [19]. Area under disease progress curve (AUDPC) was calculated as described by Jorge *et al*. [19]. The area under disease progress curve (AUDPC) was calculated on a single plant basis by the trapezoidal integration over the whole observation period using the formula:

AUDPC=$\sum_i[(D_i +D_{i-1})*(T_i-T_{i-1})]/2$, where D = is the disease score using the 1–5 severity scale above; T = represents the number of days after inoculation, *i* = (7, 14 or 21) are the days of evaluation [19].

One gram of leaf samples above and below the point of inoculation were collected from each plant for quantification of bacteria. The bacterial population was determined at 0, 7, 14 and 21 days post inoculation (dpi). The leaves were macerated in sterile double-distilled water. Three serial dilutions were spread on semi-selective YPGA-CC medium and incubated at 28 °C. After 48 hours, *Xam* colonies formed were counted. Non inoculated plants were used as controls. All the assays were performed in three biological replicates [20]. The best inoculation method based on AUDPC, bacteria count and wilt incidence of inoculated plants was used for evaluation of cassava cultivars for resistance to *Xam* using *in vitro* plants.

Optimization of inoculation method in the greenhouse was done using 8-week old potted plants of cultivars TMS60444 and TME14. Plants were covered with sterile polythene bags soaked in distilled water for 2 days before inoculation. After inoculation the plants were covered again with polythene bags for 1 day. Six plants were used for each of the inoculation methods namely leaf clipping, leaf puncturing and stem puncturing as described for *in vitro* plants and the experiment was repeated twice. The best inoculation method was determined based on AUDPC, bacteria count and wilt incidence of inoculated plants as described for *in vitro* plants. The best method was used for evaluation of cassava cultivars for resistance to *Xam* in the glasshouse.

2.4. Evaluation of Cassava Cultivars Using *in vitro* Plants

Twenty one cassava cultivars (Serere, Ebwanatereka, Numbari, Albert, AR40-6, Kiroba, TME7, Kibandameno, Kibaha, Shibe, Mzalauka, Tajirika, 30572, Karibuni, TME419, 92/0326, 98/0505, 98/0581, 01/1371, Namikonga and Muzege) were tested for susceptibility to *Xam* by leaf clipping inoculation method as described above. Ten plants of each cassava cultivar were inoculated, whereas control plants were inoculated with sterile water. The experiment was repeated two times. Plants were assessed every day for 3 weeks for appearance of disease symptoms of chlorosis or necrosis of leaves, and complete wilting of plants. Wilt incidence was measured as number of wilted plants from total number of plants inoculated. The relative resistance of cultivars to CBB was evaluated 3 weeks after inoculation based on AUDPC as described by Jorge *et al*. [19]. The pathogenic bacteria were re-isolated from wilted plants and identified as *Xam* on the basis of their characteristic morphology.

2.5. Evaluation of Cassava Cultivars Using Potted Plants in the Greenhouse

Eleven cassava cultivars (Kibaha, Albert, AR40-6, Namikonga, 30572, TME7, TME419, 92/0326, 98/0505, 98/0581 and 01/1371) were selected based on *in vitro* screening experiment, established in pots containing sterile soil and tested for susceptibility to CBB in the greenhouse. Ten plants per cultivar were inoculated in each experiment using stem puncturing method as described above. Ten plants of each cultivar inoculated with sterile water were used as the control. The experiment was repeated two times. Plants were assessed every day for 4 weeks for appearance of disease symptoms of chlorosis or necrosis of leaves, and complete wilting of plants. Wilt incidence was measured as number of wilted plants from total number of plants inoculated. The relative resistance of cultivars to CBB was evaluated 4 weeks after inoculation based on AUDPC as described by Jorge *et al*. [19].

2.6. Statistical Data Analysis

The time interval between inoculation and appearance of disease symptoms, complete wilting and AUDPC of cassava cultivars inoculated with *Xam* using *in vitro* and in vivo methods were analyzed using analysis of variance (ANOVA) in Gen-stat 14th edition. Values were log transformed before ANOVA was carried out in order to stabilize coefficient of variation. Least Significant difference was tested at P = 0.05.

3. Results

3.1. Optimization of Inoculation Methods

Inoculated *in vitro* plants of cultivars TMS60444 and TME14 produced disease symptoms of chlorosis and necrosis at 5, 7 and 8 days after inoculation using stem puncturing, leaf puncturing and leaf clipping methods, respectively. For both cultivars at 21 days after inoculation, wilt incidence of 20% was recorded for both leaf and stem puncturing methods, whereas 80% wilt incidence was observed for leaf clipping method. The symptoms observed on plants using the three methods of inoculation ranged from chlorosis of leaves, wilting and dieback of plants. There was no significant difference in AUDPC values among leaf clipping (1.606 ± 0.07), leaf puncturing (1.522 ± 0.04) and stem puncturing (1.604 ± 0.04) methods of inoculation (F = 0.40). Using bacteria population, no significant differences were observed among leaf clipping, leaf puncturing and stem inoculation methods at 7 days after inoculation for cultivar TME14. There were significant differences (F < 0.001) in bacteria count among the three methods at 14 and 21 days post-inoculation, and respectively for both cultivars TMS60444 and TME14 (Figure 1). Leaf clipping was higher than leaf

puncturing and stem puncturing by 1 and 2 log values, respectively, at 14 days after inoculation. At 21days after inoculation leaf clipping was 3 log values greater than leaf puncturing and stem puncturing (Figure 1).

For optimization of inoculation method using potted plants in the greenhouse, there were significant differences (F = 0.006) for AUDPC values among the three inoculation methods. The AUDPC values obtained for leaf clipping, leaf puncturing and stem puncturing were 1.404 ± 0.098, 1.594± 0.098 and 1.622± 0.098, respectively. Leaf puncturing and stem inoculation were 1 and 1.5 log values, respectively, more effective in introducing *Xam* into plants than leaf clipping in the greenhouse. Stem inoculation was about 0.1 log value superior than leaf puncturing. Based on bacterial population isolated from inoculated plants, there were significant differences in bacteria count among the three inoculation methods at 14 (F = 0.014), 21 (F = 0.007) and 28 (F=0.028) days after inoculation (Figure 2). Stem puncturing recorded a significantly higher bacteria population at 14, 21 and 28 days after inoculation than leaf clipping and leaf puncturing methods for both TMS60444 and TME14 (Figure 2).

3.2. Evaluation of Cassava Cultivars Using *in vitro* Plants

For the 21 cultivars of cassava tested, control plants inoculated with water did not show any disease symptoms while the plants inoculated with *Xam* isolates developed leaf chlorosis or necrosis. Symptoms of bacterial blight in all cassava cultivars appeared 5 to 19.5 days after *Xam* inoculation and differed significantly among the cultivars at 5% level of significance. In this study, symptoms were observed first on Ebwanatereka, TME7, Muzege, Serere, 98/0581, Kiroba, 92/0326, Tajirika, Numbari and Kibandameno (5 days after inoculation) followed by AR40-6 and Mzalauka (6 days after inoculation), TME419, Albert, 30572, Kibaha, Karibuni, Shibe, Namikonga and 01/1371 (8 days after inoculation) while longest duration was taken by 98/0505 (19.5 days after inoculation) (Table 1). This confirms earlier reports by Boher and Verdier (1994) that intracellular and vascular multiplication of *Xam* is slow in resistant cultivars. Plants of all the cultivars tested except for TME419, 30572, 98/0505 and Kibaha wilted. The bacteria were recovered from all the wilted plants and circular cream mucoid colonies of *Xam* appeared on semi-selective medium confirming that symptoms were due to the bacteria that was used as inoculum. No plants of cultivars TME419, 30572, 98/0505 and Kibaha wilted and eventually these plants resembled healthy control plants.

The cultivars varied significantly (F < 0.001) in AUDPC values (Table 1). Based on the mean AUDPC values of the 21 cassava cultivars, a scale of mean AUDPC value was proposed to categorize the genotypes into 4 resistance levels (Table 2). Among them, four cultivars TME419, 30572, 98/0505 and Kibaha were resistant while two cultivars Kiroba and Numbari fell into the highly susceptible category (Table 2). Four cultivars fell under moderately resistant (Albert, Ebwanatereka, Karibuni and 92/0326) and 11 under susceptible (Muzege, Serere, 98/0581, Tajirika, Namikonga, Kibandameno, Mzalauka, TME7, AR40-6, Shibe and 01/1371) category. This study shows that cassava cultivars vary in their reaction to bacterial blight.

Table 1. Comparison of 21 cassava cultivars for appearance of first disease symptoms, wilt incidence and AUDPC values after inoculation with *Xam* using *in vitro* plants

Cultivar	No. of days to appearance of disease symptoms*	Wilt incidence (%)	Log of AUDPC value*	Disease rating
TME419	8.00[c]	0	1.50±0.07[ab]	R
Albert	8.00[c]	0	1.56±0.12[abcd]	MR
Ebwanatereka	5.00[a]	25	1.61±0.09[bcde]	MR
TME7	5.00[a]	70	1.67±0.05[bcdef]	S
30572	8.00[c]	0	1.50±0.02[ab]	R
AR40-6	6.00[b]	25	1.67±0.07[bcdef]	S
Muzege	5.00[a]	75	1.79±0.04[ef]	S
Serere	5.00[a]	50	1.71±0.05cd[ef]	S
98/0505	19.50[c]	0	1.38±0.12[a]	R
Kibaha	8.00[c]	0	1.52±0.08[abc]	R
98/0581	5.00[a]	50	1.77±0.05[ef]	S
Karibuni	8.00[c]	30	1.63±0.04[bcde]	MR
Kiroba	5.00[a]	75	1.82±0.02[ef]	HS
92/0326	5.00[a]	25	1.63±0.12[bcdef]	MR
Shibe	8.00[c]	25	1.67±0.02[bcdef]	S
Tajirika	5.00[a]	100	1.79±0.03[ef]	S
Namikonga	8.00[c]	50	1.73±0.05[def]	S
Numbari	5.00[a]	100	1.83±0.02[f]	HS
01/1371	8.00[c]	75	1.67± 0.03[bcdef]	S
Kibandameno	5.00[a]	80	1.77±0.05[ef]	S
Mzalauka	6.00[b]	75	1.77±0.01[ef]	S
TMS60444 (susceptible check)	5.00[b]	100	1.82[f]	
SEm	0.25		0.12	
LSD (0.05)	0.50	0.1102	0.17	
CV (%)	7.6	11.2	7.2	
Probability	<0.001	<0.001	<.001	

HS: Highly susceptible, S: Susceptible, MR: Moderately resistant and R: Resistant. *Means followed by the same letters in the same column are not significantly different at 5% level of significance; SEm (±) indicates standard error of mean.
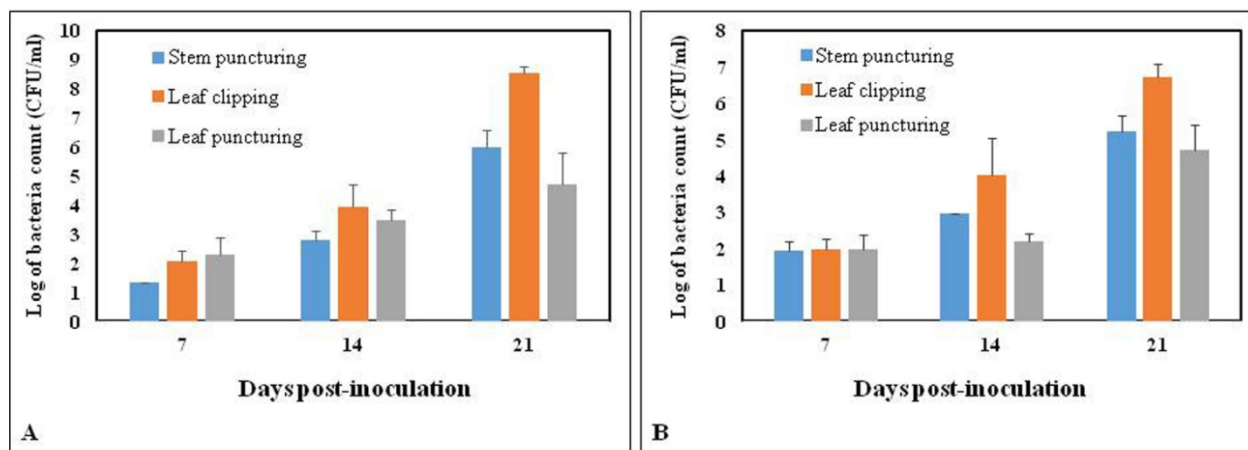
Figure 1. Bacterial growth analysis upon leaf puncturing, leaf clipping and stem puncturing inoculation of cassava cultivars (**A**) TMS60444 and (**B**) TME14 at 7, 14 and 21 days post-inoculation using *in vitro* plants. Bars represent standard errors
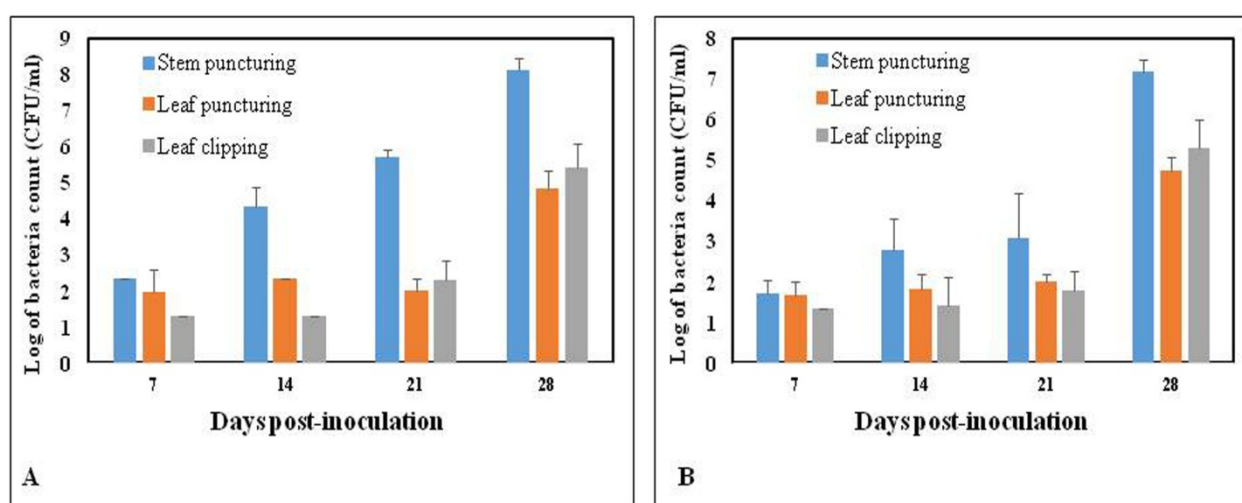
Figure 2. Bacterial growth analysis upon leaf puncturing, leaf clipping and stem puncturing inoculation of cassava cultivars (A) TMS60444 and (B) TME14 at 7, 14 and 21 days post-inoculation using potted plants. Bars represent standard errors

Table 2. Proposed resistance category of different cassava cultivars

Log of mean AUDPC	Resistance category	Code
<1.56	Resistant	R
1.56 - 1.66	Moderately resistant	MR
1.66 - 1.8	Susceptible	S
> 1.8 1	Highly susceptible	HS

3.3. Evaluation of Selected Cassava Cultivars Using Potted Plants in the Greenhouse

A total of 11 selected cassava cultivars were evaluated using potted plants in the greenhouse to confirm the results obtained from *in vitro* screening. The response of all 11 cultivars tested varied in a manner which was very similar to results produced by the *in vitro* screening method (Table 3). The incubation period for the appearance of symptoms and AUDPC values varied significantly (F < 0.001) among the cultivars (Table 3). Cultivars Namikonga having minimum number of days for first appearance of disease symptoms showed the highest log of mean AUDPC value (1.93). So this genotype appeared as maximum susceptible genotype to CBB among all tested genotypes in the greenhouse.

Similarly cultivar 98/0505 having maximum appearance of disease (15 days after inoculation) showed lowest log of mean AUDPC (1.18). Thus, the cultivar 98/0505 was the most resistant to CBB than other screened genotypes (Table 3). Based on the log of mean AUDPC values, all the cassava cultivars tested except TME7 and Namikonga using potted plants were classified to the same groups of resistance/susceptibility as previously using *in vitro* plants (Table 3). This confirms that both leaf clipping and stem puncturing used for inoculation of *in vitro* and potted plants, respectively, were efficient in the transfer of *Xam* pathogen into plant tissues. The resistant cultivars were 98/05, 30572, Kibaha and TME419. These four resistant cultivars should be considered as resistant cultivars to the pathogen and they could be used as a bacterial blight management component.

Table 3. Comparison of 11 cassava cultivars for appearance of first disease symptoms and AUDPC values after inoculation with *Xam* using potted plants

Cassava cultivars	No. of days to appearance of disease symptoms*	Log of AUDPC value*	Disease rating
TME419	13.00[cd]	1.35±0.07[abc]	R
Albert	5.00[ab]	1.58±0.00[de]	MR
Kibaha	13.33[cd]	1.29±0.05[abc]	R
30572	15.00[e]	1.27±0.06[ab]	R
AR40-6	13.67[de]	1.68±0.00[abc]	S
98/0505	15.00[e]	1.18±0.03[a]	R
98/0581	5.00[ab]	1. 82±0.07[bcd]	HS
92/0326	5.67[b]	1.57±0.10[de]	MR
TME7	12.00[c]	1.61±0.02[cd]	MR
01/1371	5.33 [b]	1.79±0.11[bcd]	S
Namikonga	3.67[a]	1.93±0.00[e]	HS
TMS60444 (susceptible check)	5.00[ab]	1.91[e]	HS
SEm	0.71	0.10	
LSD (0.05)	1.46	0.18	
CV (%)	9.3	7.4	
Probability	F=<0.001	F=<0.001	

HS: Highly susceptible, S: Susceptible, MR: Moderately resistant and R: Resistant. *Means followed by the same letters in the same column are not significantly different at 5% level of significance; SEm (±) indicates standard error of mean.

4. Discussion

Host plant resistance is an important factor in the integrated control of CBB. Therefore, continuous evaluation of cassava germplasm for disease resistance is one of the basic requirements for effective and sustained implementation of integrated disease management programmes. Screening for resistance also requires a robust system of inoculation to unambiguously discriminate resistant and susceptible cultivars. To establish a method for screening cassava cultivars for *Xam* resistance in this study, we first studied three inoculation methods (leaf puncturing, leaf clipping and stem puncturing) previously reported in the literature for cassava and/or other pathosystems [21,22,23,20]. Two cassava cultivars namely TMS60444 and TME14 susceptible to CBB were used to optimize inoculation method using *in vitro* and potted plants. Based on wilt incidence and bacterial population, leaf clipping was determined to be a reliable method for screening cassava resistance against bacterial blight using *in vitro* plants. The higher wilt incidence and bacteria count observed in leaf clipping compared to other methods of inoculation could be due to efficient entry of the pathogen hence faster multiplication and spread of the bacteria in plants. The results from this study are in agreement with previous reports by Zinsou [24], Akhtar *et al.* [25] and Ruz *et al.* [26], who found leaf clipping as the best method of inoculation in determining resistance to bacterial disease in cassava, pear and rice plants, respectively.

The reference method to evaluate cassava resistance to CBB is through area under the disease progress curve (AUDPC) [21]. Based on AUDPC values and bacterial population, stem puncturing was optimal method for screening resistance to CBB using potted plants in the greenhouse. The results from this study suggest that stem inoculation method provides a reliable method for screening of germplasm resistance against bacterial blight using potted plants in the greenhouse. Stem-inoculation has been reported as a suitable method to screen cassava cultivars for resistance to CBB [20,21,22,27]. This could be due to the fact that bacteria introduced through an incision at the stem could easily access entry into the vascular system and this represents the more natural method of *Xam* spread in the crop [22]. *Xam* is a vascular pathogen that colonizes the xylem [14].

The response to artificial inoculation with *Xam* varied among the cassava cultivars tested. The symptoms produced in the *in vitro* screening tests were very similar to the potted screening tests and represented those due to CBB under natural field conditions. The appearance of disease symptoms occurred earliest in susceptible and highly susceptible cultivars both *in vitro* and potted plants. The commonly grown cultivars such as Kibandameno, Namikonga, Serere, Muzege, Kiroba, Numbari, Tajirika, and Mzalauka were found to be susceptible and highly susceptible. The resistant cultivars were 98/05, 30572, Kibaha and TME419. This study confirms previous report by Zinsou *et al.* [28], that cassava cultivar 30572 is resistant to CBB. In the resistant cultivars identified in this study, symptoms of necrosis and browning occurred only at the inoculation point, did not spread to other parts of the plant and the plants appeared healthy and no *Xam* was recovered from these plants. The necrotic and browning symptoms on the inoculated points could probably be due to a hypersensitive reaction [29] characterized by the rapid death of individual plant cells which come into contact with pathogenic bacteria. This is characteristic of the hypersensitive response (HR), which is thought to be an important defence response to prevent further multiplication and to restrict the spread of the pathogen to other parts of the plant [30].

Disease development in the *in vitro* plants and potted plants were well correlated and cultivars were consistently ranked using AUDPC values except for TME7 and Namikonga. Area under disease progress curve values (AUDPC) gives a quantitative measure of disease development and intensity of disease [31] and the variety having the lowest AUDPC value were categorized as the most resistant, while susceptible one has higher values. The cassava populations were grouped into four groups namely resistant (19.05%), moderately resistant (19.05%), susceptible (52.38%) and highly susceptible (9.52%) based on their resistance/susceptibility level to CBB. This is in agreement with studies in Nigeria and Togo that there exists variation in resistance/susceptibility level of cassava cultivars to CBB [12, 16]. The differences in reaction of the cultivars to *Xam* inoculation could be due to the genotypic variations as a result of differences in the secretion of phenolic compounds, lignification, callose deposition, suberization and vessel occlusion (tylosis), antimicrobial compounds in the vascular activities and physiological activities in the different cassava cultivars [27,32]. The presence of additional mechanisms of resistance (types and amount of wax) at the leaf level has also been suggested by Zinsou *et al*. [28] and Wydra *et al.* [22].

The cultivars that exhibited resistance to CBB infection in this study could be developed for mass deployment to areas where there is high level of disease pressure, although caution must be exercised in such deployment since it has been established that some cassava varieties show regional adaptability [33]. Such resistant cassava cultivars should be examined in the areas where the deployment is intended before mass production could be embarked upon. In addition, these cultivars could be used as the sources of resistant germplasm in breeding programs and could be used for developing new resistant varieties.

Nine out of eleven cassava cultivars tested using potted plants were classified to the same groups of resistance/susceptibility similar to *in vitro* plants. This confirms the reliability and robustness of leaf clipping for discriminating CBB-resistant and susceptible cultivars *in vitro*. The screening procedure using *in vitro* plants offers huge potential for the rapid testing of large number of cultivars for resistance to CBB and other bacterial diseases. The response to CBB can be demonstrated within 3 weeks using this novel technique and highly susceptible germplasm can be identified and eliminated from further evaluations. Developing resistant/tolerant cassava cultivars is one of the best approaches in the management of CBB, cheaper to the farmers and safer to environment. Attempts at developing cassava genotypes resistant to CBB using conventional breeding techniques face significant hurdles mainly because most cultivars have low fertility, and unsynchronized flowering [34]. Therefore, promising resistant clones can be developed through non-conventional breeding techniques such as genetic engineering, mutations and somaclonal variations and could be screened using the *in vitro* bioassay directly. In addition, considering cassava germplasm is often exchanged or released as *in vitro* conserved plantlets, the bioassay would also have application in assessment of newly introduced accessions.

The majority of the cultivars evaluated in this study were susceptible or highly susceptible to *Xam*. This highlights the need for development of resistant cultivars for CBB disease control and such need is particularly required in farmer-preferred cultivars.

5. Conclusions and Recommendations

In the present study, 21 cassava cultivars were evaluated for their reaction to *Xam* pathogen using artificial inoculation of *in vitro* and potted plants. The cultivars varied in their reaction to the pathogen, including incubation period, wilt incidence and AUDPC value. Among the 21 cassava cultivars evaluated, four (98/05, 30572, Kibaha and TME419) were resistant, four namely Albert, Ebwanatereka, Karibuni and 92/0326 were moderately resistant, while eleven cassava cultivars namely Muzege, Serere, 98/0581, Namikonga, Kibandameno, Mzalauka, TME7, AR40-6, Tajirika, Shibe and 01/1371 were categorized as susceptible. The other two cassava cultivars (Kiroba and Numbari) were found to be highly susceptible to *Xam* pathogen. The present study identified four resistant cassava cultivars to the pathogen. This demonstrates the existence of resistance cultivars against CBB within Kenyan cassava germplasm that could potentially be used for breeding resistant cultivars. Farmers should be encouraged to incorporate these resistant clones in combination with other effective control measures into their farming systems. On the other hand, this study considered only 21 cassava cultivars from Kenya. However, cassava plant is genetically diverse in different locations and zones of Kenya. Therefore, it is recommended that all cassava cultivars be collected and evaluated for their reaction to the pathogen.

In this study, only one virulent pathogenic *Xam* isolate was used. These cultivars should also be further evaluated against a large number of *Xam* isolates after being well-characterized into races or biotypes in order to broaden the findings on resistance. Additionally, the cassava cultivars that showed a resistant or tolerant reaction to the wilt pathogen should be further evaluated against *Xam* isolate under field conditions. The current work alone cannot be conclusive; it is believed that the results obtained were facilitating further works for the satisfactory control of the bacterial disease of cassava in the country. However, more research is needed considering the various cassava cultivars from the different cassava-growing regions and the future use of molecular techniques to produce markers linked to tolerance in cassava cultivars.

Competing Interests

The authors declare no conflict of interests.

References

[1] FAO (2013). FAOSTAT database collections. Food and Agriculture Organization of the United Nations. Rome. Access date: 2016-12-22. URL: http://faostat.fao.org.

[2] Jansson, C., Westerbergh, A., Zhang, J. Xinwen Hu, S. C, "Cassava, a potential biofuel crop in (the) People's Republic of China," *Applied Energy*, 86:S95-S99, 2009.

[3] Lukuyu, B., Okike, I., Duncan, A., Beveridge, M., Blummel, M, "Use of cassava in livestock and aquaculture feeding programs," ILRI Discussion Paper 25. Nairobi, Kenya: International Livestock Research Institute.

[4] Latif, S., Muller, J, "Potential of cassava leaves in human nutrition: a review," *Trends in Food Science and Technology*, 44: 147-158, 2015.

[5] Schlenker, W., Lobell, D.B, "Robust negative impacts of climate change on African agriculture," *Environmental Research Letters* 5:014010, 2010.

[6] Jarvis, A., Ramirez-Villegas, J., Herrera Campo, B.V, Navarro-Racines, C, "Is cassava the answer to African climate change adaptation?," Tropical Plant Biology 5: 9-29, 2012.

[7] Roudier, P., Sultan, B., Quirion, P., Berg, A, "The impact of future climate change on West African crop yields: what does the recent literature say," *Global Environmental Change-Human and Policy Dimensions*, 21: 1073-1083, 2011.

[8] Rosenthal, D.M., Slattery, R.A., Miller, R.E., Grennan, A.K., Cavagnaro, T.R., Fauquet, C.M., Gleadow, R.M., Ort, D.R, "Cassava about-FACE: greater than expected yield stimulation of cassava (*Manihot esculenta*) by future CO_2 levels," *Global Change Biology*, 18: 2661-2675, 2012.

[9] Herrera-Campo V, Hyman G, Bellotti A (2011), "Threats to cassava production known and potential geographic distribution of four key biotic constraints," *Food Security* 3: 329-345.

[10] Hillocks, R.J., Wydra, K, "Bacterial, fungal and nematode diseases," *Cassava: Biology, Production and Utilization*, 261-280, 2002.

[11] Banito, A., Kpemoua, K. E., Wydra, K, "Expression of resistance and tolerance of cassava genotypes to bacterial blight determined by genotype x environment interactions," *Journal of Plant Diseases and Protection*, 115(4):152-161, 2008.

[12] Wydra, K., Banito, A., Kpémoua, K.E, "Characterization of resistance of cassava genotypes to bacterial blight by evaluation of leaf and systemic symptoms in relation to yield in different ecozones," *Euphytica*, 155(3):337-348, 2007.

[13] Moses, E., Asafu-Agyei., J.N., Adubofuor, K., Augustine, A, "Guide to identification and control of cassava diseases," *Ashmetro prints,* Kumasi, Ghana, *http://www.isppweb.org/fstf/Cassava%20Diseases%20Guide.pdf*.

[14] Verdier, V., Ojeda, S., Mosquera, G, "Methods for detecting the cassava bacterial blight pathogen: a practical approach for managing the disease," *Euphytica*, 120(1): 103-107, 2001.

[15] Xin, L., Chaoping, L., Tao, S., Guixiu, H, "Bactericide Screening against *Xanthomonas axonopodis* pv. *manihotis*," *Plant Diseases and Pests*, 5(1): 1-3, 2014.

[16] Ogunjobi A.A., Fagade, O.E., Dixon, A.G.O., Bandyopadhyay, R, "Assessment of large population of cassava accessions for resistant to cassava bacterial blight infection in the screen house environment," *Journal of Agricultural Biotechnology and Sustainable Development*, 2(6): 87-91, 2010.

[17] Ndung'u, J.N., Wachira, F.N., Kinyua, M.G., Lelgut, D.K., Njau P., Okwaro, H.,. Obiero, H, "Genetic diversity study of Kenyan cassava germplasm using simple sequence repeats," *African Journal of Biotechnology*, 13(8): 926-935, 2014.

[18] Chege. MN, "Phenotypic and genotypic diversity of *Xanthomonas axonopodis* pv. *manihotis* causing bacterial blight disease of cassava in Kenya," MSc Thesis, Jomo Kenyatta University of Agriculture and Technology, 2017.

[19] Jorge, V., Fregene, M.A., Duque, M.C., Bonierbale, M.W., Tohme, J., Verdier, V, "Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz)," *Theoretical and Applied Genetics*, 101(5-6): 865-872, 2000.

[20] Muñoz Bodnar, A., Gómez, C., Mariel, L., Bernal, A., Szurek, B., López Carrascal, C.E, "Comparing Inoculation Methods to Evaluate the Growth of *Xanthomonas axonopodis* pv. *manihotis* on Cassava Plants," *Acta Biológica Colombiana*, 20(2): 47-55, 2015.

[21] Jorge, V., Verdier, V, "Qualitative and quantitative evaluation of cassava bacterial blight resistance in F1 progeny of a cross between elite cassava clones," *Euphytica*, 123(1): 41-48, 2002.

[22] Wydra, K., Zinsou, V., Jorge, V., Verdier, V, "Identification of pathotypes of *Xanthomonas axonopodis pv. manihotis* in Africa and detection of quantitative trait loci and markers for resistance to bacterial blight of cassava," *Phytopathology*, 94(10): 1084-1093, 2004.

[23] Kim, J., Taylor, K., Hotson, A., Keegan, M., Schmelz, E.A., Mudgett, M, "XopD SUMO Protease Affects Host Transcription, Promotes Pathogen Growth, and Delays Symptom Development in *Xanthomonas*-Infected Tomato Leaves," *Plant Cell*, 20(7): 1915-1929, 2008.

[24] Zinsou, A.V, "Studies on host plant resistance to cassava bacterial blight in combination with cultural control measures in ecozones of West Africa," Doctoral thesis. University of Hannover. Germany, 2003.

[25] Akhtar, M. A., Rafi, A., Hameed, A, "Comparison of methods of inoculation of *Xanthomonas oryzae pv. oryzae* in rice cultivars," *Pakistan Journal of Botany*, 40(5): 2171-2175, 2008.

[26] Ruz, E.L., Moragrega, G.C., Montesinos, S.E, "Evaluation of four whole-plant inoculation methods to analyze the pathogenicity of *Erwinia amylovora* under quarantine conditions," *International Microbiology*, 11(2): 111-119, 2008.

[27] Banito, A., Kpémoua, K. E., Wydra, K, "Screening of cassava genotypes for resistance to bacterial blight using strain × genotype interactions," *Journal of Plant Pathology*, 92(1): 181-186, 2010.

[28] Zinsou, V., Wydra, K., Ahohuendo, B., Schreiber, L, "Leaf waxes of cassava (*Manihot esculenta Crantz*) in relation to ecozone and resistance to *Xanthomonas* blight," *Euphytica*, 149(1-2): 189-198, 2006.

[29] Atkinson, M.M., Huang, J.S., Knopp, J.A, "The Hypersensitive Reaction of Tobacco to Pseudomonas syringae pv. pisi," *Plant Physiology*, 79(3): 843-847, 1985.

[30] Chen, C.H., Lin, H.J., Ger, M.J., Chow, D., Feng, T.Y, "The cloning and characterization of a hypersensitive response assisting protein that may be associated with the harpin-mediated hypersensitive response," *Plant Molecular Biology*, 43: 429-438, 2000.

[31] Jeger, M.J., Viljanen-Rollinson, S.L.H, "The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars," *Theoretical and Applied Genetics*, 102: 32-40, 2001.

[32] Lozano, J.C., Labcrry, R, "Screening for resistance to cassava bacterial blight," *Growth*, 1(2):3, 1982.

[33] Dixon, A.G.O., Ngeve, J.M., Nukenine, E.N, "Genotype X environment effects on severity of cassava bacterial blight disease caused by *Xanthomonas axonopodis* pv. *manihotis*," *European Journal of Plant Pathology*, 108: 763-770, 2002.

[34] Ceballos, H., Iglesias, C.A., Perez, J.C., Dixon, A.G.O, "Cassava breeding: opportunities and challenges," *Plant Molecular Biology*, 56: 503-516, 2004.