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The Response of Some Cassava (*Manihot esculenta* Crantz). Cultivars to Infection by *Meloidogyne incognita* Race 1 in Calabar, Nigeria

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Abstract: Twenty-one cassava cultivars from International Institute of Tropical Agriculture's (IITA) germplasm collections were evaluated for their responses to natural and artificial infection by *Meliodogyne incognita* race 1 in Calabar humid forest zone, Nigeria. In the screenhouse trial, individual plants were inoculated with 5,000 eggs of the nematode species. Uninoculated plants served as the control. For both experiments, infected plants were scored for galling and egg mass production on a 0-5 rating scale. Galls were observed only in feeder root and none on tubers. The cassava cultivars significantly ($p \leq 0.05$) differed in their severity of galling and egg mass production. Five cultivars (96/1642, 99/2123, 91/02324, TMS 30572 and 92B/0061) had no galls and were rated immune with a Gall Index (GI) = 0. Four cultivars (97/3200, 95/0166, 94/0039 and 96/1089A) were rated resistant with GI$\leq$2. The nematode infection decreased stalk height, top fresh and dry weights and fresh root weight in all the cassava cultivars. Fresh storage root weight reduction ranges from 1.06% (95/00379) to 34.04% (94/0026). Five cultivars (91/02342, 4(2)1425, 95/00379, 82/00058 and 97/3200) were rated tolerant with severe root gallings but insignificant storage root weight decrease. These results indicate that promising source materials are available for improving cassava cultivars against root-knot nematodes.

Key words: Cassava cultivars, *Meloidogyne incognita*, resistance, tolerance, susceptible, IITA

INTRODUCTION

Cassava (*Manihot esculenta* Crantz.) is the second most important staple food crop after maize in sub-Saharan Africa and provides food for more than 200 million Africans (IITA, 1990). Its role as a major economic and food security crop has generated significant research interest at the national and international levels over the last two or so decades. Cassava provides a cheap source of Calories for both human and livestock consumption as well as serves as a raw material for agro-allied industries World-wide (Obioha and Anikwe, 1982; Olorunda, 1991). Apart from it being a staple food crop, of late, the revenue obtained from cassava export could possible rank it as an important cash crop in Nigeria. With an annual output of about 34 million metric tonnes, Nigeria is rated as the world's leading cassava producer (FAO, 2004).

Although cassava is considered a hardy crop, its yield is limited by some biotic and abiotic stress factors. Amongst the biotic factors, pests and diseases have seriously constrained the production of this crop. A vast array of pathogenic organisms (nematodes, bacteria and viruses) has caused severe yield reduction both in quantity and quality of tuber. Root-knot nematode (*Meloidogyne* sp.) is an important pest attacking cassava. Two species have been identified to be associated with cassava in Nigeria (Dickson, 1978; Caveness, 1981; Atu, 1988) and these include *M. incognita* and *M. javanica*. *M. incognita* predominates the southern humid forest, while *M. javanica* is a dominant species in the Sudan Savannah of Northern Nigeria (Olowe, 2004). Conservative yield losses throughout the tropics is estimated at 6%, equivalent to 6 million metric tones (Caveness, 1982). Symptoms of attack include formation of galls on the feeder roots with a consequent reduction in weight of storage root, stalk height and stalk weight (Caveness, 1982; Atu, 1988). The management of root-knot nematodes with chemical nematicide is the most effective means of control but its adverse effects on the environment, non-target organisms, high mammalian toxicity and prohibitive cost have discouraged its use by farmers. The use of resistant plants appears to be an economically viable alternative method of control. This approach is eco-friendly and with no added cost. Atu (1988) observed a wide variation in resistance among twenty cassava cultivars from National Root Crops Research Institute (NCRI), Umudike Southeast of Nigeria to *M. incognita* race 2.

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This experiment was carried out to assess the response of twenty-one promising cassava cultivars from International Institute of Tropical Agriculture (IITA) germplasm collections to infection by an indigenous population of *Meloidogyne incognita* (Kofoid and White) ChitWood, race I.

MATERIALS AND METHODS

The experiment was conducted in the screenhouse of the Department of Crop Science, University of Calabar, Calabar, Nigeria between October, 2005 and April, 2006. Calabar is located within latitude 5°00' and 5°40' N and longitude 8°04' and 8°62' E). This part of Nigeria has a total rainfall that ranges from 1,500-3000 mm, relative humidity of 65-90% and temperature of 22.2-38.2°C. Twenty-one cassava cultivars (97/3200, 96/1642, 99/2123, 91/02342, 94/0561, 4(2)1425, 95/0166, 94/0026, 95/0289, 95/00379, 91/02324, 96/1565, 82/00058, TMS/30572, 96/0603, 94/0039, 92/0067, 97/2205, 92B/0061, 96/1089A and 92/0326) were obtained from IITA germplasm collections, Ibadan Nigeria and used for the study. Ten-litre plastic bags were filled with 9 kg steam-sterilized sandy-loam top soil. Two 20 cm long cuttings from each of the 21 cassava cultivars were planted in each bag and later thinned to one during nematode inoculation. An indigenous population of *M. incognita* race I isolated from a cowpea plant in Calabar and maintained as a pure culture on begonia plants (*Begonia rexcultorum*) served as an inoculum source. This population was multiplied on Indian Spinach (*Bassela alba*) in the greenhouse. Root-knot nematode eggs were extracted from the heavily galled roots of the Indian Spinach using sodium hypochloride (NaOCl) technique of Hussey and Barker (1973). Thirty milliliter of the inoculum suspension contained approximately 5,000 eggs by count. Two weeks after planting, each bag was inoculated with 5,000 eggs of *M. incognita* race I by adding the inoculum in depression made in the soil around each plant. Uninoculated plants of each cultivar served as the control. The experiment was a Completely Randomized Design (CRD) with four replicates. The bags were watered once daily with 300 mL of tap water during the first 2 months of growth but later increased to 500 mL in the remaining part of the trial. A complete fertilizer N. P. K Mg 12: 12: 17: 2 was applied at the rate of 1.8 g per bag (400 kg ha^{-1}) a month after planting. Plants were grown at a mean day temperature of 28°C and mean night temperature of 20°C for approximately 6 months. Data were collected on number of galls on feeder roots per plant, fresh weight of tuber and top as well as stalk height. For egg mass count, fresh feeder root was stained with phoxine B (0.15 g L^{-1}) for 15 min (Daykin and Hussey, 1985). The top was dried in a hot air oven at 60°C for 48 h to determine the dry weight. Root gall or egg mass index was determined on a 0-5 scale rating used in the International Meloidogyne Project (IMP, 1978): 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls or egg masses per plant. Gall and egg mass scores were also made on cassava germplasm plants harvested from naturally infested field plots of Crop Science Teaching and Research Farm of the University of Calabar during the 2005 IITA multilocational cassava germplasm trial. Five cassava stands were randomly sampled out of the ten stands per plot. The identity of the root-knot nematode species that infected plants in the field was ascertained through perineal pattern examination of ten randomly picked adult females (Hartman and Sasser, 1985). The data collected were statistically analyzed using the Analysis of Variance (ANOVA). Means were separated using Duuncan's New Multiple Range Test (DNMRT) at 5% level of probability. The uninoculated controls were compared with the inoculated treatments using the student's t-test.

RESULTS AND DISCUSSION

The cassava cultivars significantly differed ($p = 0.05$) in their severity of galling and egg production (Table 1). Eight cassava cultivars were not attacked by *M. incognita* race I and had gall or egg mass index of zero and were rated immune. They include: 97/3200, 96/1642, 99/2123, 95/0166, 91/02324, TMS/30572, 92B/0061 and 96/1089A. One cultivar (94/0039) was rated resistant with a gall index (GI) of 2. The intensity of galling and egg production was moderate to severe in the rest of the cassava cultivars. Natural infection of the Cassava cultivars by the same root-knot nematode species in the field almost followed the trend of the screen house trial (Table 2). There was a highly significant ($p = 0.01$) positive correlation between the screen house and field trials in galling ($r = 0.91$) and egg mass production ($r = 0.88$). However, three cultivars out of the 8 rated immune in the screen house trial were rated resistant as few galls were observed in their feeder roots. They include; 97/3200, 95/0166 and 96/1089A. Egg production by the nematode species also followed this trend.

Generally, the nematode species reduced stalk height of all the cassava cultivars when compared with their respective controls, with the exception of 94/0039 and 96/1089A (Table 3). However, this reduction was only significant in 94/0026, 95/00289, 96/1565, 96/0603, 92/0067 and 97/ 2205. The cassava cultivars inoculated with the root-knot nematode species significantly ($p \leq 0.05$) differed

Table 1: Effect of *Meloidogyne incognita* race 1 on susceptibility of some IITA cassava Cultivars

Cassava cultivar	No of Galls/Root system	No of Egg masses/Root system	Gall index	Egg mass index	Resistant status
97/3200	0.00f	0.00g	0.00e	0.00e	I
96/1642	0.00f	0.00g	0.00e	0.00e	I
99/2123	0.00f	0.00g	0.00e	0.00e	I
91/02342	131.67a	96.67a	5.00a	4.33a	T
94/0561	61.67cd	44.33de	4.00b	4.00ab	S
4(2)1425	65.00cd	45.33de	4.00b	4.00ab	T
95/0166	0.00f	0.00g	0.00e	0.00e	I
94/0026	126.67a	101.67a	5.00a	4.33a	S
95/0289	76.67bc	62.33c	4.00b	4.00ab	S
95/00379	37.00e	23.33f	3.67c	2.67c	T
91/02324	0.00f	0.00g	0.00e	0.00e	I
96/1565	61.67cd	44.67de	4.00b	4.00ab	S
82/00058	51.67de	35.67e	4.00b	3.67b	T
TMS/30572	0.00f	0.00g	0.00e	0.00e	I
96/0603	70.00c	50.67d	4.00b	4.00ab	S
94/0039	8.00f	2.33g	2.00d	1.33d	R
92/0067	90.00b	72.67b	4.00b	4.00ab	S
97/2205	60.00cd	45.33de	4.00b	4.00ab	S
92B/0061	0.00f	0.00g	0.00e	0.00e	I
96/1089A	0.00f	0.00g	0.00e	0.00e	I
92/0326	71.67c	48.33d	4.00b	4.00ab	T

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% probability level. I = Immune, Gall Index (GI) = 0. R = Resistant, Gall Index (GI) = 1 Or 2. S = Susceptible, Gall Index (GI) = > 2

Table 2: Field response of some IITA cassava cultivars to natural infection by *M. incognita*

Cassava cultivar	No of Galls/root system	No of egg masses root system	Gall index	Egg mass index	* Resistant status
97/3200	3.00f	1.00e	2.00d	1.00d	R
96/1642	0.00f	0.00e	0.00f	0.00e	I
99/2123	0.00f	0.00e	0.00f	0.00e	I
91/02342	172.37a	98.33a	5.00a	4.00ab	S
94/0561	45.33d	32.67d	4.00b	3.67b	S
4(2)1425	86.00bc	55.00c	4.00b	4.00ab	S
95/0166	5.00f	1.33e	2.00d	1.00d	R
94/0026	84.67bc	48.67cd	4.67a	4.00ab	S
95/0289	115.33b	82.33ab	5.00a	4.33a	S
95/00379	15.67e	3.67e	3.00c	2.33cd	S
91/02324	0.00f	0.00e	0.00f	0.00e	I
96/1565	92.33b	70.67b	4.67a	4.00ab	S
82/00058	63.67cd	40.00d	4.33a	4.00ab	S
TMS/30572	0.00f	0.00e	0.00f	0.00e	I
96/0603	45.67d	30.00d	4.00b	3.00c	S
94/0039	4.00f	1.00e	2.00d	1.00d	R
92/0067	78.00c	61.33bc	4.33a	4.00ab	S
97/2205	83.33bc	52.67c	4.67a	4.00ab	S
92B/0061	0.00f	0.00e	0.00f	0.00e	I
96/1089A	2.00f	1.00e	1.00e	1.00d	R
92/0326	67.67c	46.00d	4.00b	4.00ab	S

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% probability level. * I = Immune, Gall Index (GI) = 0. R = Resistant, Gall Index (GI) = 1 or 2. S = Susceptible, Gall Index (GI) = > 2

in stem height. TMS/30572 was the tallest while 91/02324 was the shortest. Result of the fresh storage root weight of the cassava cultivars is presented in (Table 4). Nematode infection reduced fresh root weight in all the cultivars compared with their uninoculated counterparts. Fresh storage root weight reduction ranges from 1.06% (95/00379) to 34.04% (94/0026). However, the reduction was only significant in those cultivars whose stalk height were also significantly reduced besides cultivars 94/0561. There were significant ($p = 0.05$) differences in fresh tuber weight among the cassava cultivars inoculated with the nematode species. Cultivar 96/1089A had the highest fresh tuber weight, while 95/0289 had the lowest. Nematode inoculation also caused a decrease in top fresh weight of all the cassava cultivars relative to the control (Table 5). In addition to those cultivars whose fresh tuber weight were significantly reduced due to nematode infection, 91/02342 had their top fresh weights significantly reduced due to nematode parasitism. The cassava cultivars inoculated with the nematode species significantly ($p \leq 0.05$) differed in top fresh weight. Cultivar 96/1089A had the highest fresh top weight while 94/0026 had the lowest. The result of top dry weight is presented in Table 6 and it followed the trend of top fresh weight.

Evidence from this study showed that symptom of root-knot nematode infection which is characterized by

Table 3: Effect of *Meloidogyne incognita* race 1 on stalk height (cm) of some IITA cassava Cultivars

Cassava cultivar	Inoculated	Uninoculated (Control)
97/3200	69.00de	71.15
96/1642	65.33ef	66.33
99/2123	69.00de	70.67
91/02342	70.00d	74.33
94/0561	80.67b	85.67
4(2)1425	60.00g	67.33
95/0166	49.33i	50.67
94/0026	54.67h	** 65.33
95/0289	48.00i	** 59.00
95/00379	72.33cd	74.67
91/02324	42.33j	43.67
96/1565	54.00h	* 62.33
82/00058	82.33b	85.67
TMS/30572	88.00a	89.33
96/0603	54.00h	* 60.67
94/0039	72.00cd	70.33
92/0067	75.33c	** 83.67
97/2205	75.67c	* 80.00
92B/0061	55.67h	58.67
96/1089A	62.00fg	61.33
92/0326	65.33ef	66.67

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% probability. * and ** significantly different from their inoculated counterparts at 5% and 1% probability level, respectively, using the t-statistic

Table 4: Effect of *Meloidogyne incognita* race 1 on fresh root weight (kg)/plant of some IITA Cassava cultivars

Cassava cultivar	Inoculated	Uninoculated (Control)	+
97/3200	0.70abcd	0.74	5.40
96/1642	0.50abcd	0.53	5.66
99/2123	0.59abcd	0.62	4.84
91/02342	0.86abc	0.91	5.49
94/0561	0.36bcd	* 0.40	10.00
4(2)1425	0.56abcd	0.61	8.20
95/0166	0.41bcd	0.43	4.65
94/0026	0.31cd	** 0.47	34.04
95/0289	0.26d	** 0.38	31.58
95/00379	0.93abc	0.94	1.06
91/02324	0.35bcd	0.38	7.89
96/1565	0.28cd	* 0.34	17.65
82/00058	0.59abcd	0.63	6.35
TMS/30572	0.86abc	0.88	2.27
96/0603	0.62abcd	** 0.81	23.46
94/0039	0.71abcd	0.74	4.05
92/0067	0.58abcd	** 0.75	22.67
97/2205	0.87abc	* 0.96	9.38
92B/0061	0.34bcd	0.35	2.86
96/1089A	1.07a	1.09	1.83
92/0326	0.72abcd	0.78	7.69

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% level of probability. * and ** significantly different from their inoculated counterparts at 5% and 1% probability level, respectively, using t-statistic. + Percentage (%) decrease in tuber weight due to nematode infection

Table 5: Effect of *Meloidogyne incognita* race 1 on top fresh weight (kg)/plant of some IITA cassava Cultivars

Cassava cultivar	Inoculated	Uninoculated (Control)
97/3200	0.97cd	0.98
96/1642	0.72def	0.75
99/2123	0.85cde	0.86
91/02342	1.38ab	* 1.46
94/0561	0.62def	* 0.71
4(2)1425	0.80de	0.85
95/0166	0.71f	0.73
94/0026	0.37f	** 0.51
95/0289	0.55ef	* 0.62
95/00379	1.37ab	1.41
91/02324	0.60def	0.62
96/1565	0.42f	** 0.58
82/00058	1.09bc	1.17
TMS/30572	1.41ab	1.40
96/0603	0.94cd	** 1.22
94/0039	0.86cde	0.87
92/0067	0.70def	** 0.84
97/2205	1.39ab	* 1.52
92B/0061	0.89cde	0.91
96/1089A	1.70a	1.72
92/0326	0.94cd	0.98

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% level of probability * and ** significantly different from their inoculated counterparts at 5% and 1% probability level, respectively, using the t-statistic

Table 6: Effect of *Meloidogyne incognita* race 1 on top dry weight (kg)/plant of some IITA cassava Cultivars

Cassava cultivar	Inoculated	Uninoculated
97/3200	0.33abcd	0.34
96/1642	0.24bcd	0.26
99/2123	0.33abcd	0.35
91/02342	0.46abcd	* 0.55
94/0561	0.24bcd	* 0.32
4(2)1425	0.26bcd	0.28
95/0166	0.18d	0.21
94/0026	0.14d	** 0.22
95/0289	0.20cd	** 0.30
95/00379	0.48abcd	0.50
91/02324	0.23bcd	0.26
96/1565	0.18d	** 0.27
82/00058	0.39abcd	0.41
TMS/30572	0.56abc	0.57
96/0603	0.39abcd	** 0.48
94/0039	0.35abcd	0.37
92/0067	0.30bcd	** 0.42
97/2205	0.59ab	* 0.67
92B/0061	0.36abcd	0.38
96/1089A	0.70a	0.72
92/0326	0.37abcd	0.41

Means followed by the same letter within a column are not significantly different based on DNMRT at 5% level of probability * and ** significantly different from their inoculated counterparts at 5% and 1% probability level, respectively, using the t-statistic

root galling was observed on cassava feeder roots but not on the tubers. This corroborates the findings by earlier investigators (Caveness, 1982; Atu, 1988). Based on root-gallings and nematode egg production, 8 and 5 cultivars were rated immune, while one and four cultivars were rated resistant in the screen house and field trials, respectively.

The rest of the cultivars could possibly be rated susceptible to *M. incognita* race I. Caveness (1978) observed that TMS/30752 and TMS/3055 were highly susceptible to *M. incognita* race 2 in Ibadan, southwestern, Nigeria; while Atu (1988) reported that the 2 cultivars were moderately resistant and resistant, respectively to the same race of *M. incognita* in Southeastern Nigeria. However, our study shows that

TMS/30572 was immune to attack by *M. incognita* race 1. Furthermore, our finding contradicts the report by Atu (1988) that cultivar 4(2) 1425 was resistant to *M. incognita* race 2. Disparity in experimental results could occur if workers use different races of a nematode species or if the environment impacts differently on crop cultivars (Roberts and Thomason, 1986). Three cultivars were rated immune in the screen house trial but were rated resistant (very few galls) in the field trial. This could be attributed to the difference in environmental conditions or possibly population density of the nematode species. Perhaps, high inoculum density due to spotting factor and favourable environmental condition favoured pathogenesis in the field (Norton, 1978).

Nematode infection caused a decrease in above-ground fresh and dry weights, stem growth and storage root weight in cultivars rated immune, resistant, tolerant and susceptible. However, the decrease was statistically significant in those cultivars rated susceptible. Root galling by root-knot nematodes reportedly impair vital physiological processes in the plant like water, nutrient transport and photosynthesis (Khan and Khan, 1987; Melakeberhan *et al*., 1990). This could possibly account for the significant growth and yield decrease in the susceptible cultivars. Significant reduction in cassava stalk height and weight by *M. incognita* and *M. javanica* on cassava had been reported by Caveness (1981). On the other hand, the decrease in growth and yield by the nematodes species in cultivars rated immune and resistant could be attributed to the type of resistance mechanisms utilized by those cultivars (Giebel, 1982). Severe hypersensitive reaction at nematode feeding site leading to necrotic tissues instead of giant cells could also deter nutrient and water uptake with a consequent growth and yield reduction. Five cultivars: 91/02342, 4(2) 1425, 95/00379, 82/00058 and 97/32000 were severely galled but were rated tolerant. Their growth, top fresh and dry weights and storage root weight were not significantly reduced relative to the uninoculated control. A plant that survives and gives satisfactory yield at a level of nematode infection that causes economic loss on other varieties of the same species is tolerant (Fassuliotis, 1979). Canto-Saenz (1985) defines a tolerant plant as an efficient host that suffers no statistically significant growth and yield reduction. Hence, these cultivars could be judiciously utilized as planting stock in soils infested with *M. incognita* race 1.

The importance of root-knot nematodes as pests of cassava is not fully appreciated because the tuber which is the economic portion is usually not galled by the nematodes as it is the case with yam (*Dioscorea* sp.) (Ogbuji, 1978). However, this study shows that cassava cultivars differ in their resistance to the most common root-knot nematode species (*M. incognita*) in Southern Nigeria. Knowledge of the host status of the common cassava cultivars against the various species and races of this important pest would be useful in planning nematode pest management programmes in fields where the nematode is a problem.

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