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Root Knot Resistance in Sugarcane Varieties and Histological Changes in the Infested Roots

E.O.Salawu,
Sugar Research Institute,
P. M. B. 1515,
University of Ilorin,
Ilorin, Nigeria.

ABSTRACT

All the seven varieties of sugarcane *(Saccharum* spp.*)* namely:B 41227,B 60267,B 59162,B 62163, B 61110,B 60163and B4360 screened for resistance against a root-knot nematode *Meloidogyne incognita*,in a screenhouse were found susceptible, but the last three evidently showed comparative tolerance to the nematode infection. The nematode caused galling of the root, induced multinucleate giant cells, and severe necrotic cortical cells.

INTRODUCTION

Like other agricultural crops sugarcane *(Saccharum officinarum* L) is affected by several diseasses. Plant-parasitic nematodes also are known to cause reduction in cane yield (Jensen, *et. al*, 1959 ; Apt and Koike, 1962 ; Williams, 1970 ; Rao and Swarup, 1974). In recent years, efforts have been intensified to obtain nematode resistant and high yielding varieties of sugarcane so as to increase raw sugar production.

Although root-knot nematodes *(Meloidogyne* spp.) are known to be pests of sugarcane (Goodey *et. al*. 1965), there is limited information on the resistance of sugarcane to these nematodes. Legon and Razjivin (1980) reported My 5715, C 334-64, My 54129, B 60267, Ja 64-11, C 79-67, Ja 64-20 varieties tolerant to *Meloidogyne incognita* with three other varieties susceptible.

Damages caused by the root-knot nematodes include abnormal swollen roots as well as destruction of root tissues. Endo (1975) in a review indicated that root-knot nematodes are capable of inducing syncytia as well as causing proliferation of tissues resulting in organised galls on roots. In addition, syncytia induced by the nematodes are multinucleate units derived from plant tissues stimulated by the nematodes. Some workers (Krusberg and Nielsen, 1958 ; Dropkin and Nelson, 1960), however, considered cell wall dissolution and endomytosis without subsequent cell division (cytokinesis) as the mechanism for syncytial formation. Little is known about histological changes in roots of sugarcane by root-knot nematodes.

This work reports on the resistance of sugarcane varieties to root-knot nematode, *Meloidogyne incognita*, and histological changes induced in their roots.

MATERIALS AND METHODS

Screening experiment : Seven sugarcane varieties namely B 41227, B 60267, B 59162, B 62163, B 61110, B 60163 and B 4360 were used in the investigation. Sugarcane setts, from single bud cuttings of each variety were surface-sterilized in 1.5%sodium hypochlorite(NaOCl) solution for ten minutes, and were later established in steam-sterilized sandy loam top soil mixed with pure sand in ratio 2:1, in 70 x 45 x 20 centimeters wooden troughs. The plants were irrigated with tap water, daily.

Four weeks after emergence, seedlings of equal heights from each variety were transplanted into perforated 10- litre plastic buckets filled with similar sterile soil as prepared before. The seedlings were randomly arranged on benches in the screenhouse and irrigated daily. Soil temperature at 15 centimeter soil depth was recorded at 9.00 and 3.00 p. m . using a soil thermometer.

Each cane seedlings was inoculated with second stage larvae of *M. incognita,* extracted from egg masses on *Celosia argentea* using extraction tray method (Whitehead and Hemming,1965) at the rate of 5000 larvae per seedling when the seedling were five weeks old. Both the inoculated and uninoculated seedlings of each variety were replicated three times.

Cane plants were harvested from the pots 120 days after inoculation. The fresh root and shoot weights, the cane heights (from ground level to the top visible dewlap, TVD) as well as cane diameter were recorded. The degree of infestation and varietal resistance were determined by estimating the number of galled roots per plant, after the roots had been washed free of soil particles in tap water, and mopped with cloth towel following the grading shown in Table 1. Tolerance of sugarcane plants to *M. incognita* was based on the ability of the nematode to feed and reproduce without affecting the growth and yield of the crop (Sasser, 1972).

Histopathological studies : A local sugarcane variety LSI-033 known to be susceptible to *M. incognita* was used in this investigation. Sugarcane setts, from single bud cuttings, were each planted in the same type of soil as for varietal screening in perforated 5-litre plastic pots. Two weeks after emergence, the seedlings were similarly inoculated with approximately 5000 larvae per plant. Roots were selected and examined in early and advanced gall developmental stages. Uninoculated seedlings were used for comparison.

Galled roots for histological work were fixed in Formalin-Acetic-Alcohol (FAA) solution, dehydrated in ethyl alcohol, infiltrated in chloroform-Alcohol solution and embedded in paraffin. Sections were cut to a thickness of 15 microns with a rotary microtome, affixed to glass slides with egg-albumin adhesive, stained with Safranin-Fast Green and mounted in Canada Balsam (Beryln and Miksche, 1976).

RESULTS AND DISCUSSION

The result of the investigation on the varietal reaction of *Meloidogyne incognita* is presented in Table 2. All the seven varieties infected with the nematode were susceptible. Infected B 60267, B 59162 and B 62163 were shorter with lesser root and shoot weights than uninfected plants. The infected B 60163 and B 4360 were taller with more fresh shoot and root weights than the control plants. Similarly, the infected B 41227 and B 61110 were taller than the control plants, and their fresh shoot and root weights were less than the control plants respectively. The fresh shoot and root weights of B 61110 and B 41227 were also more than the control plants. More than 40 galls per root were present in roots of B 60267, B 59162 and B 41227, whereas between 20 and 25 galls occurred on the roots of B 62163, B 61110, B 60163 and B 4360 sugarcane varieties.

Microscopic examination of galled roots, showed that the swollen roots contained larval and adult female stages of *M. incognita.* The degree of infestation is as shown in Table 3. The soil temperature during the investigation was between 24^{0}C and 32^{0}C.

Table 1. Evaluation of the degree of infestation and resistance to *M. incognita*.

Root-Knots per plant	Degree of infestation	Degree of resistance
0	0	Immune
1—2	1	Resistant
3—10	2	Moderately Resistant
11—30	3	Moderately Susceptible
31 and above	4	Susceptible.

Histopathological studies: Slight root gall development occurred on sugarcane roots fifteen days after inoculation. The differentiation and maturation regions of roots were the areas largely galled. The galls were first sickle- shaped, and as the root diameter increased at inspection intervals, the infected roots were club- shaped. All the galls formed were terminal in position (Fig. 1). Egg masses occurred 35 to 37 days after inoculation of the sugarcane varieties.

Examination of stained serial sections revealed *M. incognita* associated with each swollen root. The number of giant cells associated with each nematode was 3 or 4. Usually more than one giant cell was present adjacent to another with the nematode head always against or between giant cells (Fig. 2 and 3). Giant cell nuclei were dispersed inside the cell and cytoplasmic content within giant sells were more dense than those inside normal cells. Cell wall dissolution was not observed in the induced giant cells.

The nematode most commonly infected pholem elements as well as the cortical cells. It was noted that both the inner and outer cortical cells were necrotic in more than 80 percent of the root sections examined.

Roots of plants susceptible to root-knot nematodes are usually galled as a result of nematode-host interaction (Bird, 1962 ; Balasubramanian and Rangaswami, 1962). Legon and Razjivin (1980) reported seven sugarcane varieties to be tolerant to *M. incognita* infection. In this investigation all the seven varieties of sugarcane tested were susceptible to the nematode. The reduction in cane yield in B 41227, B 60267, B 59162 and B 62163 compared with the controls evidently showed that these sugarcane varieties were not tolerant to *M. incognita* infection. Wallace (1973) had indicated that stunting and reduced rate of growth may be caused by any one change in the plant physiological functions like respiration, photosynthesis, transpiration, translocation and mineral uptake. The infected sugarcane variety B 61110, B 60163 and B 4360 increased in growth and yield compared with the controls, and had highly proliferated roots (except B 61110 plant). Similar stimulation of growth had been reported by Apt and Koike (1962), who indicated that the growth of sugarcane seedling was higher at the infection levels of 100 *Trichodorus christie* than 0,500 and 1000 nematodes per plant. They concluded that increases in the root weight of the plant was due to the production of lateral roots and thus increased their water and mineral intake.

Table 2. Varietal reactions of some selected sugarcane varieties to *M. incognita* (3 replicates).

Sugarcane variety		**Height (cm)	DIAMETER Top (cm)	DIAMETER Middle (cm)	DIAMETER Bottom (cm)	FRESH WEIGHTS Shoot (g)	FRESH WEIGHTS Root (g)
B41227	Infected	50.50 ± 3.04	4.06 ± 0.35	11.07 ± 0.64	8.20 ± 0.67	226.80 ± 0.82	236.67 ± 3.33
	Uninfected	45.07 ± 0.33	4.33 ± 0.17	11.17 ± 0.08	8.17 ± 0.17	231.44 ± 4.73	210.00 ± 1.16
B60267	Infected	88.17 ± 2.53	4.67 ± 0.33	12.33 ± 0.33	8.10 ± 0.36	415.80 ± 0.55	381.67 ± 1.67
	Uninfected	90.33 ± 0.33	5.50 ± 0.29	12.33 ± 0.17	6.67 ± 0.33	519.56 ± 4.72	430.66 ± 0.67
B59162	Infected	82.00 ± 2.00	4.17 ± 0.17	8.57 ± 0.23	6.40 ± 0.21	269.49 ± 0.62	226.67 ± 1.67
	Uninfected	84.66 ± 0.33	4.67 ± 0.33	9.17 ± 0.17	6.33 ± 0.17	288.12 ± 4.73	270.50 ± 0.19
B62163	Infected	89.00 ± 2.08	4.83 ± 0.17	11.00 ± 0.50	7.17 ± 0.17	420.53 ± 0.19	241.67 ± 1.67
	Uninfected	100.50 ± 0.29	3.67 ± 0.17	9.67 ± 0.33	7.17 ± 0.17	486.50 ± 4.73	274.67 ± 0.33
B61110	Infected	91.00 ± 0.58	4.67 ± 0.33	8.67 ± 0.17	6.33 ± 0.17	373.28 ± 1.27	380.00 ± 2.89
	Uninfected	85.67 ± 0.17	4.83 ± 0.17	8.17 ± 0.17	6.17 ± 0.17	349.53 ± 4.73	404.33 ± 0.33
B60163	Infected	74.33 ± 2.34	3.67 ± 0.33	6.67 ± 0.17	4.83 ± 0.17	132.30 ± 0.55	438.33 ± 4.41
	Uninfected	68.67 ± 0.33	3.67 ± 0.17	5.17 ± 0.17	4.33 ± 0.17	103.91 ± 4.73	320.33 ± 0.33
B4360	Infected	61.33 ± 1.45	4.33 ± 0.17	8.83 ± 0.17	4.67 ± 0.33	278.78 ± 2.90	516.67 ± 1.67
	Uninfected	58.17 ± 0.17	4.33 ± 0.17	8.17 ± 0.17	6.33 ± 0.17	231.44 ± 4.73	510.33 ± 0.33

**From ground level to the top visible dewlap (TVD) ;

± Standard Error of means.

Table 3. The effects of *M. incognita* on the degree of infestation and tolerance of sugarcane varieties.

Variety	Degree of infestation	Tolerance of Sugarcane varieties to *M. incognita*
B 41227	4	Susceptible, intolerant
B 59162	4	Susceptible, intolerant
B 62163	3	Susceptible, intolerant
B 61110	3	Susceptible, intolerant
B 60163	3	Susceptible, tolerant
B 4360	3	Susceptible, tolerant
B 60267	4	Susceptible, intolerant

* 3 = Moderately susceptible, * 4 = Susceptible.

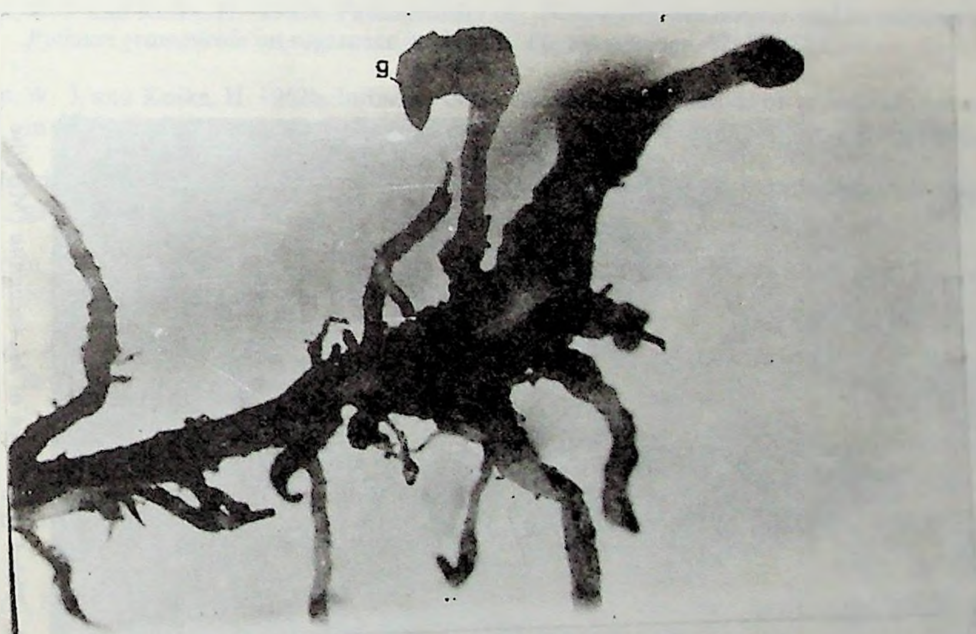

Figure 1. Sugarcane roots 25 days after inoculation with *M. incognita* [the sickle-shape gall (g) and extensive malformation of roots]

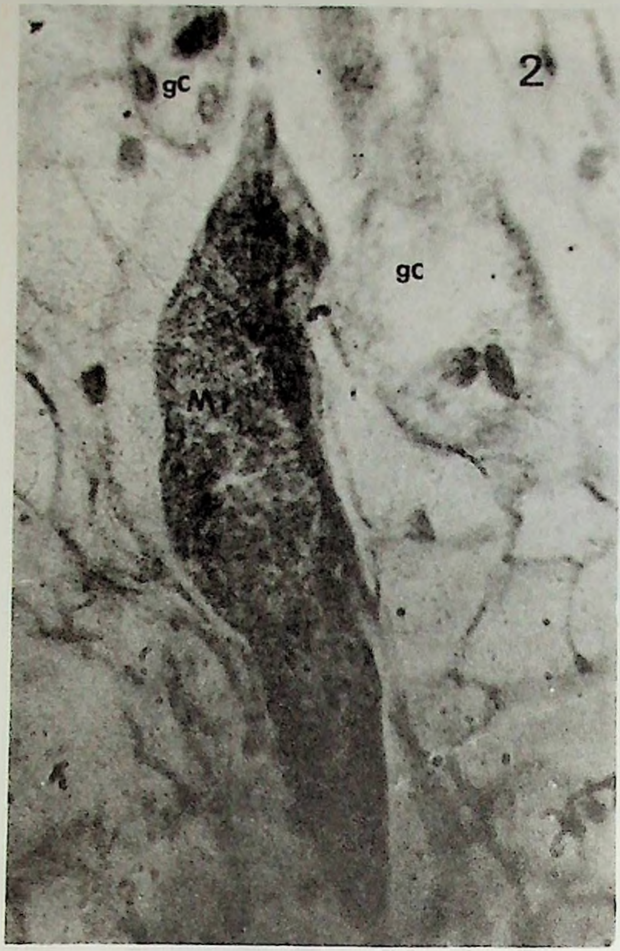

Figure 2. Third stage larva of *M. incognita* (Mi) [the nematode head between two giant cells (gc)].

Figure 3. Adult female of *M. incognita* (Mi). The nematode head is between multinucleate giant cells (gc) [the dense cytoplasmic contents of the giant cells].

The development of giant cells and severe injury to the cortical and endodermal tissues are significant. Giant cell formation in roots of plants infected by root-knot nematode species has been extensively discussed by several workers (Dropkin and Nelson, 1960 ; Huang and Maggenti, 1969 ; Paulson and Webster, 1970 ; Jones and Northcote, 1972 ; Bird, 1973). Huang and Maggenti (1969), Paulson and Webster (1970), Jones and Northcote (1972) agreed that multinucleate state of giant cells was the result of cell wall expansion and nuclear duplication without cytokinesis. Bird (1973), however, indicated that giant cells were formed partly by incorporation of cells where adjacent cells were dissolved. In this investigation, giant cells with dense cytoplasmic inclusions frequently occurred. Since no cell wall dissolution occured in these cells it thus appears that the cells only expanded as a result of increased cytoplasmic contents. Nuclear duplication without cytokinesis which was frequently encountered in root sections confirmed the previous reports by Huang and Maggenti (1969), Paulson and Webster (1970) and Jones and Northcote (1972). Gipson *et.al.*(1971), with an electron microscope, had shown that in soybean infected with *Heterodera glycine*, the cytoplasm in giant cells became dense as result of increase in the number of plastids and endoplasmic reticulum-like materials. In this investigation, cytoplasmic inclusions were not evident with the use of light microscope.

Swollen sugarcane root-tips was largely caused by the nematode developmental stages feeding and developing at the same site as well as inducing giant cells. The devitalization of the root-tips by the nematode is certainly detrimental to effective water and nutrient uptake from the soil by the plants.

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