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Evaluation of cassava clones for resistance to anthracnose disease using phytotoxic metabolites of *Colletotrichum gloeosporioides f. sp. manihotis* and its correlation with field disease reactions

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ABSTRACT

***Colletotrichum gloeosporioides f. sp. manihotis*, the causal agent of cassava *(Manihot spp)* anthracnose disease produces toxic metabolites in culture. Symptoms induced on cassava leaves and stem cuttings by the toxic metabolites are similar to those induced by the pathogen. Twenty one *M. esculenta* clones were screened under field conditions using natural infection and with the phytotoxic metabolites of the pathogen. Results indicated that 8 cassava clones were resistant while the other 13 clones were susceptible at varying degrees. Results from *in vitro* screening of cassava using phytotoxic metabolites compared favorably with field screening based on natural infection. Use of toxic metabolites appears to be a more effective assay for screening cassava clones for resistance to anthracnose disease than those based on natural disease infection.**

Key words: Anthracnose, cassava, *Colletotrichum gloeosporioides*, *Manihot esculenta*, phytotoxic metabolites.

INTRODUCTION

Cassava (*Manihot esculenta*) is the second most important staple food (maize being the first) in Sub-Sahara Africa (IITA 1988). It is a source of calories for nearly 500 million people in the world (Cock 1985) and is one of the most efficient crops in terms of carbohydrate production (De-vries *et al.* 1967). However, its cultivation is being hindered by a series of factors of which disease is the most important in Africa (Lozano and Booth 1974). Of all diseases affecting cassava, cassava anthracnose is the most devastating (Hahn *et al.* 1988; 1989).

The disease is found wherever cassava is cultivated in West Africa. It has the potential of causing high yield loss and hence has been considered to be of great economic importance (IITA 1976).

The cassava anthracnose disease is caused by *Colletotrichum gloeosporioides f. sp. manihotis* Penz. (Chevaugeon 1956) which attacks stems, twigs, fruits and leaves (IITA 1990). Hahn *et al.* (1989) reported that the most significant effect of cassava anthracnose disease is the high reduction of healthy planting materials available to farmers. Although there are several control methods to effectively manage cassava athracnose (Hahn *et al.* 1988; 1989), the most effective approach would be the selection and breeding for disease resistant varieties (Nwankiti *et al.* 1987). Most of the existing methods of screening for resistant varieties in cassava, however, are time consuming, labour intensive and require a large amount of landspace. Phytotoxic metabolites of pathogens have been reported to play a significant role in pathogenesis (Chandraskharan and Ramakrishnan 1973, Walker and Templeton 1978; Amusa 1991). Phytotoxic metabolites have been employed in screening crops for disease resistance (Wheeler and Luke 1955; Hartman *et al.* 1986; Amusa *et al.* 1994). *C. gloeosporioides* has been reported to produce phytotoxic metabolites that induce symptoms similar to those of the pathogens (Sharma and Sharma 1969; Frantzeen *et al.* 1982; Amusa *et al.* 1993). This study was therefore designed to produce in culture and extract phytotoxic metabolites of *C. gloeosporioides* and to use them in the rapid screening of clones of cassava for disease resistance and to compare the results with the regular field screening.

MATERIAL AND METHODS

Field screening

Twenty one cassava clones including 2 checks

clones Tms 30211 (resistant) and Isunikankyan (susceptible) obtained from the Tuber Root Improvement Programme of IITA Ibadan , Nigeria were planted in a completely randomized design. Each plot (in quadruplicate) measured 6 x 5m $(30m)^2$ with 30 plants/clone. There was an unplanted row between each plot and the whole land was surrounded by a border row planted to Tms 30211. Manual weeding was done three times within the growing season. Between two and nine months after planting, cassava plants were observed for the natural development of anthracnose disease symptoms. Disease severity scores were based on the scale of 1 - 5, where class 1, no observable symptoms; class 2, development of shallow cankers on the lower part of the stem; class 3, development of successive cankers higher on the plant with the older cankers becoming larger and deeper; class 4, development of dark brown lesions on green shoots, petioles and leaves, young shoots collapsing and distorted and class 5, wilting and drying up of shoots and young leaves and death of part of or whole plant.

Furthermore three cankers/plant and 10 plants/clone, were randomly selected and the size of cankers was measured. The data collected were subjected to analysis of variance with inferences drawn from the analyzed data using Duncan's multiple range test. The experiment was conducted in 1995 and repeated in 1996.

Laboratory culture of pathogen

Cassava stems showing symptoms of anthracnose were collected from the field screening plot. The samples were washed in running tap water and the infected portions were excised and cut into small pieces. These were then surface sterilized using 10% NaOCl for 30s, and rinsed in four successive changes of sterile distilled water. The surface sterilized pieces were then plated onto potato - dextrose agar and the plates were incubated for 6 days at 26° C. The causative pathogen was identified by microscopic examinations as well as comparing the isolate with standards obtained from the Cassava Pathology Laboratory, IITA, Ibadan, Nigeria.

Toxin production

Each Erlenmeyer flask (250 ml) containing 100ml of sterilized Richard's medium (Sharma and Sharma 1969) was inoculated with three fungal mycelial disks (2cm diameter) cut from the edges of 5-day-old cultures of the pathogen. The flasks were shaken at 100 rpm for 21 days at 26°C under a 12 hrs. photoperiod. Mycelial mats were removed by passing the cultures through four layers of cheesecloth and the culture filtrate was passed through 0.45 µm millipore filter after which the filtrate was reduced to 1/10th of its original volume under vacuum at 40°C.

Bioassay

The leaf puncture bioassay method of Sugawara *et al.* (1978) was adopted for the rapid detection of the phytotoxic activities of metabolite of *C.gloeosporioides .f sp.manihotis.* Leaves and stems of cassava clone Isunikankyan grown in the green house for 8 weeks, were excised with a sterile razor blade at the petiole and were covered with wet sterile cotton. Ten microlitre aliquots of the concentrated metabolites were spotted on one half of the test plant, and on the stem cuttings (10cm). On the other half of the leaves, drops of uninoculated liquid culture (Richard's medium) were placed as control. The treated leaves and stems were placed in sterile petri - dishes incubated at 28°C and the plates were observed after 24 hrs and 72 hrs.

***In vitro* screening for disease resistance**

Twenty one cassava clones were screened with toxic metabolites of *C. gloeosporioides f sp manihotis* using leaf and stem puncture bioassay techniques. Stems and leaves from 2-month old cassava plants grown in green houses showing no symptoms of infection were excised, rinsed in running tap water, dipped in 10% NaOCl for 30s, and then rinsed again in 4 successive changes of sterile distilled water. The stems were then cut into 10 cm pieces. These and the leaves were individually placed in sterile Petri dishes lined with moistened sterile filter papers. Subsequently three 10µl droplets of the concentrated phytotoxic metabolites were placed on these plant parts, which were then punctured with sterile needles at the centre of each droplet. Ten leaves and stem cuttings were inoculated per each cassava clone and these were replicated three times. The punctured leaf surfaces were encircled by petroleum jelly to avoid the spread of the metabolites and were incubated for 24 hrs, while the stem cuttings were observed after 72 hrs. Distilled water and uninoculated Richard's medium were used as control. The experiments were performed in 1995 and repeated in 1996. The determination of susceptibility and resistance of cassava clones was based on modified lesion diameter scale of Goodie (1958) and Sudi and Podhardizky (1959) as follows: 3.0 - 8.0 mm = resistant; 8.1 - 14.9 mm = moderately susceptible; >15.0 mm = susceptible. The plant

reaction category was modified by transforming the lesion diameter value obtained to $\log_e$. The data were then subjected to analysis of variance and Duncan's multiple range test was used to separate the means at $p = 0.05$.

RESULTS

Results of field screening showed that cassava clones 95/0084, 95/0076, 95/0087, 95/0045 95/0018, 95/0078, 95/0079, 95/0088 and Isunikankiyan had disease scores between 3.00 and 4.10 (Table 1). These cassava clones were regarded as susceptible. Cassava clones 95/0057, 95/0056, 95/0022, 95/0030, 95/0014, Tms 30211, 95/0027 and 95/0025 had disease scores between 1.00 and 1.60 and were regarded as resistant. The other 4 clones which had disease scores between 2.00 and 2.90 were regarded as moderately susceptible.

The results of the field experiments also revealed that 8 cassava clones had not more than 6 necrotic lesions (canker) per plant. These clones were regarded as resistant. Another 3 cassava clones with 6.10 to 14.00 necrotic lesions per plant were regarded as moderately susceptible. The other 10 cassava clones had more than 14 necrotic lesions per plant and these were regarded as susceptible to the disease (Table 1).

The metabolites of *C. gloeosporioides f. sp. manihotis* in culture were phytotoxic to cassava leaves and stem cuttings. The symptoms produced by the metabolites were similar to those induced by the pathogen on cassava plants.

Results of *in vitro* screening for resistance using toxic metabolites revealed that cassava clones 95/0057, 95/0056, 95/0022, 95/0020, Tms 30211, 95/0014, 95/0013 and 95/0027 produced necrotic lesions of not more than 8.00 mm in diameter and were therefore considered resistant. The other clones were susceptible to varying degrees (Table 1). Moreover, there were positive and significant correlations between the field disease screening and the *in vitro* screening method (Table 2).

Table 1. Response of 21 cassava clones to natural infection by *Colletotrichum gloeosporioides* f. Sp *manihotis* and to the toxic metabolites of the pathogen *in vitro*.

Clones	Field screening[v]				*In vitro* screening[r]			
	Disease score		Canker number		Lesion size on leaves, mm		Lesion size on stem cuttings, mm	
95/0084	3.60a	S	20.00a	S	20.00a	S	18.17a	S
95/0076	3.40ab	S	18.94ab	S	19.11ab	S	16.33b	S
95/0087	3.40ab	S	18.67ab	S	16.00cd	S	15.00c	S
95/0045	3.40ab	S	16.67c	S	16.00cd	S	15.33bc	S
95/0018	3.20ab	S	16.50bc	S	16.00cd	S	16.33b	S
95/0078	3.20ab	S	15.67cd	S	17.33bc	S	16.83ab	S
Isunikankiyan	3.00ab	S	17.00bc	S	15.67cd	S	15.33bc	S
95/0079	3.00ab	S	16.93c	S	18.00b	S	15.33cd	S
95/0088	3.00ab	S	12.00d	MS	15.67cd	S	15.00c	S
95/0065	2.80b	MS	17.03bc	S	14.67d	MS	12.50d	MS
95/0069	2.80b	MS	16.27c	S	15.50cd	S	15.33bc	S
95/0073	2.40bc	MS	7.00e	MS	10.50d	MS	8.33e	MS
95/0010	2.40bc	MS	6.47e	MS	10.67e	MS	11.17d	R
95/0025	1.60cd	R	4.00f	R	7.50f	R	7.67e	R
95/0027	1.60cd	R	2.00g	R	7.00f	R	7.00e	R
Tms 30211	1.60cd	R	2.00g	R	8.00f	R	7.00e	R
95/0014	1.40d	R	2.00g	R	7.00f	R	7.00e	R
95/0030	1.40d	R	2.00g	R	3.00d	R	4.00e	R
95/0022	1.20d	R	0.99gh	R	8.00f	R	8.00e	R
95/0056	1.00d	R	0.00h	R	6.67f	R	5.88f	R
95/0057	1.00d	R	0.00h	R	7.00f	R	5.19g	R

[v]Each value within the same column is a mean of three replicates (30 plants) and is transformed from the log of the value

[r]Each value with in the same column is a mean of three replicates (10 leaves/stem cuttings/cassava clones) and is transformed from the log of the original value. Mean values followed by the same letter in a column are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test.

S = Susceptible; MS = Moderately susceptible; R = Resistant

Table 2. Pearson correlation coefficient of the response of cassava clones to natural infections of *C. gloeosprioides* f sp *manihotis* and to the toxic metabolites.

	Canker sizes	Mean lesion sizes on leaves	Mean lesion sizes on stems (*in vitro*)
Field disease score	0.973**	0.953**	0.958**
Mean canker size		0.945**	0.935**
Mean lesion size on- - leaves (*in vitro*)			0.944**

** Values are significant at $p < .01$ ($n = 21$)

DISCUSSION

Results of this study showed that the phytotoxic metabolites were produced by *C. gloeosporioides f sp. manihotis* in culture. This is in consonance with reports that *Colletotrichum* species produce phytotoxic metabolites in culture (Sharma and Sharma 1969; Fratzeen *et al.* 1978; Amusa 1991). Toxic metabolites have also been extracted from *Colletotrichum* infected leaves (Amusa *et al.* 1993). The 21 cassava clones screened using natural infections reacted differentially to the induction of anthracnose (canker) by the pathogen. Cassava clones 95/0057, 95/0056, 95/0022, 95/0030, 95/0014, Tms 30211, 95/0027 and 95/0025 were regarded as resistant based on field disease scores. The others were susceptible at varying degrees. The clone Tms 30211, used as the resistant check is known as one of the most resistant clones (IITA1985, Ikotun and Hahn1991). Considering the sizes of cankers, it was observed that 8 cassava clones had necrotic lesion sizes of less than 6 mm in diameter. The other cassava clones including Isunikankiyan were susceptible at varying degrees. The resistant clones though developed necrotic lesions (cankers), their development depended mostly on the activity of insect vector *Pseudoptheraptus devastans* rather than the effect of the pathogen (Muimba 1982). *P. devastans* has been reported to induce necrotic lesions (cankers) of up to 6 mm in diameter on cassava clones without the presence of *C.*

gloeosporioide (Muimba 1982). Therefore, the 8 clones with lesion diameter of less than 6 mm can be regarded as resistant. A local clone Isunikankiyan known to be very susceptible to anthracnose (Ikotun and Hahn1991) was confirmed as susceptible in our field screening.

Screening for resistance using phytotoxic metabolites of *C. gloeosporioides f. sp manihotis* revealed that 8 cassava clones were resistant to induction of necrotic lesions. Ten cassava clones were susceptible while 3 other clones were moderately susceptible. The disease reactions of these clones were similar to those obtained from field screening tests using natural infection. For instance the clone, Tms 30211 was reported to be resistant to cassava anthracnose disease while Isunikankiyan, a local clone is known to be susceptible to the disease under natural infection (IITA 1985).

Results also revealed that highly positive correlations exist between the size of canker in the field, the field disease score based on natural infection and the canker size from *in vitro* screening technique using toxic metabolites. The *in vitro* screening experiment was completed and terminated within 72 hrs of inoculation. Thus, thousands of cassava clones can be screened within a short period by this method. The success of the experiment may be due to the use of virulent strains of the pathogen. Since the metabolites affect the tissue directly and also because resistance to anthracnose is genetically controlled, several environmental factors that normally affect the conventional screening methods are eliminated. The success of the conventional methods largely depend on the abundance and the activity of *P. devastans*. Evidence abound that *C. glocosporioides* being a weak pathogen, does not on its own induce cankers but only colonize already formed wounds (Chevougeon 1956; Muimba 1982). Therefore the disease development is dependent on favorable environmental conditions for the development of the vectors and the availability of propagules of the pathogen in abundance. The conventional methods are also time consuming and need at least two planting seasons for any reasonable inference to be made. Moreover, the cost in terms of labor and land is also high. Since phytotoxic metabolites have been found to be useful in screening other crops for disease resistance (Wheeler and Luke 1955; Hartman *et al.* 1986; Rines and Luke 1988; Amusa *et al.* 1994), results of this study suggest that screening for disease resistance in cassava with toxic metabolites obtained from *C. gloeosporioides f sp manihotis* can be an efficient and reliable tool in breeding.

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