Reaction of Selected Coffee Germplasm to Root-Knot Nematodes in Kenya

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Abstract

Coffee is one of the most important cash crops in Kenya and a leading export earner. Nematodes are among the most important biotic constraint in coffee production in Kenya and crop improvement work has mainly been breeding for resistance to diseases such as coffee berry disease and coffee leaf rust. However resistance has been used successfully in other coffee producing countries and it is one of the most economical and practical nematode management strategies. A greenhouse study was conducted to test the response of local and exotic coffee germplasm to root knot nematodes (RKNs). Ten (10) cultivars provided by Coffee Research Foundation (CRF) were tested for resistance to *Meloidogyne incognita* under greenhouse conditions (25 ± 2^oC). Nematodes were extracted from the roots using Modified Baermann Technique and enumerated using Cobbs slide. After 90 days of plant growth, the disease severity was evaluated and the experiment repeated twice. Galling indices (GI), egg mass indices (EMI) and nematode populations recovered from soil samples indicated a range of responses from resistant to highly susceptible. Three breeder's lines including Robusta tree 1, Robusta tree 2 and Robusta tree 3 were rated resistant with galling indices of 1.2-3.0. This study has demonstrated the potential of host resistance as a strategy in the management of nematodes in coffee for increased productivity. Field evaluation needs to be conducted to confirm these findings. The identified resistance sources can be utilized to deploy resistance genes to improve existing varieties that have high commercial value but lack resistance to nematodes.

Key words: Resistance, susceptible, galling indices, nematode population, cultivars

Introduction

Breeding of coffee cultivars with resistance to nematodes may be the most economical and practical option for sustainable nematode management (Maredia *et al.,* 2003; Rosskopf *et al.,* 2005). Nematode resistant cultivars have been shown to reduce root damage by 32% (Campos and Villain, 2005; Castillo *et al.,* 2009). However, since coffee is often attacked by a diverse community of plant parasitic nematodes, breeding for combined resistance is a challenging task. Loss of resistance to pests may be attributed to; breeding done in ignorance of the basic principles of genetics, traits such as unpalatable or toxic chemicals being reduced during domestication, yet these same chemicals may deter colonization by pests, or lead to pest adaptation through development of new races of pests (Dent, 2000, Starr *et al.,* 2002).

The varieties of coffee grown in Kenya have been previously tested for high yields, ecological adaptation and resistance to major diseases of coffee namely, coffee berry disease caused by *Colletotrichum kahawae* and coffee leaf rust caused by *Hemileia vastatrix* (MoA, 2006) . Other coffee lines and crosses are either being developed or on adaptive trial for various desirable traits but not for resistance to nematodes. In countries like Brazil, varieties resistant to nematodes have been developed and are in commercial use (Wintgens, 2009). Robusta coffee is mainly grown in the neighboring country of Uganda. However, CRF is undertaking trials using the Robusta as the rootstock since studies have revealed that *Coffea canephora* (Robusta) is resistant to nematodes (Campos & Villain*,* 2005). Arabica coffee is the most commonly grown coffee in Kenya and it is popular for its superior quality. The Arabica coffee cultivars grown in Kenya include K7, SL 28, SL34, Ruiru 11 and Batian and characteristics of these varieties are as shown in Table 1.

Table 1: Characteristics of commercial coffee cultivars grown in Kenya

Variety	Area/ecology	Characteristics
K7	Low altitudes (selected at Muhoroni)	Produces fair yields, resistant to coffee leaf rust and does well in low altitude areas
SL 28	Medium altitudes, dry areas without leaf rust (selected at NARL, Kabete)	Fairly high yielding, good quality, drought resistant, susceptible to CBD and fairly resistant to coffee leaf rust
SL34	Medium to high altitudes with good rainfall	High yielding, susceptible to CBD and coffee leaf rust
Ruiru 11	All coffee growing areas (bred at CRF, Ruiru)	Resistant to coffee berry disease (CBD) and coffee leaf rust (CLR)

Key: CBD – Coffee berry disease, CLR – Coffee leaf rust

Most of the farmers in Kenya cultivate the traditional Arabica coffee cultivars despite the development of Ruiru 11, a more superior high yielding variety resistant to the major diseases in Kenya namely coffee berry disease and coffee leaf rust. Root knot and lesion nematodes frequently cause serious damage to Arabica coffee (Castillo *et al.,* 2009). CRF has developed several varieties for disease resistance making a breakthrough in coffee berry disease and coffee leaf rust management, but there has been no varieties developed for resistance to nematodes yet. Management of nematode problems has focused mainly on the use of healthy planting materials and soil fumigation (Wintgens, 2009). Nematode resistant cultivars are thus important complementary and sustainable options. This study was carried out with the aim of evaluating the available coffee germplasm in Kenya for resistance to the most damaging root knot nematodes, *Meloidogyne* spp.

Materials and methods
Three-month-old potted coffee seedlings from ten different varieties namely K7, Blue Mountain, Robusta – Tree 1, Robusta – Tree 2, Robusta – Tree 3, Ruiru 11, SL28, Selection 6, Selection 5A, and CR30 were obtained from Coffee Research Foundation (CRF) and challenged with *Meloidogyne* spp. at three inoculum levels of 1000, 2000 and 5,000 eggs/juveniles per pot. Inoculation was done by pipetting 10 ml aliquots carrying the required concentrations of eggs and pouring into four pencil-size holes around each seedling at a depth of 2-3 cm. The controls received only 10ml of distilled water. A completely randomized design with eight replicates was used and the experiment repeated three times. Nematode inoculum (*Meloidogyne* spp) was multiplied *in situ* in potted spinach plants.
Nematode egg inoculum from the galled plants was prepared following the technique described by Hooper et al., (2005). Galling was assessed using a rating scale adopted from Luc et al., (2005) as follows; 1= no eggs or galls, 2= 1-10% galled roots, 3= 25% roots galled, 4= 50% roots galled, 5 = 75% galling and 6= 100% galling with dysfunctional roots/ plant withered.
Ninety days after inoculation, the experiment was terminated and data on nematode numbers in soil, galling and egg mass index, shoot and root fresh weight recorded. Data was collected by taking the various measurements by weighing fresh root after carefully washing off the soil and shoot (aerial) parts. Roots from sample plants were taken to the laboratory for extraction using Modified Baermann funnel technique as described by Hooper et al., (2005). In the laboratory, roots were washed and examined for galling, then stained with Phloxine B to assess the egg masses. Thereafter, roots were chopped into 1cm segments and macerated using a blender, sieved with nested nematode sieves and using a known volume of the filtrate, nematode counts were determined using the nematode counting slide.
Data collected was subjected to analysis of variance (ANOVA) using GENSTAT Release 7.2 software. Means, when significantly different, were separated using the Fisher' protected LSD test at 5% probability level.

Results

Significant (P≤0.05) differences were observed among the coffee varieties in galling, egg mass indices and juvenile counts. Galling and egg mass indices for the 10 cultivars ranged from 1.6 - 4.4 (Table 2). Varieties SL28, Selection 5A, Selection 6 and CR.30 were rated as susceptible with galling indices in the range of 3.4-4.4

(Table 3). Ruiru 11 and Blue Mountain were rated as moderately resistant with galling and egg mass indices of 2.3-2.8. K7, Robusta Trees 1, 2 and 3 with mean galling index of 1.6 – 2.1 were rated as highly resistant to *M. incognita*.

Table 2: Galling and egg mass indices of coffee cultivars challenged with root knot nematode (*Meloidogyne* spp.) 120 days after inoculation.

Cultivar	Galling index	Egg mass index	Susceptibility Rate
Selection 5A	4.2a	3.9a	Susceptible
SL 28	3.4b	3.7a	Susceptible
Selection 6	4.4a	3.9a	Susceptible
CR.30	3.6b	3.4a	Susceptible
Ruiru 11	2.3d	2.8b	Moderately resistant
Blue Mountain	2.8c	2.6b	Moderately resistant
K7	1.6e	2.2bc	Resistant
Robusta - Tree3	2.1d	2.8b	Resistant
Robusta - Tree 2	1.6e	2.4bc	Resistant
Robusta – Tree 1	2d	2.7b	Resistant
L.S.D (P=0.05)	0.4333	0.5260	

All data are means of 8 replicates. Means followed by the same letter within each column are not significantly (P=0.05) different.

At higher nematode inoculum doses, egg mass indices were highest for Selection 5A, Selection 6 and SL 28 (Table 3). Robusta tree 2 supported the lowest nematode counts with the least galling indices compared to SL28 and Selection 5A, which had the highest. The susceptible to moderately resistant cultivars (CR 30, Selection 6 and Blue Mountain) showed the highest root damage at higher inoculum intensities with symptoms of reduced lateral root system characterized by rotting and necrotic lesions. This interfered with nutrient and water uptake, resulting to stunted plants/unthrifty plants as depicted by poor growth, short internodes, small leaves and defoliation (Plate 1).

Table 3: Egg mass indices on roots of coffee cultivars infested with different levels of root knot nematode inoculum.

Germplasm	Inoculum density (eggs and juveniles)		
	1000	2000	5000
Blue mountain	2.3c	2.7b	3.0c
CR 30	4.0a	3.7b	3.3a
Selection 5A	3.7ab	3.7b	4.3a
K7	2.3c	1.7f	2.6ab
Robusta - Tree 1	2.0c	3.0d	2.7ab
Robusta - Tree 3	2.7bc	3.3c	3.0ab
Robusta – Tree 2	2.3c	2.3e	2.3b
Selection 6	4.3a	4.3a	4.0a
Ruiru II	3.3b	3.0d	3.0a
SL 28	3.0b	4.0a	4.3a
L.S.D (P=0.05)	0.4445	0.2435	0.7699

All data are means of 8 replicates. Means followed by the same letter within a column are not significantly (P=0.05) different
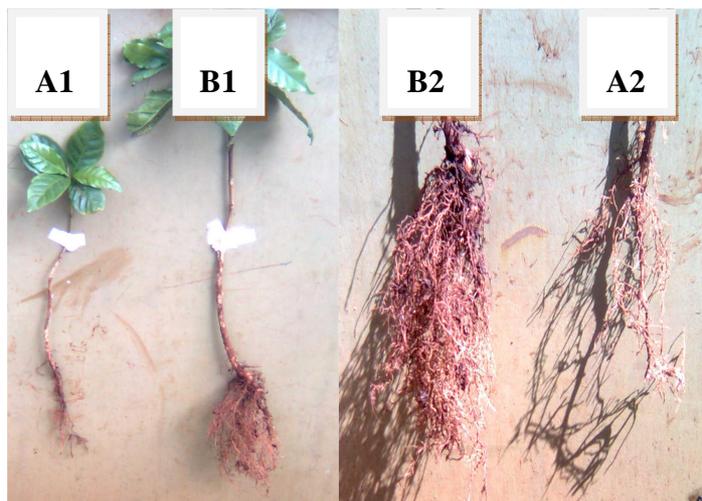
Plate 1: Root system of coffee seedlings inoculated with *Meloidogyne incognita* eggs (A1 and A2) and control (B1 and B2)

The analysis of variance showed that there was a high variation due to genotypes. The value of sum of squares due to inoculation level in relation to G x I sum of squares indicated substantial differences (P=0.05) in genotype response on different inoculation levels (Table 4).

Table 4: Analysis of variance of egg mass index for inoculation experiments

Source	df	SS	MS	F	P
Main Effects					
Genotype (G)	9	31.7889	3.5321	11.35	<0.001 **
Inoculation level (I)	2	0.8667	0.4333	1.39	0.256
Interaction (G x I)	18	11.5778	0.6432	2.07	0 .019 **
Residual	60	18.67	0.3111<		
Total	89	62.9000			

The highest nematode counts (3344) were found in the soil sample obtained from pots planted with cultivar CR30 while the lowest (50) was recovered from soils planted with Robusta tree 3 (Table 5). Ruiru 11, K7 and Selection 5A moderately suppressed nematode numbers with mean populations of, 541, 376 and 728 respectively. Robusta tree 3 had the least percentage root weight reduction followed by Robusta Tree 1 and Tree 2. Cultivars: Selection 6, Blue Mountain, SL28 and CR 30 had the highest differences. The differences in reaction to nematode among the different cultivars could also be seen in the root masses of the various treatments (Plate 2)

Table 5: Galling, egg mass indices, juvenile counts and % reduction in root mass of cultivars infested with *Meloidogyne* species (5000 eggs).

Cultivar	Galling index	Egg Mass index	Juvenile count/200 cm^3 soil	% Root weight reduction	Reaction
Robusta -Tree 2	1.6	1.3c	80d	14	R
K7	3.9	4.0b	376c	24	R
Ruiru 11	4.8	4.4b	541c	25	MR
Selection 5A	4.8	4.6a	728c	25	S
SL 28	4.6	4.6a	1370b	47	S
CR. 30	4.9	4.6a	3344a	48	S
Blue Mountain	4.0	4.6a	1696b	50	MR
Robusta -Tree 1	1.5	1.4c	102d	12	R
Robusta -Tree 3	1.4	1.1c	50d	5	R
Selection 6	5.2	5.1a	1840b	41	S
L.S.D	0.5366	0.4752	435.8		

All data are means of 8 replicates. Means followed by the same letter within a column are not significantly (P=0.05) different.

Key:

R   - Relatively resistant;
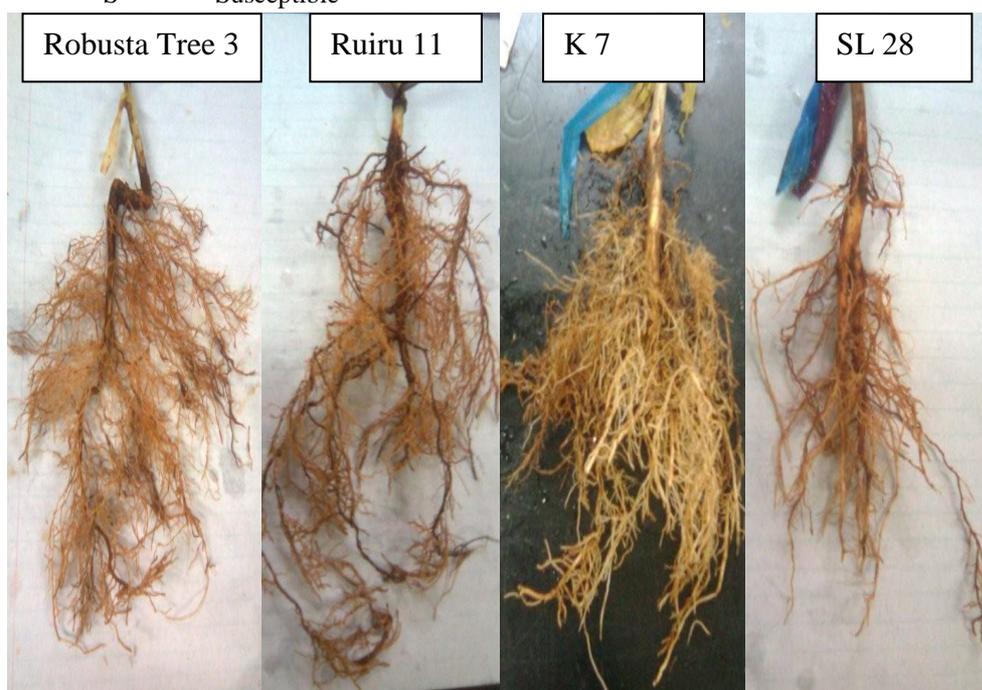MR   - Moderately resistant
S   - Susceptible

Plate 2: Root masses of various coffee cultivars inoculated with *M. incognita* 5000 eggs

When challenged with higher inoculum intensities of 5000 and 10,000 eggs and juveniles of *M. incognita*, Robusta trees 1 and 2 were significantly (P=0.05) different in egg mass indices from Robusta tree 3 (Table 6). However, at very high rates of nematode inoculum, resistance seems to break down even for the Robusta crosses as was demonstrated by the high J2 counts from these treatments. Treatment means of the three Robusta trees were significantly (P, 0.05) different from the mean of SL 28, a susceptible cultivar used as the control at 5% probability level (Table 6).

Table 6: Galling, egg mass indices, juvenile counts and % root damage of coffee cultivars challenged with higher rates of *Meloidogyne* inoculum.

Cultivar/ Color code	Inoculation @ 5000 eggs			Inoculation @ 10,000 eggs		
	Galling index	Egg mass index	J2s/ 200cm^3 soil	Galling index	Egg mass index	J2s/ 200 cm^3 Soil
Robusta -Tree 2	3.2b	2.7b	380d	4.1b	3.5b	1047c
Robusta -Tree 3	2.5c	1.7c	588b	3.4d	2.3c	1313c
Robusta -Tree 1	3.1b	2.3b	470c	3.9c	3.4b	1675b
SL 28 control	4.5a	4.5a	2175a	5.4a	5a	3265a
L.S.D	0.3873	0.4262	79	0.2360	0.2465	198.9

Means followed by the same letter within a column are not significantly (P=0.05) different.

Discussion

The host suitability study of the ten coffee cultivars to *Meloidogyne incognita* showed that most of the Arabica cultivars were susceptible, except Ruiru 11. Other cultivars had different responses to *M. incognita* and this is in line with reports of other workers where differential reaction of varieties to nematode attack has been documented (Starr *et al.,* 2002). Root necrosis affected the gall index-egg mass relationship. It has been shown that root tissue necrosis does not allow normal root-knot nematode reproduction and subsequently there is less galling (Dent 2000). Resistance seemed to breakdown when the cultivars were challenged with high concentration of inoculum, possibly due to high initial pathogen population of nematodes resulting to greater damage of the tender roots before the plant established itself as also indicated by Luc *et al.,* (2005).

Studies have also shown that *Coffee arabica* is relatively susceptible to many species of *Meloidogyne* species (Zhang and Schmitt; 1995; Starr *et al.,* 2002; Campos and Villain, 2005; Castillo *et al.,* 2009). Past screening studies in coffee identified resistance to *Meloidogyne* spp. in C*offea canephora*, specifically var. Robusta (Whitehead, 1998; Campos *&* Villain, 2005; Castillo *et al.,* 2009; De'Souza, 2008). So far, grafted and resistant Robusta hybrids with considerable resistance to many *M. incognita* pathotypes have been developed elsewhere and they include: Robusta breed T3561X T3751 in El Salvador, Nemaya variety whose ancestors are T3751 and T3561 and Apoata in Brazil (Bertrand *et. al.,* 2001; Campos & Villain, 2005; Castillo *et al.,* 2009; Cabos *et al.,* 2010). 'Romex', a Mexican Robusta is currently being used in Mexico where clones R34, R37 and R48 have shown a high tolerance to "corchosis" (Castillo *et al.,* 2009; Wintgens, 2009).

Other studies revealed that Arabica coffee cv. Iapar 59 is also resistant since it contains the Mex-1 resistance gene that confers high resistance to *Meloidogyne spp.* through a mechanism referred to as hypersensitivity (rapid necrosis reaction of affected cells) (Sayan *et al*., 2008). Arabica coffee cv. Caturra is believed to be more susceptible than cv. Iapar since it contained more nematodes than those found in cv. Iapar 59 root systems six weeks after inoculation (Bertrand *et. al.,* 2001, Wintgens, 2009). Other coffee cultivars reported as being resistant to root knot nematodes include: Timor hybrid, an interspecific hybrid between *C. arabica* and *C. canephora* which is also a variety of R11 as its female parent (Gichuru, 2007).

Despite the development of resistance against nematodes for developed economies, when resistant crop cultivars are available to farmers in the tropics, the adoption is still very low as observed by Roberts (2002). Many factors have to be taken into account such as acceptability based on preferences and quality characteristics, growing period and harvesting time among others. Cup tests conducted with coffee from all grafts with *C. canephora* revealed that coffee quality is not compromised; hence the lines conferring resistance to *M. incognita* provide useful germplasm for crop breeding in the tropics (Bertrand *et. al.,* 2001). In addition, rootstock of Funukaga cultivar (*Coffea liberica*) has been found to improve resistance to nematodes when the susceptible *Coffea arabica* is used as scion (Bittenbender *et. al.,* 2001*).* The resulting crop has been shown to retain good cup quality typical of Arabica coffee, high yields and nematode resistance (Bittenbender *et. al.,* 2001).

Conclusions and recommendations

The study identified three coffee germplasm namely Robusta tree 1, Robusta tree 2 and Robusta tree 3 as having considerable resistance to root knot nematodes. *Coffea canephora* has resistance to root knot nematodes and thus it can be used as root stock for *Coffea arabica*.

Further research work is needed to screen these cultivars under field conditions and undertake economic analysis to assess those of high commercial value in terms of yield and quality. The highly significant G x I interaction obtained in this study indicates the necessity of testing nematode resistant coffee varieties more than once through field trials for accurate characterization of genotypic performance. The three Robusta lines provides potential sources of resistance for breeding and grafting to address nematode problems in Kenya

Acknowledgements

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