EARLY SCREENING OF CASSAVA FOR RESISTANCE TO ROOT-KNOT NEMATODES

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ABSTRACT

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A method was developed for early screening of cassava for resistance to root-knot nematodes. One-node cassava cuttings were planted in plastic disposable beverage cups filled with sterile 1:1 (by volume) sand-soil mixture. Two weeks after planting, the plantlets were infested with a 10 ml suspension containing approximately 1 000 *Meloidogyne* spp. eggs. One month after inoculation, the cassava root systems were evaluated for damage and rated for gall index. Densities of *Meloidogyne* females in the roots were established by differential staining, followed by counting of the female root-knot nematodes under a stereomicroscope. Significant differences in gall index and number of established females, but not in reduction in fresh root weight, were observed among varieties. While some confirmatory field surveys are still required, findings from preliminary surveys carried out in two areas of Uganda suggest that the relative susceptibility of a variety in the field can adequately be predicted by using one-node cuttings.

Key words: Cassava, *Meloidogyne* spp., resistance screening, Uganda.

RESUMEN

Talwana L. A. H., P. R. Speijer, E. Adipala y N. R. Maslen. 1997. Selección temprana de yuca resistente al nematodo agallador (*Meloidogyne* spp.). Nematrópica 27:19-25.

Se desarrolló un método, para la selección temprana de yuca, resistente al nematodo agallador. Cortes de nodos simples de yuca, se sembaron en vasos plásticos desechables, rellenos con una mezcla estéril (50:50) de arena y suelo. Dos semanas después del plantío, las plantulas fueron infestadas con 10 ml de una suspensión conteniendo aproximadamente 1 000 huevos de *Meloidogyne* spp. Un mes después de la inoculación los sistemas de raíces de la yuca, fueron evaluados en relación al daño y medidos por el índice de agallamiento. Las densidades de las hembras de *Meloidogyne* en las raíces, fueron establecidas por tinción diferencial, seguido por el conteo en el microscopio stereoscopico. Diferencias significativas en las variedades, fueron observadas para el índice de agallamiento y para el numero de hembras establecidas, pero no en la reducción del peso fresco de la raíz. Aunque aun se requieren algunos muestreos en el campo, los resultados de los muestreos preliminares, realizados en dos áreas de Uganda, sugieren que la susceptibilidad relativa de una variedad en el campo, puede ser adecuadamente pronosticada, usando el corte de nodos simples.

Palabras claves: Yuca, *Meloidogyne* spp., selección, Uganda.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major food crop in Africa and other tropical and subtropical countries (FAO, 1989). The importance of cassava is increasing due to its high yielding capacity, ability to grow in marginal soils, flexibility in differ-

ent farming systems and resistance to local pests and diseases (FAO, 1989). Nevertheless, the principal factors limiting cassava production remain biotic factors such as pests and diseases including nematodes (IITA, 1990). Until recently, the study of nematodes as pests of cassava has received little attention (Caveness, 1982). Although comprehensive lists of nematode pests of cassava and their distributions have been compiled (Bridge *et al.*, 1991; Caveness, 1980; Hogger, 1971; Jatala and Bridge, 1990; McSorley *et al.*, 1983; Namaganda *et al.*, 1994), nematodes are often disregarded as constraints to cassava production. This is due, in part, to the fact that the damage they cause regularly goes unnoticed because of the naturally 'knobbly' and rough texture of the roots, which can disguise the nematode damage.

Nevertheless, nematological studies on cassava have now begun to receive more attention, which is justified given the importance of the crop. Much of the pioneering work was done at the International Institute of Tropical Agriculture, Ibadan, Nigeria (McSorley *et al.*, 1983) but no particular method was described for effective and efficient early screening of cassava germplasm for nematode resistance. Microplot and pot trials take a relatively long time to produce results, require ample space and the work is tedious. This limits the number of progenies and replications that can be included in an investigation. The present study was aimed at developing a rapid and efficient early screening method for testing the resistance of cassava progenies to root-knot nematodes (*Meloidogyne* spp.). To confirm the validity of the improved technique, preliminary comparisons for different varieties were made between the responses in early screening experiments and those in field plots with cassava plants grown for over 10 months.

MATERIALS AND METHODS

One-node cuttings of five cassava varieties Nase 1, Nase 2, Bukalasa 11, Ebwanateraka and Bao were planted in disposable plastic beverage cups filled with a sterile 50:50 (by volume) sand-top soil mixture. The cups were arranged in a randomized complete block design with six replications, each replicate comprising one to be inoculated and one non-inoculated plant of every variety. The plants were placed outdoors. After approximately 2 weeks from planting when the roots were well developed, they were inoculated. A suspension of about 1 000 root-knot nematode (*Meloidogyne* spp.) eggs was poured into a hole made in the soil around the base of the plant and a thin layer of soil added. The inoculum was collected from infested cassava roots and cultured on tomatoes (*Lycopersicon esculentum* Mill. cv. Moneymaker) and consisted of a mixed population of *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood in approximate ratio of 4:1 (identification by J. Machon of the International Institute of Parasitology, St. Albans, UK). The eggs were extracted from tomato plants by the method described by Hussey and Barker (1973). Four weeks after inoculation, the plants were harvested and their roots hand picked from the soil. In the 4-week period, the root-knot nematodes were expected to have completed 1-2 life cycles (Orton-Williams, 1972; Eisenback and Triantaphyllou, 1991). The roots were examined for galling and fresh roots were weighed. To enable counting of *Meloidogyne* spp. females in roots, a 1 g sample of fresh roots from each of the infected plants was cut into about 2 cm pieces, washed clean and tied in a muslin cloth to keep each sample separate. The bundles were placed in a boiling solution of equal volumes of lactic acid (DL-Lactic acid),

glycerol and distilled water with 0.01% acid fuchsin stain for about 2-4 minutes and allowed to cool in the staining solution. The roots were then placed for about 24 hours in a clearing solution consisting of equal volumes of glycerol and distilled water. The clearing solution removed the stain from the plant tissues but not from the nematode tissues (Hunt and Bridge, 1991). Root samples from each infected plant were observed under a stereomicroscope (× 40 magnification) and dissected to expose the nematodes. The number of female *Meloidogyne* spp. with and without egg masses per gram of roots and the number of galls per gram of roots were recorded, with coalesced galls counted as one. The gall index was scored according to the method of Sasser *et al.* (1984).

Three trials were conducted with similar results and the data were pooled for analysis. Fresh root weights of the infected and non-infected plants were subjected to analysis of variance (ANOVA) and means were separated by a Least Significant Difference (LSD) test. Means of the infected and non-infected plants were compared by a t-test. The distribution of the experimental units among categories 1-4 of gall index as given by Sasser *et al.* (1984) was tested for difference among varieties by Chi-square. Total number of female root-knot nematodes was transformed to a logarithmic scale. An angular transformation (arcsine) was done on the percentages of the number of females with egg masses and reduction in fresh root weight. Transformation was to bring the data to near-normality as required by ANOVA (Gomez and Gomez, 1984).

A limited survey was carried out in fields that had been planted with cassava for more than 10 months at Serere Agricultural and Animal Production Research Institute (SAARI) in Soroti district and at Ikulwe District Farm Institute (DFI) in Iganga district, Uganda. The objective of the survey was to make comparisons between varietal responses in early screening and in the *in situ* field situation. The varieties were planted using stem cuttings, which are considered to be free of root-knot nematodes. Root samples were collected from 10 plants per variety selected randomly within a field, with each plant used as a replication. The samples were processed in the same way as for the early screening trials. Two varieties Nase 1 and Nase 2 sampled at SAARI were the same as those used in the pot trials while one, Migyera (TMS 30572), was included in the survey because it is grown widely in the area, as an African Cassava Mosaic Virus (ACMV) disease-resistant variety. At Ikulwe District Farm Institute, four varieties used in the early screening trials, Nase 1, Nase 2, Ebwanateraka and Bao, were sampled.

RESULTS

All varieties tested developed symptoms in response to infection by the root-knot nematodes, though with varying degrees of severity. Nase 1 was less affected ($P \leq 0.05$) than Nase 2, Bao, Ebwanateraka and Bukalasa 11 (Table 1). The gall index for root-knot nematode damage on roots of different varieties corresponded well with the number of females that developed in the root system (Table 2). Nase 1 supported the least number of females while Bao and Bukalasa 11 supported the highest number, and had a moderate to severe gall index.

A considerably larger percentage of females on Nase 1 did not produce egg masses, which may indicate the unsuitability of this variety for root-knot reproduction in comparison to other varieties. Analysis of variance comparing varieties showed differences ($P \leq 0.01$) in percentage females with egg masses in root-knot

Table 1. Gall index scores of cassava four weeks after inoculation with 1 000 *Meloidogyne* spp. eggs.[z]

Variety	Gall index category			
	1	2	3	4
Nase 1	1 (3.2)	6 (15.1)	11 (5.8)	0 (11.2)
Nase 2	0 (0.2)	0 (1.4)	2 (2.0)	16 (2.0)
Bukalasa 11	0 (0.2)	0 (1.4)	1 (3.4)	17 (3.0)
Ebwanateraka	0 (0.2)	1 (0.1)	6 (0.1)	11 (0.0)
Bao	0 (0.2)	0 (1.4)	6 (0.1)	12 (0.1)
Expected value for χ^2	0.2	1.4	5.2	11.2

[z]Values of 18 plants; figures in parenthesis are calculated Chi square values.

nematodes which developed four weeks after inoculation and in total numbers of females (Table 2). Percentage females with egg masses was lower ($P \leq 0.001$) for variety Nase 1 than for the other four varieties.

Fresh root weights of inoculated plants were less ($P \leq 0.05$) than those of non-inoculated plants for each variety. Root-knot nematodes caused a reduction in root weight of 32-43% but the reduction was not significantly ($P > 0.05$) different among varieties (Table 2).

Although Chi-square analysis of gall index data revealed no differences ($P > 0.05$) in gall indices between varieties after infection with natural populations of root-knot nematodes in fields at Serere, there were varietal differences ($P \leq 0.001$) at Iganga (Table 3). At Iganga, Nase 1 was the least galled variety ($P \leq 0.01$), while Nase 2,

Table 2. Reproduction and damage of root-knot nematodes *Meloidogyne* spp. on cassava four weeks after inoculation with 1 000 eggs.[z]

Variety	Female root-knot nematodes		Fresh root weight (g)		
	Total number of females	Percentage females with egg masses	Clean	Infested	Percentage reduction in fresh root weight
Nase 1	30	40	2.3	1.4	37.9
Nase 2	135	69	3.1	2.1	32.1
Bukalasa 11	133	77	2.2	1.2	42.8
Ebwanateraka	108	74	2.7	1.6	38.6
Bao	107	74	3.0	1.9	35.1
LSD ($P \leq 0.05$)	24	8	0.3	0.4	ns
CV (%)	35	19	19.0	32.5	53.7

[z]Means from 18 plants.

Table 3. Reaction of five cassava varieties to natural populations of root-knot nematodes, at Serere and Iganga, Uganda.[z]

Variety/Location	Total number of females	Percentage females with egg masses	Gall index category			
			1	2	3	4
SERERE						
Nase 1	27	39	1 (1.6)	4 (2.0)	5 (0.3)	0 (3.7)
Nase 2	111	81	0 (0.3)	0 (2.0)	4 (0.0)	6 (1.4)
Migyera	95	68	0 (0.3)	2 (0.0)	3 (0.3)	5 (0.5)
LSD ($P< 0.05$)	46	17				
CV (%)	63	30				
Expected values for χ^2			0.3	2	4	3.7
IGANGA						
Nase 1	28	50	0 (0.0)	3 (9.6)	6 (12.1)	1 (5.9)
Nase 2	116	67	0 (0.0)	0 (0.6)	0 (1.6)	10 (0.6)
Ebwanateraka	105	69	0 (0.0)	0 (0.6)	0 (1.6)	10 (0.6)
Bao	82	71	0 (0.0)	0 (0.6)	0 (1.6)	10 (0.6)
LSD ($P\leq 0.05$)	22	0.2				
CV (%)	29	9.1				
Expected values for χ^2			0	0.6	1.6	7.8

[z]Means from 10 plants; figures in parenthesis are calculated Chi square values.

Ebwanateraka and Bao were severely galled with almost every observation in category 4. Chi-square tests between Nase 1 and Nase 2, Nase 1 and Migyera, and Nase 2 and Migyera showed that Nase 1 was significantly less galled than Nase 2 but not Migyera, and that there were no significant differences between Nase 2 and Migyera at Serere (χ^2 values were 11.12, 7.16 and 2.24, respectively compared to a tabulated $\chi^2_{(3\ df,\ 0.05)}$ of 7.82).

The percentage females with egg masses for Bao was higher ($P\leq 0.05$) than those of all other varieties at Iganga. The total number of females was lower in Nase 1 ($P\leq 0.05$) compared to the other varieties. Similarly, at Serere, Nase 1 had a lower ($P\leq 0.001$) female fertility and total number of females than Nase 2 and Migyera.

DISCUSSION

The screening method revealed a considerable difference in host plant reaction to root-knot nematode infection for the different cassava varieties. Variety Nase 1 which had poorest host plant qualities for root-knot nematodes in the pot trials was also observed to be the least suitable host in the field. The low female nematode fertility in Nase 1 could imply that the nematode population will develop slowly on it compared to the other varieties.

A reduction in root weights due to root-knot nematode infection was observed, but the extent to which this translates into loss of production under field conditions needs further evaluation.

Bukalasa 11 had a numerically higher reduction in fresh root weight and Nase 2 had a numerically lower reduction in fresh root weight, compared to the other varieties. Based on reduction in fresh root weight, a clear distinction in reaction to root-knot nematode infection among varieties cannot be drawn. Reductions in fresh tuber weights and fresh fibrous root weights of about 50% resulting from root-knot nematode damage have been reported for cassava (Caveness, 1982; Gapasin, 1980; McSorley *et al.*, 1983) and in root weights of other plants (Taylor and Sasser, 1978). Coyne and Namaganda (1994) and Namaganda *et al.* (1994) reported that root-knot nematodes occur widely on cassava in Uganda and are potentially pests of major importance, especially in areas of high population density, where new cassava varieties are introduced in sandy soils where highly nematode-susceptible crops such as tobacco are grown earlier in the rotation. Root-knot nematodes did not cause yield loss to cassava varieties at on-farm trial sites in Uganda (Talwana, 1994). Nevertheless, yields were numerically lower at sites with high gall index scores.

Because root-knot nematodes infect mostly feeder roots (Gapasin, 1980), it was possible with the new technique to reduce the time required for each early screening study from about four months in the case of pot trials using field-planting size cuttings to about six weeks. Moreover, the performances of the four varieties Nase 1, Nase 2, Ebwanateraka, and Bao in field surveys were consistent with the results of the one-node cutting trials. While additional field surveys and field trials are required, the findings of the current study suggest that the relative susceptibility of a cassava variety to root-knot nematode infestation in the field can be predicted by testing one-node cuttings. This can save considerable time and will allow more progenies to be evaluated in future.

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