



Original Research Article

Reaction of sugarcane genotypes to root-knot nematodes (*Meloidogyne* spp.) in Kenya

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^{1,2,*}Chirchir Alexander Kipkorir, ²John W. Kimenju, ²Emmanuel S. Ariga, ³George M. Kariuki and ⁴Chispine O. Omondi

¹Department of Crop Development, KA LRO - Sugar Research Institute, P.O. Box 44-40100, Kisumu Kenya.

²Department of Plant Science and Crop Protection University of Nairobi, P.O. Box 29053-00625, Kangemi, Kenya.

³Department of Agric Science and Technology, Kenyatta University, P.O. Box 43844-00100 Nairobi.

⁴Department of Plant Breeding, KA LRO - Coffee Research Institute, P.O. Box 4-00232, Ruiru, Kenya.

*Corresponding Author

E-mail: alkchirchir@yahoo.com
Tel.: +254 722 206921

Resistance/tolerance to some parasitic nematodes has been found to exist in various sugarcane cultivars in varying degrees. This trait has not been fully exploited especially in Kenya. Fifteen sugarcane cultivars were randomly selected and grown under a glass house in a completely randomized design with three replications over two seasons. Single-budded setts were inoculated with 2000 J₂ root-knot nematodes (*Meloidogyne* spp.). Infestation of root-knot nematodes (RKN) on Co 421 reduced fresh root weight by 33%, reduced tillering of Co421, KEN82-62 and KEN98-530 by 70%, 46% and 33% respectively, but did not affect those of KEN00-13 and KEN82-121. Uniquely, nematode inoculation promoted tillering of EAK 70-97 and N14 which increased by 21% and 30% respectively. Co421 and D8484 hosted the highest nematode population numbers with mean egg mass indices of 3.0 and 2.5 respectively. Conversely, KEN83-737 and Co945 had lowest nematode numbers with mean egg mass indices of 0.8. This study demonstrated that sugarcane cultivars do exhibit varying degrees of host resistance or tolerance to RKN. The resistant/ tolerant cultivars may be incorporated into integrated nematode management packages.

Key words: Resistance, root-knot nematodes, sugarcane cultivars, susceptibility, tolerance.

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is a widely cultivated crop in the tropical and subtropical countries for its numerous benefits that include its use in foodstuffs, as fiber and production of bio-fuel (Santos et al., 2012). In Kenya, sugarcane is an important cash crop earning small-scale farmers approximately US\$ 100 million annually (Government of Kenya (Gok), 2010). However, over the last decade there has been a steady decline of cane yields, falling from 91 ton ha⁻¹ in 1996 to 63 ton ha⁻¹ in 2010 (Gok, 2010; Mulwa et al., 2011). Probable causes for this reduction in productivity include the widespread use of low quality sugarcane varieties, poor agricultural and land management practices, and pests and diseases. Among

pests and diseases plant parasitic nematodes have been reported to cause significant yield loss in sugarcane production (Gok, 2010; Nzioki and Chirchir, 2010).

Worldwide over 310 species representing 48 genera of ecto- and endoparasitic nematodes have been reported to be associated with sugarcane root rhizosphere (Cadet and Spaul, 2005; Adesiyun et al., 1990). Root-knot nematodes, *Meloidogyne* spp., are widely distributed in tropical, subtropical and warm temperate regions of the world, are serious pests of a broad range of food and fibre crops, including cotton, soybean, cowpea, mung bean, peanut, tomato, potato, capsicum, cucurbits, tobacco, pineapple, banana, papaya and sugarcane (Luc et al., 1990). In

sugarcane fields, high nematode population densities of *Meloidogyne incognita* and *M. javanica* are usually found when crops are grown in light-textured soils, as these soils are ideally suited to nematode reproduction (Stirling, 2006). According to Stirling and Blair (2000), these species have been cited by various authors in different sugarcane producing regions as important pests of the crop. Cadet and Spaul (2005) have also reported that generally plant parasitic nematodes are among the most common pests that build-up over time and thus contribute to the yield decline.

Root-knot nematodes (RKN) are widely spread in cane fields and are most injurious to sugarcane roots during early growth (Dick, 1996). Damage caused by RKN on sugarcane results in reduced primary and secondary tillering, shorter stalks and lower yields (Spaul and Cadet, 1990). It decreases cane yields and reduces the number of consecutive ratoon crops (Cadet and Spaul 2003). In some tropical areas where the infestation is severe, nematodes are responsible for the desertion of hundreds of hectares of sugarcane plantations (Adesiyan et al., 1990).

Once nematodes are present in a field, it is nearly impossible to eradicate them. According to Berry et al. (2011), the best way to handle the infested field is to manage the nematode problem. There are several recommended practices for sugarcane farmers to manage this problem in their fields. Planting tolerant cultivars (Cook and Evans, 1987; Spaul and Cadet 2003; Spaul et al., 2005) has been identified as one of the sustainable and environmental friendly approaches in management of nematodes in sugarcane fields. Several field trials have shown that certain varieties of sugarcane are more tolerant of nematodes than others (Moberly and Clowes, 1981; McArthur and Spaul, 1995; Cadet and Spaul, 2003).

In recent years, the world sugar industry has begun to move away from use of chemical nematicides towards a farming approach that includes use of conventional farming systems that are environmental friendly and sustainable in management of pests and diseases. Some of the strategies include use of intercropping between sugarcane cycles and exploring of resistance in sugarcane cultivars (Spaul and Cadet, 2003). This drift has been driven by observations that the impact of yield decline can be reduced by use of resistant genotypes and by the desire to cut down production costs incurred through purchase of nematicides as well as environmental concerns (Stirling et al., 2001).

Compared to recent advances in plant-pathogen interactions as in the case of *Arabidopsis thaliana* (Sijmons et al., 1991; Boiteux et al., 1999; Vercauteren et al., 2001; Gheysen and Fenoll, 2002) and *Lotus japonicus* (Lohar and Bird, 2003; Lohar et al., 2004), no information is available on the interaction between RKN and sugarcane cultivars grown in Kenya. This study was therefore conducted to evaluate the relative susceptibility or tolerance to RKN among selected sugarcane cultivars grown in Kenya.

MATERIALS AND METHODS

Fourteen sugarcane cultivars (Co421, Co617, Co945, CB38-22, D8484, EAK70-97, KEN00-13, KEN82-121, KEN82-216, KEN82-472, KEN82-493, KEN82-62, KEN83-737 and KEN98-530) were selected by use of stratified random sampling and compared against cv. N14 maintained as the standard. The experiment was conducted in a glass house under completely randomized design with three replications over two seasons.

Single-budded setts of each cultivar were subjected to hot water treatment at 50°C for 2 hours. The setts were then pre-germinated in germination boxes and a single sett was planted per pot. Potting soil, collected from sugarcane fields, was sieved to remove debris and homogenized, then mixed with sand at a ratio of 2:1. The mixture was autoclaved after which 3 kilogrammes were placed in 5-litre pots of 15 cm diameter. At planting and thirty days after planting, each pot was fertilized with 20 g diammonium phosphate and 20 g urea, respectively.

The nematode inoculum (*Meloidogyne* spp.) was extracted from galls with eggmasses of infested sugarcane roots by use of the modified Baermann funnel technique (Hooper et al., 2005). It was then reared on young tomato plants. Two weeks after transplanting, cane seedlings were inoculated with 2,000 second stage juveniles (J₂). Inoculation was done by slowly dispensing 25 ml of the previously prepared nematode suspension into holes made in the soil around the plant and as close to the roots as possible. Control pots were inoculated with 25 ml distilled water. The potted plants were uprooted 120 days after planting and soil was gently shaken from the root system. Shoot height, fresh and dry shoot weight, fresh root weight and numbers of tillers were measured. Nematodes were extracted from 200 cm³ soil and 10 g of roots (fresh weight) by the Hooper et al. (2005) method and the egg mass index was assessed using the scale ranging from 1-5 as illustrated by Coyne et al. (2007). Data was subjected to analysis of variance (ANOVA) and means separated by least significant difference using GenStat statistical package (GenStat, 2011) version 14 (VSN International).

RESULTS

Inoculation of sugarcane with root-knot nematodes did not affect plant height and fresh and dry shoot weights for all cultivars (Table 1). Though infestation of the cultivars by RKN did not show any effect on fresh root weight in the first season, there was significant difference ($P \leq 0.05$) on the fresh root weight of Co 421 infested by the nematode as it had its fresh root weight reduced by 33% in the second season compared to the non-inoculated one (Table 2). However, among all the other cultivars the means of fresh root weight for inoculated plants did not significantly differ

Table 1. Response of sugarcane genotypes to inoculation of root -knot nematode in season 1

Genotypes	Shoot height		Fresh shoot weight		Dry shoot weight		Fresh root weight		Tillers number		Nematode Count/ 200 cm ³ soil and 10 g of roots	Egg mass index
	In	NI	In	NI	In	NI	In	NI	In	NI		
CB38-22	30.0	30.8	34.8	36.0	9.1	8.8	28.1	29.4	1.3	1.7	25.0	1.7
Co421	33.2	36.2	77.4	88.2	17.9	26.8	16.8	45.3	1.7	4.3	83.3	3.0
Co617	39.5	39.8	62.2	66.0	15.7	16.1	34.8	35	4.0	4.0	16.7	1.3
Co945	23.7	24.0	36.1	42.1	9.1	12.7	31.3	31.9	2.7	2.7	12.5	1.0
D8484	40.8	47.3	103.2	100.9	32.0	34.3	38.8	37.8	3.7	3.0	87.5	3.0
EAK70-97	43.5	42.2	80.4	88.9	25.7	26.1	50.0	56.1	4.3	4.3	16.7	1.3
KEN00-13	34.5	33.3	91.2	93.2	27.3	29.6	37.5	39.0	3.7	3.7	41.7	2.7
KEN82-121	35.5	33.7	54.8	56.9	16.0	17.4	32.1	34.5	1.3	1.3	54.2	2.3
KEN82-216	33.8	32.8	74.0	78.6	20.7	22.6	33.9	35.4	3.0	3.3	12.5	1.0
KEN82-472	34.7	36.2	89.2	91.1	24.0	24.2	29.3	30.8	2.0	2.3	33.3	1.7
KEN82-493	36.8	34.0	72.0	73.1	19.9	19.3	69.6	69.7	3.0	3.7	33.3	2.7
KEN82-62	33.3	36.4	84.0	72.7	22.3	19.6	57.0	59.6	2.0	5.0	20.8	1.7
KEN83-737	32.3	33.5	60.0	60.8	23.2	23.9	42.8	43.7	4.0	4.3	16.7	1.3
KEN98-530	46.3	49.7	106.3	111.2	34.7	35.5	77.0	62	3.3	3.3	41.7	2.3
N14	33.3	33.7	75.3	77.4	29.7	30.2	47.8	47.7	2.7	2.7	50.0	2.0
Grand Means	35.4	36.2	73.4	75.8	21.8	23.1	41.8	43.9	2.8	3.3	36.39	1.9
L.S.D (0.05)		10.204 ^{ns}		29.38 ^{ns}		8.339 ^{ns}		19.76 ^{ns}		1.40 ^{ns}	13.157*	0.6712*
CV (%)		15.7		20.3		14.4		27.3		29.9	44.1	43.6

In = inoculated; NI = non-inoculated; L.S.D = least significant difference at $P \leq 0.05$; CV = coefficient of variation; *, ^{ns} = Significant, not significant respectively at $P \leq 0.05$.

Table 2 Response of sugarcane genotypes to inoculation of root knot nematode in season 2

Genotypes	Shoot Height		Fresh shoot weight		Dry shoot weight		Fresh root weight		Tillers number		Nematode Count/ 200 cm ³ soil and 10 g of roots	Egg mass index
	In	NI	In	NI	In	NI	In	NI	In	NI		
CB38-22	35.3	33.5	79.2	84.1	13.8	11.5	20.3	20.3	1.0	1.0	33.3	2.0
Co421	33.3	36.3	81.3	83.4	16.9	25.4	31.3	46.6	1.3	4.3	100.0	3.0
Co617	48.0	48.0	91.6	91.3	18.3	19.9	42.6	43.3	3.3	3.7	16.7	1.3
Co945	22.7	25.7	38.1	34.8	15.2	14.2	26.8	28.0	2.3	3.0	12.5	0.7
D8484	41.2	42.8	113.7	118.8	32.1	33.6	50.7	53.8	3.7	4.0	54.2	2.0
EAK70-97	34.0	32.7	50.7	53.6	22.9	24.7	13.5	14.4	4.0	3.3	16.7	1.7
KEN00-13	30.0	30.3	80.9	82.3	23.2	24.2	32.8	33.5	3.7	3.7	29.2	1.3
KEN82-121	31.5	32.3	59.5	61.5	15.1	15.5	12.7	14.8	2.0	2.0	62.5	1.3
KEN82-216	35.5	34.2	83.2	83.7	21.9	21.4	30.1	31.0	2.7	3.3	12.5	1.3
KEN82-472	28.2	29.3	88.5	88.3	17.4	17.8	20.4	21.6	2.0	2.7	37.5	1.3
KEN82-493	37.5	38.2	55.8	60.6	20.5	21.6	22.4	23.4	3.0	4.0	29.2	2.0
KEN82-62	32.5	33.2	80.6	80.2	20.8	21.15	32.5	32.9	2.0	3.7	16.7	1.3
KEN83-737	47.5	48.0	120.7	123.5	22.8	23.6	43.3	44.4	3.3	4.3	4.2	0.3
KEN98-530	47.2	47.7	120.9	124.1	34.2	35.8	50.3	51.3	2.7	4.0	37.5	2.0
N14	40.3	43.3	132.3	130.9	20.1	20.2	17.0	18.7	3.0	2.3	50.0	2.3
Grand Means	36.3	37.0	85.1	86.7	21.0	22.0	29.8	31.9	2.7	3.3	34.2	1.6
L.S.D (0.05)		4.245 ^{ns}		6.269 ^{ns}		5.469 ^{ns}		5.34*		1.1892*	11.51*	0.7193*
CV (%)		6.5		3.9		16.4		8.4		24.5	43.6	57.4

In = inoculated; NI = non-inoculated; L.S.D = least significant difference at $P \leq 0.05$; CV = coefficient of variation; *, ^{ns} = Significant, not significant respectively at $P \leq 0.05$.

($P \leq 0.05$) compared with the non-inoculated.

In the first season, inoculation by RKN on sugarcane had no effect on the number of tillers produced. However parasitism by RKN significantly ($P \leq 0.05$) affected prolificacy of tillering of cultivars in the second season. The tillering of Co421, KEN82-62 and KEN98-530 cultivars was reduced by 70%, 46% and 33% respectively. On the other hand, inoculation had no effect on the tillering of varieties KEN00-13 and KEN82-121. Uniquely, however, inoculation seemed to promote tillering for varieties EAK 70-97 and N14, with their tillering increasing by 21% and 30% respectively.

All the sugarcane genotypes tested were found to be host to root-knot nematode. However, the mean populations possessed by each cultivar significantly differed ($P \leq 0.05$) across the genotypes. Genotypes Co421, D8484 and KEN82-121 predominantly exhibited the highest mean population whereas genotypes KEN83-737, KEN82-216 and Co945 had the lowest mean population of nematodes over both seasons.

There was significant difference ($P \leq 0.05$) in nematode egg mass index among the sugar cane genotypes in first and second seasons. Over both seasons, genotypes Co421 and D8484 proved to be the preferable hosts that had the highest overall mean egg mass index of 3.0 and 2.5 respectively. Conversely, genotypes KEN83-737 and Co945 had the least overall mean egg mass index of 0.8 over both seasons.

DISCUSSION

The description of the terms 'susceptible' and 'resistance' in this study is adopted from Stirling (2006): susceptible varieties are described as those that are capable of supporting nematode reproduction, whereas resistant ones are those where multiplication is limited. Stirling (2006) adopted this scale in a study to rate susceptibility of sugarcane varieties to root-knot nematode species (*M. javanica* and *M. incognita*) in Australia. The results, however, provide no information on the capacity of the tested varieties to resist attack from the nematodes but rather withstand infestation, a property that is usually referred to as 'tolerance'. Tolerance to damage is independent of resistance and relates to the ability of a host genotype to withstand or recover from the damaging effects of nematode attack and to yield well (Trudgill, 1991). The use of crop resistance approach against pest infestation such as root-knot nematode is one of the principles of crop protection and has become important in pest management in recent years following environmental hazards caused by chemical control measures (Olowe, 1992; Mangala and Mauria, 2006). It is apparent from the result of this investigation that various sugar cane cultivars have different degrees of resistance to *M. incognita* infestations

with evidence in variation of plant growth, vigor and reproduction (Stirling, 2006).

Generally, all the inoculated sugar cane cultivars showed reduced shoot height, shoot weight and root weight compared to non-inoculated because all the genotypes were susceptible host allowing root-knot nematode to survive and parasitize cane resulting in reduced physiological processes due to deprived nutrients flow as they attack the roots affecting their uptake ability. According to Hussey (1989), root-knot nematode parasitizes the host plant by affecting on its nutrients: the infective second stage juvenile (J_2) penetrates the host root near the root tip, then initiates a feeding site after which it migrates to the developing vascular cylinder. The damaged root and vascular system limits the ability of the plant to access moisture and nutrients, resulting in slower plant growth and consequently reduced crop yield (Nicol et al., 2011; Stirlin et al., 2003).

In the non-inoculated sugar cane cultivars, the numbers of tillers were higher compared to the inoculated ones. This result shows that root-knot nematode reduce tillering ability in sugarcane. This trend was also observed in fresh shoot and root weights of inoculated cane. These observations agree with those reported by Stirling et al., 2003, Brigde et al., 2005 and Nicol et al., 2011 in their studies on rice where they demonstrated reduced growth and number of tillers in rice infested by RKN.

High tolerance to root-knot nematode was observed in KEN83-737, KEN82-216, Co945 and Co617: though they were infested by the nematode, they could withstand its effects and remained healthy unlike Co421 and D8484 whose susceptibility resulted in evident reduction in growth and high nematode population. In addition, cultivars tolerant to the nematode maintained their high tillering ability compared to the most susceptible genotypes. Nematodes affect reproduction ability of cane since its growth vigour and reproduction is basically dependent on nutrition (Trudgill, 1991; Jacquet et al., 2005; Nicol et al., 2011, and Stirling et al., 2001)

KEN83-737, KEN82-216, Co945 and Co617 were more resistant to root-knot nematode and were a less preferable host and therefore had less nematode population counts compared to Co421 and D8484 which were a more susceptible host thus harboring higher nematode populations. This finding confirms that of Trudgill (1991) where he found that less nematode populations were counted in more resistant crops compared to susceptible genotypes. Since the objective of every farmer is to make a profit by increasing yields and reducing cost of production incurred in the control of nematodes, they are most likely to prefer cultivars that possess higher levels of resistance or tolerance when faced with soils having high nematode infestation.

Fewer egg masses were counted in the tolerant varieties compared to the susceptible varieties possibly because the

former inhibited reproduction; as a result the females of root-knot nematode in tolerant cultivars couldn't produce many eggs as compared to susceptible host. This observation confirms the finding made by Trudgill (1991).

The mechanism of resistance to RKN in crop plants seems to vary between crops, and among cultivars of a crop and may also manifest as either pre- or post-infection (Dhandaydham et al., 2008). Pre-infection resistance was clearly evident on cucumber and peanut studies conducted by Haynes and Jones (1976) and Bendezu and Starr (2003) respectively. This resistance is due to lack of nematode entry into the plant and is possibly due to the presence of pre-formed chemicals in the plant that are toxic and antagonistic to the nematodes (Huang, 1985). Additionally, post-infection resistance mechanisms exist and are manifested after the penetration of the nematode in the host and, in some cases, are associated with a classical hypersensitive response (HR) (Dhandaydham et al., 2008). The HR is typically explained by the gene-for-gene-model in which a virulence gene product from the pathogen is specifically recognized by the resistance gene product of the host (Bent, 2011; 1996). The number of genes controlling resistance to RKN seemed to differ among hosts and even among varieties. For example, a single gene controls resistance in soybean cultivar 'Forrest' (Luzzi et al., 1994a), whereas multiple genes control resistance in soybean lines PI96354 and PI417444 (Luzzi et al., 1994b). Therefore, there could be multiple genes that control resistance in sugarcane but they differed in all the screened 15 test cultivars.

CONCLUSIONS AND RECOMMENDATION

The majority of sugarcane cultivars tested showed moderate to high level of tolerance to RKN. Four varieties, KEN83-737, KEN82-216, Co945 and Co617 showed a high level of tolerance while nine varieties, N14, EAK70-97, KEN98-530, CB38-22, KEN00-13, KEN82-121, KEN82-472, KEN82-493 and KEN82-62 showed moderate tolerance. Only two varieties, Co421 and D8484 were susceptible. This study demonstrated that sugarcane cultivars grown in Kenya possess varying levels of resistance and tolerance to root-knot nematode. Cook and Evans (1987) have reported successes in developing resistant cane cultivars using conventional breeding procedures. Therefore resistance/tolerance to plant parasitic nematodes should be incorporated in the variety improvement programmes as part of an integrated pest management strategy.

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