

## REACTION OF INTRODUCED YELLOW CASSAVA TO A CASSAVA BACTERIAL BLIGHT ISOLATE IN PORT HARCOURT, NIGERIA

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### Abstract

Two Ghanaian lines and five IITA unreleased lines of yellow cassava were tested for their susceptibility to a cassava bacterial blight (CBB) disease. They were evaluated together with two local white lines which were known to be highly susceptible to the disease. Two trials each were conducted in the greenhouse under artificial irrigation and on the field under rain-fed conditions. The greenhouse work was done between January and July, 1986 while the field experiments were performed from April to November, 1986. In both the greenhouse and field experiments conducted during the period when the disease was reported to affect cassava in Nigeria (April to September), one of the introduced yellow lines showed a high susceptibility level (3.8) similar to the two local white lines (3.0 and 3.6). The six other introduced lines were moderately resistant (2-2.4). In the field trial conducted between August and November, however, all the lines including the local white ones showed high resistance (1.0-1.3).

### Introduction

Cassava (*Manihot esculenta* Crantz) is an important tropical root crop. In terms of the colour of the root pulp, two major varieties, white and yellow, exist worldwide (Oduro, 1981). Whereas only the white variety exist in Port Harcourt area of Rivers State of Nigeria, both white and yellow varieties exist elsewhere especially Ghana where yellow 'gari' and 'fufu' of the latter variety have been popular for a long time (Oduro, 1981; Oduro, 1984).

Unlike the white cassava, yellow cassava is reported to have a high betacarotene content and, therefore, is rich in vitamin A (Maravalhas, 1964; McDowell & Oduro, 1983; Safo-Kantanka *et al.*, 1984). Attempts were, therefore, made to introduce this variety to Port Harcourt, Nigeria by the principal author.

An important cassava disease in Port Harcourt area was cassava bacterial blight (CBB). This study was thus carried out to ascertain the susceptibility or otherwise of the yellow cassava lines introduced into the area.

### Experimental

#### *Planting materials*

A field and a greenhouse (pot) experiments were conducted on seven lines of the yellow cassava variety which had not been previously cultivated in the Port Harcourt area and two locally-cultivated white cassava lines. The latter were known to be susceptible to the cassava bacterial blight (CBB), and were included in the study to assess the degree of susceptibility of the introduced yellow cassava. They were RWG (white cassava with green petiole in Rivers State) and RWR (white cassava with pink/red petiole in Rivers State).

Five of the yellow cassava were unreleased lines from the International Institute for Tropical Agriculture (IITA). These were CB 1981, CB 71673, CB 71693, CBR5-10-80411 and 82/01256 code named IITA<sub>1,3</sub>, IITA<sub>5</sub> and IITA<sub>8</sub> respectively in this study. The two other yellow cassava lines were BB<sub>4</sub> and BB<sub>5</sub> (Oduro, 1981) from Ghana, code-named respectively GBB<sub>1</sub> and GBB<sub>2</sub> in this study. The pulp characteristics of all the nine lines are presented in Fig. 1.

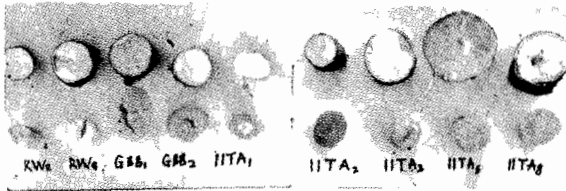


Fig.1. Cross section of fresh (above) and boiled (below) root pulps of the introduced yellow cassava lines and the two local white lines in Port Harcourt, River State, Nigeria, harvested 9 months after planting. Root pulps were boiled separately for 30 min in beakers containing distilled water. RWR and RWG = Two local white varieties; GBB<sub>1</sub> and GBB<sub>2</sub> = Two yellow cassava from Ghana; IITA<sub>1-3</sub>, IITA<sub>4</sub> and IITA<sub>5</sub> = Five yellow cassava from IITA.

### Planting

The two greenhouse trials were conducted from January to April, and from April to July, 1986. In both experiments, cuttings were planted in 16 cm × 30 cm black polyethylene bags filled with heat-sterilized loamy garden soil. There were 10 plots (replicates) per trial. In each plot there were two bags per line spaced 45 cm apart giving a total of 20 plants per line in each trial. The spacing between lines was 60 cm. All the bags which were to be given the same inoculation treatment in a plot were kept in the same column to prevent cross-infection from the inoculated to the non-inoculated plants. The positions occupied by the different lines were randomly determined in each plot. Watering in the greenhouse was done twice a week.

Two field trials were also carried out from April to July and from August to November, 1986 in the Rivers State University of Science and Technology (RSUST) Research Garden. The planting plan was the same as in the greenhouse/pot experiment; i.e. 45 cm between plants of the same line and 60 cm between plants of different lines within a plot. Ten plots were used in each experiment

and randomization of the lines was done for each plot. The cuttings to be inoculated were again paired with those to be used as controls. The field trials were rain-fed with no supplementary watering.

### Inoculation

In both the greenhouse and field trials, the leaf clipping technique was used for doing the inoculations 6 weeks after planting. The technique involved the clipping of the third- and fourth- leaf margins of the plants with sterilized scissors that had been dipped into a 48-h-old culture of bacterium, *Xanthomonas manihotis*, suspended in sterile distilled water (SDW) to give a concentration of  $2 \times 10^9$  cells/ml. The bacterium was isolated from diseased local white cassava and cultured on nutrient agar slants. The control plants were clipped with sterile scissors dipped in SDW. Immediately after treatment, all the plants in the greenhouse were humidified for 48 h by covering the leaves with transparent polyethylene bags moistened with SDW. Field inoculations, however, were done only in the evenings without covering the plants to prevent the leaves from getting scorched by the afternoon hot sun.

### Disease scoring

Observations of symptoms were made from the 5th day after inoculation. Scoring of the treated plants was based on CBB disease assessment 1-5 scale (Perreux, Terry & Persley, 1978) where

- 1 = No symptom
- 2 = Angular leaf spot
- 3 = Extensive wilting
- 4 = Defoliation and partial die-back
- 5 = Death of the entire plant

Mean scores which were used in rating the lines, were obtained from the 10 replicates of each line. The underlisted standard scale (Perreux, Terry & Persley, 1978) was used in scoring each cassava line.

Range of mean scores	Interpretation
1.0 - 1.4	Highly resistant (HR)
1.5 - 1.9	Resistant (R)
2.0 - 2.4	Moderately resistant (MR)
2.5 - 2.9	Moderately susceptible (MS)
3.0 - 3.4	Susceptible (S)
3.5 - 4 or 5	Highly susceptible (HS)

experiment, the following observations were made.

Within 7 days after inoculation in the January - April trial, water-soaked angular leaf spots were observed along the clipped margins of all the bacterial-treated plants of the nine lines. The spots enlarged and coalesced resulting in blight and abscission of all the clipped leaves within 14 days after inoculation. There was no further disease

TABLE 1

Severity of bacterial blight symptoms on various lines of cassava 70 days after artificial inoculation of *X. manihotis* by the leaf clipping method in the greenhouse (January-April 1986)

Cassava lines	Treatment	Number of plants per score of 1-5					Mean score
		1	2	3	4	5	
IITA <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>2</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>3</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>5</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>8</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>2</sub>	x	-	-	-	10	-	4.0
	w	10	-	-	-	-	1.0
RWG	x	-	-	-	10	-	4.0
	w	10	-	-	-	-	1.0
RWR	x	-	-	-	10	-	4.0
	w	10	-	-	-	-	1.0

Note: 1. Ten homogeneous clones were used for each line.

2. Disease severity was scored on a scale of 1 - 5, where 1 = No symptoms; 2 = Angular leaf spot;

3 = Extensive wilting; 4 = Defoliation and partial die-back; 5 = Death.

3. x = *X. manihotis*; w = sterile distilled water.

## Results

### Greenhouse experiment

When cassava plants in all the nine lines were inoculated in the January-April 1986 greenhouse

development in the remaining plant parts of any of the IITA and GBB<sub>1</sub> lines. However, inoculated plants of the GBB<sub>2</sub> line and the two local white lines in all the replicates, in addition, showed gummy exudates from the stem and petioles, leaf

TABLE 2  
Severity of bacterial blight symptoms on various lines of cassava 70 days after artificial inoculation of *X. manihotis* by the leaf clipping method in the field (April - July 1986)

Cassava lines	Treatment	Number of plants per score of 1-5					Mean score
		1	2	3	4	5	
IITA <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>2</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>3</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>5</sub>	x	-	8	-	2	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>8</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>2</sub>	x	-	-	2	6	2	4.0
	w	10	-	-	-	-	1.0
RWG	x	-	-	4	6	-	3.6
	w	10	-	-	-	-	1.0
RWR	x	-	-	2	8	-	3.8
	w	10	-	-	-	-	1.0

Note: 1. Ten homogeneous clones were used for each line.

2. Disease severity was scored on a scale of 1-5, where 1 = No symptoms; 2 = Angular leaf spot; 3 = Extensive wilting; 4 = Defoliation and partial die-back; 5 = Death.

3. x = *X. manihotis*; w = Sterile distilled water.

wilt and tip die-back (Table 1).

When the greenhouse experiment was repeated in April-July 1986, the results obtained were fairly comparable (Table 2). After the initial leaf spots, blight and abscission of the clipped leaves, there was no further disease development in all the IITA lines and GBB<sub>1</sub> except in GBB<sub>2</sub>, RWR and RWG. Six plants of GBB<sub>2</sub> further produced die-back symptoms (Fig. 2) while two died. Six of the RWG and eight of the RWR also showed die-back symptoms in addition (Table 2).

The mean score was 2.0 each for IITA<sub>1,3</sub>, IITA<sub>5</sub>,

IITA<sub>8</sub> and GBB<sub>1</sub> while RWG, RWR and GBB<sub>2</sub> had 3.6, 3.8 and 4.0 respectively. This indicated that the five IITA lines and GBB<sub>1</sub> were moderately resistant while RWG, RWR and GBB<sub>2</sub> were highly susceptible (Table 3).

No symptoms were observed in the SDW-treated plants in all the nine cassava lines.

#### Field experiments

The results of the field experiment in April - June 1986 were essentially similar to those of the greenhouse experiment in April-July 1986 (Table 4). All

TABLE 3  
Rating of various lines of cassava 70 days after artificial inoculation of *X. manihotis* using the leaf clipping method

Cassava Treatment lines	Mean scores in the greenhouse and rating			Mean score in the field and rating		
	Jan-Apr 1986	Apr-Jul 1986	Rating	Jul-Aug 1986	Rating	Aug-Nov 1986
HTA <sub>1</sub>	x 2.0	MR 2.0	MR	2.0	MR	1.0
	w 1.0	HR 1.0	HR	1.0	HR	1.0
HTA <sub>2</sub>	x 2.0	MR 2.0	MR	2.0	MR	1.2
	w 1.0	HR 1.0	HR	1.0	HR	1.0
HTA <sub>3</sub>	x 2.0	MR 2.0	MR	2.0	MR	1.2
	w 1.0	HR 1.0	HR	1.0	HR	1.0
HTA <sub>4</sub>	x 2.0	MR 2.0	MR	2.4	MR	1.3
	w 1.0	HR 1.0	HR	1.0	HR	1.0
IITA <sub>3</sub>	x 2.0	MR 2.0	MR	2.0	MR	1.0
	w 1.0	HR 1.0	HR	1.0	HR	1.0
GBB <sub>1</sub>	x 2.0	MR 2.0	MR	2.0	MR	1.0
	w 1.0	HR 1.0	HR	1.0	HR	1.0
GBB <sub>2</sub>	x 4.0	HS 4.0	HS	3.8	HS	1.1
	w 1.0	HR 1.0	HR	1.0	HR	1.0
RWC	x 4.0	HS 3.6	HS	3.6	HS	1.2
	w 1.0	HR 1.0	HR	1.0	HR	1.0
RWR	x 4.0	HS 3.8	HS	3.0	SS	1.3
	w 1.0	HR 1.0	HR	1.0	HR	1.0

Note: 1. Figures were averaged from 10 plants per treatment.

2. Disease rating for resistance was according to the following criteria:

1.0-1.4 = HR (Highly resistant) 2.5-2.9 = MS (Moderately susceptible)

1.5-1.9 = RR (Resistant) 3.0-3.4 = SS (Susceptible)

2.0-2.4 = MR (Moderately resistant) 3.5-4.0 = HS (Highly susceptible)

3. x = *X. manihotis*, w = Sterile distilled water



Fig. 2. Reaction of an introduced yellow cassava line GBB<sub>2</sub> to a strain of *Xanthomonas manihotis* in a greenhouse experiment. Left: Control plant treated with sterile distilled water. Right: Leaf wilt without abscission, and tip die-back of the shoot 39 days after inoculation with *X. manihotis* using leaf clipping technique.

the nine lines showed water-soaked angular leaf spots along the clipped margins of the leaves within 7 days after inoculation followed by blight and abscission of clipped leaves within 14 days. There was no further disease development on any of the lines except IITA<sub>5</sub>, GBB<sub>2</sub>, RWG and RWR.

Gummy exudates came from the stems and petioles of the leaves followed by extensive wilting in two plants each of GBB<sub>2</sub> and RWR. Four plants of RWR and two each of RWG and IITA<sub>5</sub> had partial die-back. In addition to this, the two IITA<sub>5</sub> plants developed stem necrosis (Fig. 3). Four plants each of GBB<sub>2</sub> and RWG died (Fig. 4) from the disease before the experiment was terminated 70 days after inoculation (Table 4).

The mean score each of IITA<sub>1,3</sub>, IITA<sub>5</sub> and GBB<sub>1</sub> was 2.0 while IITA<sub>5</sub> was 2.4 indicating that all the six lines were moderately resistant. On the other hand, RWR, RWG and GBB<sub>2</sub> had mean scores of 3.0, 3.6 and 3.8 respectively, indicating their susceptibility (Table 3).

When the field experiment was repeated in August-November 1986, the results were different from the previous experiments (Table 5). Only a few of the inoculated plants showed the water-soaked angular-leaf spots at the clipped edges.

These spots did not result in blight and abscission of the inoculated leaves and there was no further disease development.

The mean score of 1.0-1.3 for all the nine lines indicated that the lines were highly resistant (Table 3). In both field experiments, no symptoms were observed in the plants from all lines treated with SDW.

### Discussion

Results from the artificial inoculation of the nine cassava lines with a strain of *X. manihotis* showed that the data from the two greenhouse and one of the field experiments were generally similar.

All the five yellow IITA lines and one of the Ghanaian yellow cassava, GBB<sub>1</sub> were moderately resistant (2.0-2.4) while the other Ghanaian line GBB<sub>2</sub> was more susceptible (3.8) than the two local lines, RWR (3.0) and RWG (3.6).

The moderate resistance exhibited by the IITA lines might have been due to the fact that, those lines were bred for resistance to the cassava mosaic virus which was linked to resistance to the CBB pathogen (Hahn & Keyser, 1985). Considering the reaction of GBB<sub>1</sub> to the CBB pathogen (2.0), it might be concluded that the line was naturally moderately resistant since it was obtained from a land-race clone.

In contrast to the findings from the greenhouse and the first field trials (i.e. January-July and April-July respectively), the second/repeated field experiment (August - November) gave mean disease scores 1.0-1.3 for all the nine lines, including those found to be highly susceptible in the earlier work, making them highly resistant. According to Pacumbaba, Ezuma & Lukezo (1978), CBB infection is influenced by the amount of rainfall, relative humidity, temperature and other environmental parameters. Amount and frequency of rainfall and, consequently, relative humidity during the period of this second field experiment were much lower than during the April-July period, which marked the peak of the rainy season in the Port Harcourt area. These might have, therefore, affected the disease development and severity after artificial inoculation in the second field trial to give

TABLE 4  
Severity of bacterial blight symptoms on various lines of cassava 70 days after artificial inoculation of *X. manihotis* by the leaf clipping method in the field (April - July 1986)

Cassava lines	Treatment	Number of plants per score of 1-5					Mean score
		1	2	3	4	5	
IITA <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>2</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>3</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
IITA <sub>5</sub>	x	-	8	-	2	-	2.4
	w	10	-	-	-	-	1.0
IITA <sub>8</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>1</sub>	x	-	10	-	-	-	2.0
	w	10	-	-	-	-	1.0
GBB <sub>2</sub>	x	-	2	2	2	4	3.8
	w	10	-	-	-	-	1.0
RWG	x	-	4	-	2	4	3.6
	w	10	-	-	-	-	1.0
RWR	x	-	4	2	4	-	3.0
	w	10	-	-	-	-	1.0

Note: 1. Ten homogeneous clones were used for each line.

2. Disease severity was scored on a scale of 1-5, where 1 = No symptoms; 2 = Angular leaf spot; 3 = Extensive wilting; 4 = Defoliation and partial die-back; 5 = Death.

3. x = *X. manihotis*; w = Sterile distilled water.

the observed contrasting results. The findings also agreed with those of Terry (1976) that (1) the CBB disease was prevalent in Ibadan, Nigeria during the rainy season from April to September as the pathogen required moisture to enhance its survival and increase its chances of infecting, and (2) the disease incidence decreased and remained low from November to March.

In conclusion, cassava cultivation in Port Harcourt area was usually rain-fed so planting was expected to fall within the April - July period. Consequently, the results of these studies suggest that most of the introduced yellow cassava lines from IITA and GBB<sub>1</sub> hold promise for resistance to the CBB disease and could, therefore, be

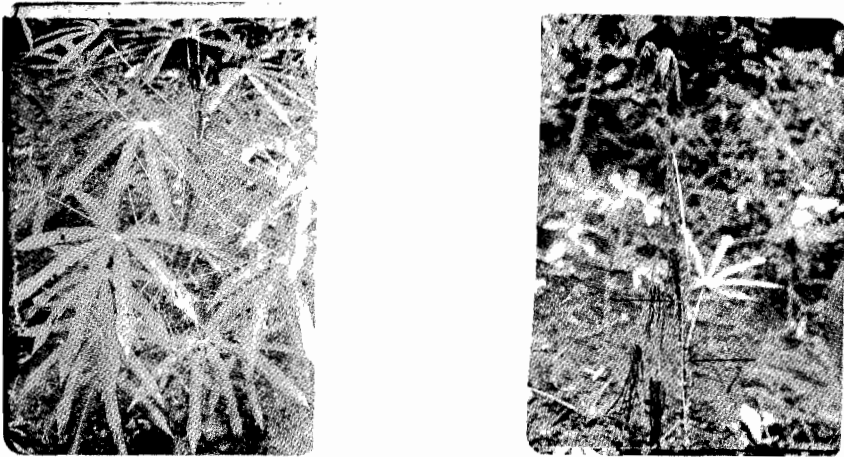


Fig. 3. Reaction of an introduced yellow cassava line, IITA, to *Xanthomonas manihotis* in field experiment. Left: Control treated with sterile distilled water. Right: Wilting, stem necrosis (arrow) and extensive defoliation 67 days after inoculation with *X. manihotis* using leaf clipping technique.



Fig. 4. Reaction of a local cassava line, RWG to the local *Xanthomonas manihotis* in the field experiment. Left: Control treated with sterile water. Right: Death of the entire shoot 51 days after inoculation with *X. manihotis* using leaf clipping technique.

investigated further to obtain the most suitable lines for the cultivation of the relatively more nutritious yellow cassava in the area.

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TABLE 5

Severity of bacterial blight symptoms on various lines of cassava 70 days after artificial inoculation of *X. manihotis* by the leaf clipping method in the field (April - July, 1986)

Cassava lines	Treatment	Number of plants per score of 1-5					Mean score
		1	2	3	4	5	
IITA <sub>1</sub>	x	10	-	-	-	-	1.0
	w	10	-	-	-	-	1.0
IITA <sub>2</sub>	x	8	2	-	-	-	1.2
	w	10	-	-	-	-	1.0
IITA <sub>3</sub>	x	8	2	-	-	-	1.2
	w	10	-	-	-	-	1.0
IITA <sub>5</sub>	x	7	3	-	-	-	1.3
	w	10	-	-	-	-	1.0
IITA <sub>8</sub>	x	10	-	-	-	-	1.0
	w	10	-	-	-	-	1.0
GBB <sub>1</sub>	x	10	-	-	-	-	1.0
	w	10	-	-	-	-	1.0
GBB <sub>2</sub>	x	9	1	-	-	-	1.1
	w	10	-	-	-	-	1.0
RWG	x	8	2	-	-	-	1.0
	w	10	-	-	-	-	1.0
RWR	x	7	3	-	-	-	1.3
	w	10	-	-	-	-	1.0

Note: 1. Ten homogeneous clones were used for each line.

2. Disease severity was scored on a scale of 1-5, where 1 = No symptoms; 2 = Angular leaf spot;

3 = Extensive wilting; 4 = Defoliation and partial die-back; 5 = Death.

3. x = *X. manihotis*; w = Sterile distilled water.

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