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Rapid screenhouse assessment of bacterial blight in cassava genotypes in Nigeria

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Bacterial blight disease caused by *Xanthomonas axonopodis* pv. *manihotis* (Berthet-Bondar) Dye was assessed in 11 artificially inoculated cassava genotypes in a screenhouse. Disease progress was estimated at intervals of 3 days by measuring the length of necrotic lesions on stems and leaves, as well as estimating the average disease score and area under disease progress curve (AUDPC). Based on the average disease scores, cassava genotypes 30572, TME 1, TME 7 and TME 9 were classified as resistant to bacterial blight, genotypes 4(2)1425, TME 2, TME 4 and TME 12 were tolerant while cassava genotypes 30001, TME 3, and TME 28 were susceptible. Direct correlations, statistically significant at $p < 0.05$, were obtained between stem necrosis, leaf necrosis, average disease scores and AUDPC in the 11 cassava genotypes. Screenhouse experiments afford rapid assessment of resistance status of cassava genotypes to bacterial blight in Nigeria.

Keywords: bacterial blight; cassava genotypes; rapid screening; screenhouse; Nigeria

Introduction

Cassava (*Manihot esculenta* Crantz) is the world seventh most important crop and constitutes a staple food for an estimated 800 million people, representing – over one-eighth of the world population (CIAT 1993; Nweke 1996).

The major constraints to stable production of cassava (*Manihot esculenta*) in Africa are diseases, insects, mites, weeds, soil and agronomic limitation and socio-economic factors. There are about 36 diseases of fungal, bacterial, mycoplasma and viral origin which attack cassava all over the world (Pillai et al. 1983). Of the economic diseases, African cassava mosaic, cassava bacteria blight and anthracnose are the most important (Mahungu et al. 1994). They cause an estimated 75% root yield reductions (Theirberge 1985).

Cassava bacterial blight (CBB) is a disease caused by *Xanthomonas axonopodis* pv. *manihotis* (formerly, *Xanthomonas campestris* pv. *manihotis*) (Vauterin et al. 1995) and losses due to this pathogen had been put at about 60% in Nigeria (Hahn et al. 1989).

It is important that any effort at improving cassava production must consider resistance or susceptibility status of cassava genotypes to diseases, especially CBB.

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Although the status of some cultivars and landraces are known, yet the status of some improved elite lines and genotypes still remain uncertain (Dixon et al. 1992). Consequently, there is need for a continuous evaluation of available cultivars and elite lines for resistance to diseases with a view to selecting promising cultivars and using them for the development of improved resistant cultivars.

The aim of this study is to conduct screenhouse assessment of bacterial blight disease in artificially inoculated cassava genotypes, and consequently, evaluate the resistance and susceptibility status of the genotypes based on their average disease scores.

Methodology

Preparation of soil

Top soil from the farmland of University of Agriculture, Abeokuta, Nigeria that had been left fallowed was collected. The soil was sterilised in the soil oven in the Department of Biological Sciences Laboratory, College of Natural Sciences (COLNAS) at 200°C for 2 h. The soil was left in the screenhouse and later packaged into 5000 cm^3 capacity polythene bags in a constructed screenhouse located near the Department of Crop Protection, College of Plant Science and Crop Production. The packaged soil was left for 2 weeks to stabilise.

Collection of bacterial isolate

Isolates of *X. axonopodis* pv. *manihotis* – the causative agent of cassava bacteria blight was collected from the stock cultures of Pathology Unit, International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria. The culture was maintained on Specific Xanthomonas (SX) medium slant at 4°C and was sub-cultured into Petri dishes when needed.

Preparation of bacterial inoculum

Specific Xanthomonas (SX) medium was inoculated with wire loops of *X. axonopodis* pv. *manihotis* and the plates were incubated at room temperature (about 28°C) for 24 h. The plates were aseptically flooded with 1 ml sterile water and the concentration of colony forming units (cfu) of the bacterium in 1 ml water was determined using UV/VIS spectrophotometer. The cells were serially diluted to 10^9 cfu/ml bacterial suspensions. The diluted bacterial suspension served as bacterial inoculum throughout the experiment.

Collection of cassava stem cuttings

Disease-free cassava stem cuttings of 11 genotypes were collected from the Crop Breeding Unit, International Institute of Tropical Agriculture, IITA, Ibadan, Oyo State, Nigeria. Stem cuttings were obtained from 10-month-old mother plants. The stems cuttings were labelled and moistened with sterile water in sterile polythene to prevent dryness of the cuttings prior to planting.

The genotypes collected were: 30001, 30572, 4(2)1425, TME 1, TME 2, TME 3, TME 4, TME 7, TME 9, TME 12 and TME 28.

At the point of planting, the cassava stem cuttings were immersed in a fungicide 0.05% solution of 0.05% benlate to control of fungal pathogens.

Design of experiment

The treated stem cuttings were planted in pre-sterilised soil in polythene bags in the screenhouse in a completely randomised design with three replicates. The pots were 0.5 m apart in a row with 10 pots per row.

Inoculation of leaves and stems of cassava plants

Inoculation was carried out in the screenhouse from the prepared inoculum. One millilitre of the bacterial suspension was inoculated onto the third and fourth leaves from the apex of the plant by direct infiltration so as to prevent mechanical damage to the leaves. Three plants per variety were inoculated. The control leaves were treated with sterile water. The plants were 6 weeks old at the time of inoculation.

For stem inoculation, sterile wooden stick was inserted into the 24-h old bacterial culture and used to stab-inoculate the cassava stem between the third and fourth leaves from the top. Sterile wooden stick inserted into sterile water was used in the control experiment. The experiment was replicated three times.

Symptom assessment

Assessment of symptom on infected stems and modified form of Restrepo's classification

Symptoms due to stem inoculations were monitored every 3 days. The disease was scored on a scale of 0 to 5, according to Restrepo et al. (2000) as shown in Table 1.

Restrepo et al. (2000) assessed plants having average disease score of 0–3 as incompatible (resistant) while those with average score of 4–5 were considered compatible (susceptible).

In this work, Restrepo's classification was modified to include those plants that could be considered as tolerant to bacterial blight. Table 2 shows the original Restrepo's classification and the modification made by this work.

Table 1. Disease scale for bacterial blight in cassava.

Symptom on infected stem	Disease score
Healthy plant, no reaction observed	0
Dark area or necrosis around point of inoculation	1
Gum exudates on stem around point of inoculation	2
Wilting of one or two leaves	3
Wilting of more than two leaves	4
Complete wilting and total dieback	5

Source: Restrepo et al. (2000).

Table 2. Classification of resistance status of cassava plants to bacterial blight.

Average disease score	Classification
Restrepo's classification[1]	
0–3	Resistant
4–5	Susceptible
Modified version[2]	
0–1	Resistant
2–3	Tolerant
4–5	Susceptible

[1]Restrepo et al. (2000); [2]Modified by the present work.

Assessment of symptoms on infected leave

Angular leaf spots around the point of inoculations were observed at intervals of 3 days. The size of the lesion on each inoculated leaf was measured with a ruler and mean values calculated per cassava genotype.

Area under disease progress curve (AUDPC)

The AUDPC was calculated for each inoculated plant from the average disease score, according to the technique of Shanner and Finney (1977). Mean values were then obtained per cassava genotype.

AUDPC formula used for calculation was:

$$\Sigma_i[(D_i + D_i - 1) \times (t_i - \mathrm{t_i} - 1)]/2,$$

where D is the disease score using the 0 – 5 severity scale, t is days after inoculation and $i = 3, 6, 9, \ldots, 30$ days after inoculations.

Statistical analysis

Data were subjected to analysis of variance (Genstat 2008). Treatment means indicating significant difference were separated using Fischer protected least significant difference (LSD) at 5% probability level.

Correlation analysis was done to establish the relationship among the various indices of disease establishment (leaf spot, stem necrosis, average disease score and AUDPC).

Results

The area of leaf lesions due to bacterial blight disease in the 11 cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis* is shown in Figure 1. Elite line 30572 and local varieties TME 1 and TME 2 had the least necrotic lesion where lesion size was lower than 0.15 cm^2. Other elite lines and local varieties had larger necrotic sizes.

Table 3 showed the mean area of leaf lesions (cm^2) induced in Cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*. The necrotic lesions were minimal in cassava elite line 30572 and local varieties TME 1 and TME 2. In these three, necrotic lesions were lower than 0.15 cm^2. All the other genotypes had

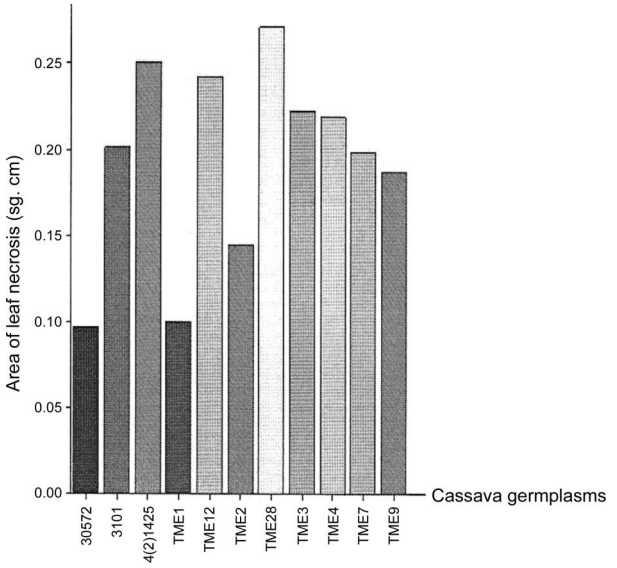

Figure 1. Area of leaf lesion in cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

Table 3. Mean area of leaf lesions (cm^2) induced in cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

	Area of leaf lesion (days after inoculation)			
Germplasm	3	6	9	Mean
30001	0.09	0.28	0.28	0.2011
30572	0.07	0.10	0.11	0.9607
4(2)1425	0.13	0.24	0.47	0.2506
TME 1	0.00	0.09	0.13	0.1000
TME 2	0.11	0.22	0.28	0.1444
TME 3	0.11	0.26	0.28	0.2222
TME 4	0.11	0.26	0.31	0.2189
TME 7	0.13	0.28	0.28	0.1989
TME 9	0.15	0.26	0.28	0.1867
TME 12	0.13	0.28	0.31	0.2422
TME 28	0.11	0.26	0.34	0.2711
SED				0.0349
LSD (5%)				0.0688

larger necrotic sizes. However, disease started in these germplasms as small dark brown necrotic specks on the leaves within 3 days of inoculations. Only TME 1 had no such symptom at Day 3. Also, the speck was largest in TME 9 at Day 3 with a necrotic area of 0.15 cm^2 on the leaves. By the ninth day, elite line 4(2) 1425 had the largest necrotic lesion area of 0.47 cm^2.

Reactions of cassava germplasms to stem inoculation are shown in Figure 2. Cassava elite line 30572 and local varieties TME 1 and TME 7 had the least record of

stem necrosis. Genotype 30572 and TME 1 also recorded the least AUDPC as shown in Figure 3.

Figure 3 also showed that the local cassava germplasms were more affected with stem necrosis than the elite ones with a general trend of high-lesion size in local cassava germplasms when compared with elite lines. The elite lines however became gradually susceptible as the days after inoculation increased.

Local germplasm (TME series) developed leaf necrosis faster and more in extent than elite line [30001, 30572 and 4(2)1425] as shown in Figure 4. There was however a sharp reduction in leaf necrosis 6 days after inoculation. By the ninth day, the area of necrotic leaves had fallen below the values in elite germplasm.

In Figure 5, the two sets of germplasms have similar extent of stem necrosis up till the ninth day after inoculation. The local germplasm thereafter remained higher in extent of stem necrosis while the elite germplasms had reduced stem necrosis. The

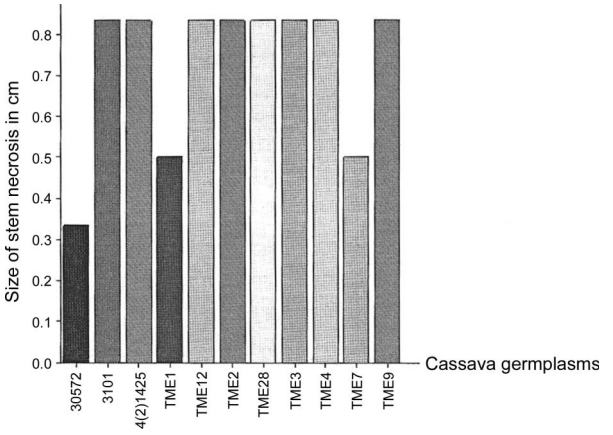

Figure 2. Stem necrosis in cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

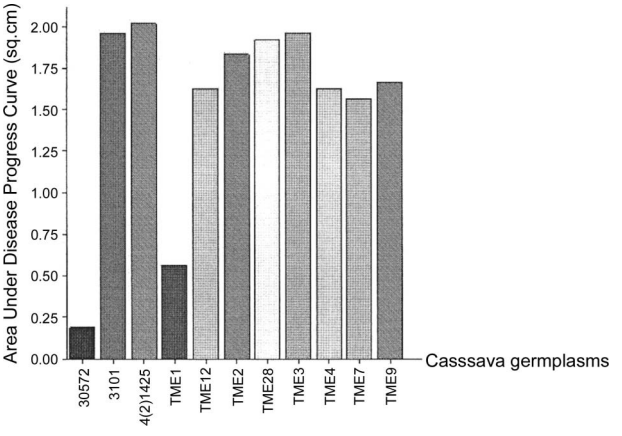

Figure 3. AUDPC of cassava germplasms inoculated with *X. axonopodis* pv. *manihotis*.

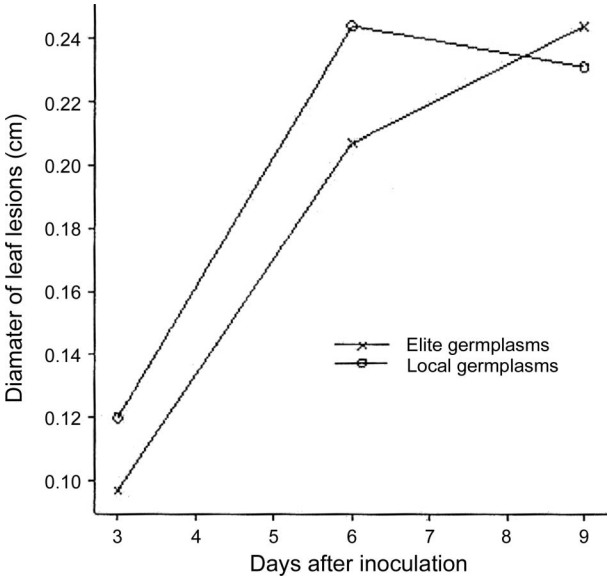

Figure 4. Development of leaf necrosis in elite and local cassava germplasms inoculated with *X. axonopodis* pv. *manihotis*.

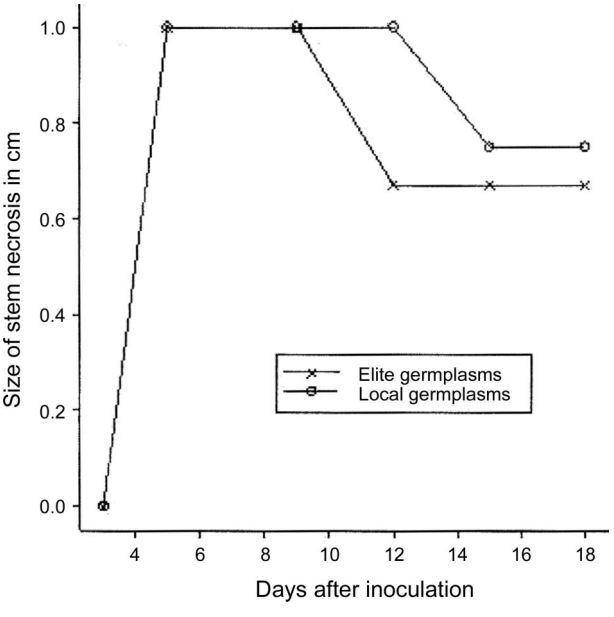

Figure 5. Development of stem necrosis in elite and local cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

difference recorded in the two sets of germplasms was more pronounced with regards to AUDPC. Local germplasms had greater AUDPC all through the experimental period (Figure 6).

Table 4 showed the various indices of bacterial blight diseases in the cassava germplasm and the LSD values at 5% for each index. The germplasm type had statistically significant effect on the indices of disease establishment. Germplasm 30572 had significantly low values for these disease indices. TME 1 followed in the record of low values.

Table 5 shows the bacterial blight rating of cassava germplasms. With this modified rating, three cassava genotypes (30001, TME 3 and TME 28) were

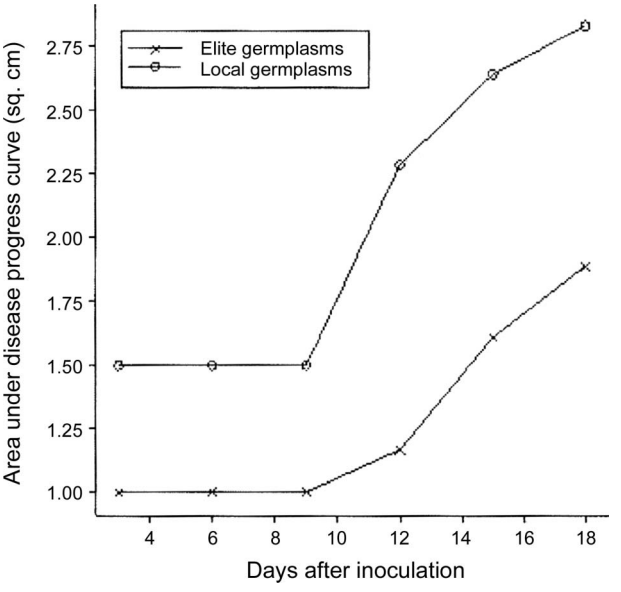

Figure 6. Area under disease progress curves for elite and local cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

Table 4. Leaf and stem necrosis, average disease score and area under disease progress of Cassava germplasms after inoculation with *X. axonopodis* pv. *manihotis*.

Cassava germplasm	Area of leaf lesion (cm^2)	Area of stem necrosis (cm^2x)	Area under disease progress curve
30001	0.2011	0.8330	1.9580
30572	0.9607	0.3330	0.1880
4(2) 1425	0.2506	0.8330	2.0190
TME 1	0.1000	0.5000	0.5630
TME 2	0.2422	0.8330	1.8330
TME 3	0.2222	0.8330	1.9580
TME 4	0.2189	0.8330	1.6250
TME 7	0.1989	0.5000	1.5620
TME 9	0.1867	0.8330	1.6660
TME 12	0.2422	0.8330	1.6250
TME 28	0.2711	0.8330	1.9160
SED	0.0349	0.9760	0.4043
LSD (5%)	0.0688	0.1919	0.8051

susceptible to bacterial blight; four (4(2)1425, TME 2, TME 4 and TME 12) were tolerant and four (30572, TME 1, TME 7 and TME 9) were resistant to bacterial blight disease caused by *X. axonopodis* pv. *manihotis*.

Table 6 showed the correlation among the indices of CBB. There was a high correlation between the AUDPC and stem necrosis ($r > 0.8$) while minimal correlation existed between the area of leaf lesion and stem necrosis ($r = 0.5$).

Table 7 showed the analysis of variance (ANOVA) of the symptoms of the cassava germplasms inoculated with *X. axonopodis* pv. *manihotis*. The ANOVA of the symptoms indicated significant differences between leaf lesion and stem necrosis. Mean square for stem necrosis was higher than that of the leaf lesion showing that there was more variation than that of the leaf lesion. The mean square of the AUDPC was the highest thus indicating that the aggressiveness of the disease was higher in the AUDPC than in leaf lesion and stem necrosis.

Table 5. Average disease score and CBB rating of cassava germplasms.

Genotype	Average disease score	CBB rating*
30001	4.000	Susceptible
30572	0.330	Resistant
4(2)1425	2.670	Tolerant
TME 1	0.133	Resistant
TME 2	2.330	Tolerant
TME 3	4.000	Susceptible
TME 4	1.330	Tolerant
TME 7	0.330	Resistant
TME 9	1.000	Resistant
TME 12	1.330	Tolerant
TME 28	4.000	Susceptible

*Rating based on Restrepo et al. (2000), modified as indicated in Table 2.

Table 6. Correlation coefficients among the indices of CBB disease rating –AUDPC, disease score, area of leaf lesion and necrotic stem.

Indices	AUDPC	Disease score	Lesion area	Necrotic stem
AUDPC	1.000000			
Disease score	0.733211	1.00000		
Lesion area	0.732129	0.517882	1.00000	
Necrotic stem	0.857325	0.73174	0.71933	1.00000

Table 7. Analysis of variance (ANOVA) of symptoms developed and AUDPC of genotypes after inoculation with *X. axonopodis* pv. *manihotis* using a general linear model.

Source	df	SS	MS	*F*-value
Genotypes	10	0.60803	0.06080	<0.001
Leaf lesion	187	2.04567	0.01094	<0.001
Stem necrosis	385	66.0000	0.17140	<0.001
AUDPC	77	50.3442	0.65380	<0.001

df, degree of freedom; SS, sum of squares; MS, mean square. Components of variance are genotypes, leaf lesion, stem necrosis and area under disease progress curve (AUDPC).

Discussion

Necrotic lesions developed within 3 days of inoculation on both leaves and stem that were inoculated with *X. axonopodis* pv. *manihotis*. The lesion kept on enlarging in genotypes that were susceptible as also reported by Maraite (1993), while it coalesced and dried up in resistant genotypes. Developed leaf lesion also led to the wilting of the leaves after 9–15 days of inoculation in six genotypes: 30001, TME 2, TME 3, TME 4, TME 7 and TME 9. In germplasm 4(2)1425, TME 12 and TME 28 the leaves wilted in 12 days. For germplasms 30572 and TME 1, it took 15 days after inoculation for the leaves to wilt.

Genotypes 30001, TME 3 and TME 28 had the highest disease score while 30572 and TME 7 had the least.

AUDPC was least in germplasm 30572 while 4(2)1425 had the highest. The AUDPC for 30001 and TME 3 and were high (2.83) from 21 days after inoculation. The curve however stopped enlarging for genotypes 30572 and TME 1 by 3 and 12 days after inoculation, respectively.

Analysis showed that there was no correlation between resistance and method of inoculation (stem or leaf). The lack of correlation between the leaf and stem inoculations is probably due to the fact that resistance mechanisms occur in stem vascular tissue, as parenchyma cells in the phloem or adjacent to the xylem play important role in resistance as reported in previous studies (Kpermoua et al. 1996). There was however correlation between the stem necrosis and AUDPC which may be due to the fact that the parameters were taken from stem inoculation.

Stem inoculation seemed to be a better method of evaluating resistance to bacterial blight as necrotic lesion on the leaves measured above 0.25 cm^2 recorded in the leaf inoculation while the stem necrosis size was as high as 0.80 cm^2. Further experiment showed greater damage to parts of the plant above the point of inoculation on the stem which could be as a result of upstream movement of water and nutrients from the root region to other parts of the plant above the ground level.

Stem inoculation technique also allowed assessment of AUDPC that showed distinctly improved line 30572 and local variety TME I being below 0.75 cm^2 while all other genotype were above 1.50 cm^2. AUDPC shows the aggressiveness of the disease reaction from one germplasm to the other (Restrepo et al. 2000), thereby allowing variance analysis to be completed.

Progressive increment was observed in the development of leaf necrosis as the days after inoculation was also increasing until the final wilting of leaf which could be due to stress inflicted on the leaf stalk.

Conclusion

The results of this study showed that cassava genotype could be screened for resistance or susceptibility within the screenhouse. The condition within the screenhouse allows for development of symptoms that can be used in the classification of cassava genotype.

It can also be concluded that within the screenhouse genotype 30572, TME1, TME 7 and TME 9 are resistant to CBB; 4(2)1425, TME 2, TME 4 and TME 12 are tolerant while 30001, TME 3 and TME 28 are susceptible.

Screenhouse evaluation of resistance to pathogen is therefore a viable and a rapid option while determining susceptibility of cassava germplasms to bacterial blight.

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