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Evaluation of selected tomato cultivars reaction to infestation with *Meloidogyne javanica* in greenhouse conditions

M.W. Mwangi[1*] J.W. Kimenju[1] R.D. Narla[1], W.M. Muiru[1], G.M. Kariuki[2]

[1]*Department of Plant science and Crop protection, University of Nairobi, Nairobi, Kenya*

[2]*Department of Agricultural Science and Technology, Kenyatta University Nairobi, Kenya*

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Abstract

Root-knot nematodes are the most important phytoparasites that decrease tomato production in Kenya. It is important to test susceptibilities of tomato cultivars to root-knot nematodes (RKN) in order to mitigate against losses, by putting in place appropriate control measures. Three week old tomato seedlings of six different tomato cultivars Prostar F1, Kilele F1, Oxly, Cal j, Rambo F1 and Roma VFN F1 were transplanted separately into 500 g plastic pots. The experiment had one treatment for each tomato cultivar where each pot was inoculated with 1000 second stage juveniles of *Meloidogyne javanica* and a control without any treatment. Each was then replicated four times and arranged in a randomized compete block design in the greenhouse. After nine weeks the experiment was terminated, root and shoot weight, number of flowers, and number of galls per root system were taken. The most susceptible cultivars were Rambo F1, Prostar F1and Roma VFN with gall indices of 4.0, 3.5 and 3.0 respectively while cultivar Oxly was the least susceptible with galling indices of 1.5. The effect on growth due to nematode infestation was determined on the basis of reduction in heights, number of flowers, shoot and root weight. Cultivars, Rambo F1, Roma F1, Prostar F1 and Cal J had high galling indices and greater reductions in dry shoot weight and number of flowers compared to the cultivars Kilele F1 and Oxly which had low galling indices. It is important to use tomato cultivars that are less susceptible to RKN to increase tomato production.

* **Corresponding Author:** M.W. Mwangi ✉ wanjirumargie00@gmail.com

Introduction

Tomato production is hindered by diseases and pests that cause significant yield losses. Bacteria, fungi and viruses are among the causes of diseases in tomato, while among the nematodes, the root knot nematodes (RKN) belonging to the genus *Meloidogyne* are the most important, causing a significant drop in yields. Root knot nematodes *(Meloidogyne* spp.), affect tomato production (Sikora and Fernandez, 2005; Serfoji *et al*., 2010) with yield losses of 30 to 40% in tropical regions (Charchar *et al*., 2003).

Tomato production relies heavily on appropriate management strategies to control diseases, nematodes and pests. One of the methods widely used by the farmers is planting of hybrids with resistance genes to diseases, nematodes and pests. Tomato cultivars with genes for resistance to Fusarium wilt, Fusarium crown, Verticillium wilt, root knot nematodes (RKN) and tobacco mosaic virus have been developed (Erb and Rowe, 1992). Resistant varieties are very popular because the varieties are high yielding, despite the high cost of purchasing the seeds. There are many tomato varieties that are resistant to RKN. These are noted in some seed catalogues and variety descriptions as being nematode resistant, or the label simply has an acroynym VFN (Verticillium, Fusarium, Nematode) meaning Verticillium, Fusarium and nematode resistant.

It has been observed that tomato cultivars vary in their level of susceptibility to *Meloidogyne* spp. Several researchers evaluated degree of susceptibility to *M. javanica* based on reproductive factor (rate of reproduction). Reproductive factor (RF) is calculated as final population divided by the initial population (Irshad *et al*., 2012). A reproductive factor greater than one is indicative of reproduction while RF of less than one implies no reproduction. Abbas *et al*. (2008) rated tomato cultivars with high reproductive factors as susceptible. Similarly on the basis of reproductive factor, Jaiteh *et al*. (2012) classified tomato cultivars as highly resistant, moderately resistant, and highly tolerant, while Esafahani *et al*. (2012) classified them as tolerant and hypersensitive.

Formation of galls on infected roots is a primary symptom to nematode attack. Several workers based the level of susceptibility on the number of galls and galling indices. Khanzada *et al*. (2012) rated tomato cultivars into different levels of susceptibility based on galling indices. Kamran *et al*. (2012) reported different number of galls for all the tested tomato genotypes that were susceptible to *Meloidogyne* spp.

Previous studies have shown that RKN and *Fusarium oxysporum* f. sp. *lycopersici* can form an interaction resulting in a disease complex that further compounds the losses incurred by the two, which is much higher than each of them individually or when their effects are added together (Bhagawati *et al*., 2000, 2007). Furthermore, fusarium resistant tomato cultivars can have their resistance broken due to the presence of nematodes. In the light of the possibility of the formation of a disease complex, it is important to test the susceptibilities of tomato cultivars to RKN. Susceptibility to RKN in a cultivar would mean that the possibility of forming a disease complex is high. The present study was done to test susceptibility of popular tomato cultivars, Prostar F1, Kilele F1, Oxly, Cal j, Rambo F1 and Roma VFN, to *Meloidogyne javanica*.

Materials and methods

The study was carried out in a greenhouse in the University of Nairobi, College of Agriculture and Veterinary Sciences (CAVS), Upper Kabete Campus field station. Ambient day temperatures ranged from 25^{o}C-34^{o}C in the greenhouse during the period of study.

Identification of Meloidogyne species used in the experiments

The initial single egg mass population of *Meloidogyne javanica* used in these experiments was obtained from Kenyatta University Plant and Microbial Sciences Department. Confirmation of identification was by cuticular markings in the perenial area of a mature female using a modification of a method by

Kariuki *et al.* (2013). The females were teased out of galled roots by use of forceps and a fine needle taking care not to puncture the body of the female. The females were stored in 0.9% sodium chloride solution to avoid osmotic effects of water. They were identified at Nematological laboratories at International Centre for Insect Physiology and Ecology (ICIPE Kenya) using an identification guide by Eisenback (1981).

Planting of tomato cultivars

Certified tomato seeds of the cultivars Prostar F1, Kilele F1, Oxly, Cal j, Rambo F1 and Roma VFN were sterilized in 1% solution of sodium hypochlorite and were then sown in steam sterilized soil and sand mixture. The seeds for the different tomato cultivars were sown separately in the seedling trays and were later transplanted into 500 g flower pots containing steam sterilized soil and sand mixture.

Preparation of the M. javanica second stage juveniles (J2s) and infestation procedures

Second stage juveniles (J2s) were extracted from Cal J tomato roots infested with *Meloidogyne javanica* using a modification of a method by Coyne *et al.* (2007). Three week old tomato seedlings of the six different tomato cultivars Prostar F1, Kilele F1, Oxly, Cal j, Rambo F1 and Roma VFN F1 were transplanted separately into 500 g plastic pots filled with sterilized sand and soil mixture. Each pot contained one plant. The seedlings were each infested with second stage juveniles (J2s) of *M. javanica* one day after transplanting. A hole was made two centimetres near each plant using a plastic spoon. Thirty millilitres of suspension containing 1000 J2s was then dispensed into the hole which was then covered.

Experimental design

The experiment had one treatment for each tomato cultivar where each pot was inoculated with 1000 J2s of *Meloidogyne javanica*. A control without any treatment was included for each tomato cultivar. The treatment and the control experiment in each tomato cultivar were replicated four times. The experiment was laid out in a randomized complete block design in the greenhouse. Plants were watered once every 24 hours and sprayed with a fungicides and pesticides once per week to control fungal diseases and pests. The fungicide Ridomil Gold was applied at the rate of five grams per two litres water. Different pesticides were used interchangeably and these included Evisect (thiocyclam SP50%) at 1g/litre and Actara (thiamethoxam) at 0.8g/litre.

Extraction of juveniles from the roots and soil

Second stage Juveniles (J2s) were extracted from infested tomato roots and soil after termination of experiment using modified Baermann tray method (Whitehead and Hemming, 1965). From a homogeneous 500 g sample of soil used in each experiment 250 g was used for the extraction of J2s.

Data collection

The test plants were harvested nine weeks after inoculation and transplanting. The plant height, the number of flowers in each plant, fresh shoot weights, dry shoot weights, fresh root weights and dry root weights were recorded. Roots from treatments inoculated with nematodes were stained with cold eosin yellow (0.1 g L^{-1} of water) for 30 minutes to facilitate the counting of galls. Gall indices (GI) were measured according to a scale by International Meloidogyne Project (1978) using the following values: O = zero gall; 1 =1 or 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls and 5 = > 100 galls per root system.

Statistical analysis

Data was analysed by one way ANOVA using GenStat statistical package (Discovery Edition 14). The infestation of *M. javanica* on the different tomato cultivars was the exploratory variable and effect on growth was the response variable.

The means obtained were separated using Fisher's protected least significant difference (L.S.D) method at 5% level of significance. All count data (counts of J2s in the roots and soil) were transformed by logarithmic transformation. The percentage reductions in growth parameters compared to control were calculated (Hussain, Mukhtar and Kayani,

2011).

Results

Identification of Meloidogyne species

Meloidogyne species used in the experiments were identified by the perenial patterns of the matured female. The nematode species had the characteristic lateral lines that separate the dorsal striae from the ventral striae and a low dorsal arch with a nearly flattened top was confirmed as *Meloidogyne javanica* (Fig.1).

Reaction of tomato cultivars to root knot nematodes

All roots of the different tomato cultivars had galls, however the cultivars Rambo F1, Roma and Prostar F1 had significantly (P<0.05) higher galling indices than the cultivars Cal J, Kilele F1 and Oxly (Table 1).

Table 1. Reproduction factor, number of galls and juvenile populations induced by treatment with *M. javanica* in six tomato cultivars in greenhouse conditions.

Cultivar	Gall index	J2s /root system	J2s/500g soil	Total J2s	Reproductive factor
Cal j	2.3ab	2342(3.37)	1074(3.03)	3416(3.53)	3.41c
Kilele F1	2.0a	1671(3.2)	1061(3.03)	2732(3.44)	2.73b
Oxly	1.5a	777(2.89)	393(2.58)	1181(3.1)	1.18a
Prostar F1	3.5cd	4078(3.61)	1911(3.28)	5989(3.78)	5.98e
Rambo F1	4.00d	4500(3.65)	1471(3.16)	5971(3.78)	5.97e
Roma VFN	3.00bc	2610(3.42)	1294(3.11)	3844(3.6)	3.84d
L.S.D (P=0.05)	0.73	0.073	0.143	0.05	0.34

Data are means of four replications. Means followed by the same letter are not significantly (P < 0.05) different according to Fisher's L.S.D test. Value in brackets are log_{10} transformed means that were compared using L.S.D.

There were also significant (P<0.05) differences in the reproductive factor of the tomato cultivars. The tomato cultivar Prostar F1 and Rambo F1 had the highest reproductive factor of 5.98 and 5.97 respectively while cultivar Oxly had the least reproductive factor of 1.2.

Effect of infestation with root knot nematodes on growth

There were reductions in both fresh and dry root weights of the cultivars inoculated with *M. javanica* compared to the un-inoculated ones (Table 2).

Table 2. Effect of infestation with *M. javanica* on the root fresh and dry weights in different tomato cultivars.

Cultivar	Root fresh weights (g)			Root dry weights (g)		
	Un-inoculated	Inoculated	% reduction	Un-inoculated	Inoculated	% reduction
Cal J	19.72 c	17.7c	11	3.45b	3.23b	6.5
Kilele F1	18.25 bc	13.7 ab	24.8	3.4b	3.23b	5.7
Oxly	20.80 c	15.35bc	26.2	3.48b	3.45b	0.71
Prostar F1	13.19a	12.39ab	6.06	2.6a	2.4a	3.84
Rambo F1	15.65ab	12.06a	22.9	2.75a	2.3a	0.97
Roma VFN	15.66ab	14.12ab	9.85	2.45a	2.18a	11.23
L.S.D (P=0.05)	3.46	3.14		0.6	0.52	

Data are means of four replications. Means followed by the same letter are not significantly (P < 0.05) different according to Fisher's L.S.D test.

The cultivar Oxly had the highest mean dry root weight and also the least reduction in the root dry weights (0.71%) due to inoculation with *M. javanica*.

Inoculation with *M. Javanica* brought about a reduction in the mean fresh shoot weight of nematode inoculated tomato cultivars compared to un-inoculated tomato cultivars (Table 3). Cultivars Roma VFN and Prostar F1 had the greatest reductions in the fresh shoot weights at 38.7% and 21.7% respectively.

Table 3. Effect of infestation with *M. javanica* on the shoot fresh and dry weights of different tomato cultivars.

Cultivar	Fresh weights (g)			Dry weights (g)		
	Un-inoculated	Inoculated	% reduction	Un-inoculated	Inoculated	% reduction
Cal J	13.84a	11.3a	18.35	4.4a	4.11b	6.59
Kilele F1	21.66ab	18.19c	16.02	5.59a	5.56d	0.54
Oxly	19.01b	15.38abc	19.01	4.71a	5.07cd	-8.63
Prostar F1	22.31b	17.46bc	21.7	5.08a	3.90ab	23.22
Rambo F1	18.11b	16.82bc	7.1	5.22a	4.84c	7.3
Roma VFN	21.69b	13.3ab	38.7	4.87a	3.4a	30.18
L.S.D (P=0.05)	4.475	4.389		1.028	0.63	

Data are means of four replications. Means followed by the same letter are not significantly (P < 0.05) different according to Fisher's L.S.D test.

There were also reductions in shoot dry weight of inoculated tomato cultivars compared to un-inoculated ones (Table 3). The greatest percentage reductions in the dry shoot weights were found in tomato cultivars Roma VFN (30.18%) and Prostar F1 (23.22 %). Meanwhile cultivar Kilele F1 had the lowest reductions (0.54%) in the dry shoot weights while cultivar Oxly had a slight increase (8.63%) in shoot dry weight, compared to the un- inoculated control tomato cultivars (Table 3).

Significant differences (P<0.05) were observed in the number of flowers in the different tomato cultivars inoculated with *M. javanica*. A reduction, in the number of flowers was observed in inoculated tomato cultivars compared to un-inoculated ones.

Table 4. Effect of infestation with *M. javanica* on number of flowers, and height in different tomato cultivars.

Cultivar	Number of flowers			Plant height (cm)		
	Un-inoculated	Inoculated	% reduction	Un-inoculated	Inoculated	% reduction
Cal J	7.75a	3.75a	52	61.2 a	53.27ab	12.9
Kilele F1	15 c	13.5d	10	63.1a	65.4 d	-3.6
Oxly	8.75a	11bcd	-25.7	58.6a	58.2 bc	0.68
Prostar F1	9.25ab	6.33a	32.4	62.7a	61.3cd	9.85
Rambo F1	14.3c	6.25ab	31.6	68 a	65.4 d	2.23
Roma VFN	12.75bc	8.0 bc	37.3	53.8a	50.3a	6.5
L.S.D (P=0.05)	3.9	2.92		9.15	5.43	

Data are means of four replications. Means followed by the same letter are not significantly (P < 0.05) different according to Fisher's L.S.D test.

The maximum percentage reduction in the number of flowers was found in cultivars Cal J (52%), followed by Roma VFN, Rambo F1 and Prostar F1 at 36.3%, 32.3% and 31.93% respectively (Table 4). The cultivar Kilele F1 had the least percentage reduction (10%) while cultivar Oxly had an increase of 25.7%.

It was observed that there was a reduction in heights of tomato cultivars inoculated with *M. javanica* compared to un-inoculated ones, with the exception of tomato cultivar Kilele F1 (Table 4). The heights of the cultivars Kilele F1 and RamboF1 were the least affected by the *M. Javanica* inoculation, and had the greatest heights that were significantly different from the other tomato cultivars Cal J, Oxly and Roma VFN.

Discussion

Susceptibility of different tomato cultivars to root-

knot nematode M. javanica

High root galling, and high reproductive factors are evidence that the tomato cultivars were good hosts for *M. javanica*. The most susceptible cultivars were cultivars, Rambo F1, Prostar F1, Roma VFN, and Cal J with galling indices of 4.0, 3.5, 3.0 and 2.3 respectively.

The differences in the galling indices and reproductive factor in the six tomato cultivars indicate different levels of susceptibility of the cultivars to *M. javanica*. Different levels of susceptibility to root-knot nematodes based on root galling indices and reproductive factor have been reported by several authors (Yazdi, 2012; Khanzada *et al.*, 2012; Jaiteh *et al.,* 2012; Esafahani *et al.*, 2012). Khan (1994) reported that the development of galls increased significantly in susceptible genotypes compared to resistant genotypes.

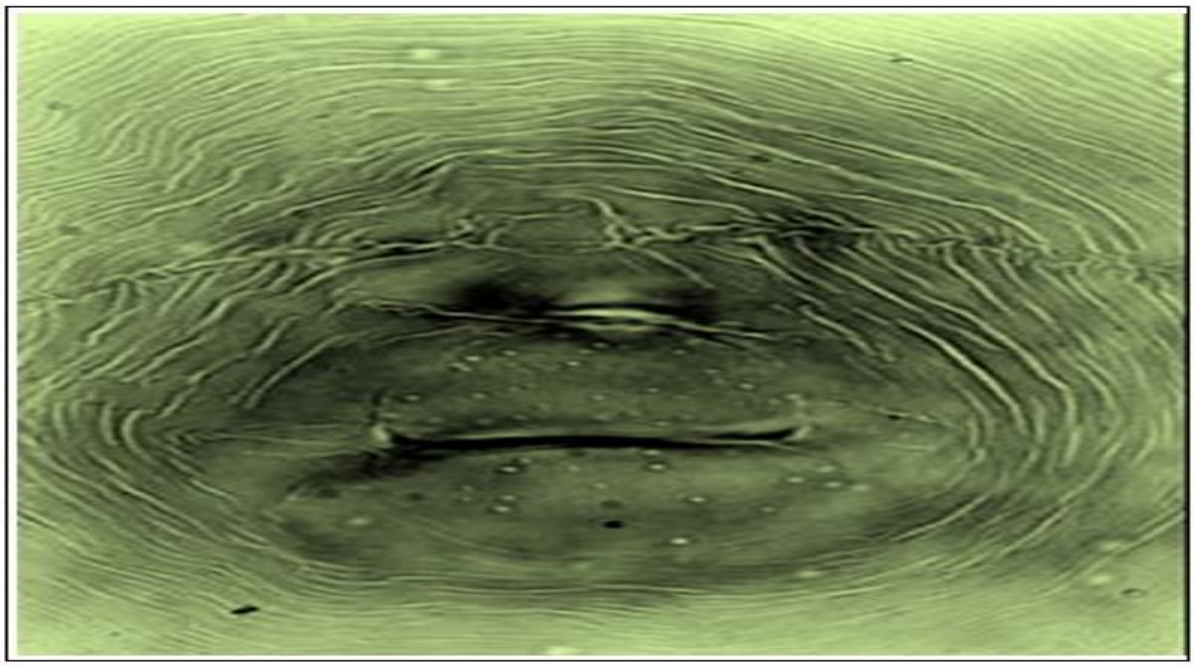

Fig. 1. Perineal pattern of *M.javanica* x40 magnification.

The variation in the susceptibility of the six tomato cultivars to infestation by *M. javanica*, might be due to genetic differences. Similarly Castagnone- Sereno (2006) and Jacquet *et al.* (2005) reported that the level of susceptibility to *Meloidogyne* spp. is controlled by the tomato genotype.

The tomato cultivars Rambo F1, Prostar F1, Cal J, Oxly and Roma VFN lacked resistance to root knot nematodes. All resistance to nematodes in *M. incognita*, *M. javanica* and *M. arenaria* is conferred by the Mi-gene and this has been the source of resistance for many years (Roberts and Thomason 1989 as cited by Verdejo-lucas *et al.*, 2009).

The cultivar Roma VFN was very susceptible to the nematode populations despite having the nematode resistance genes. The high temperatures (25°C-34°C) in the greenhouse could have rendered the Mi-gene infective. Kolashian *et al.* (1996) reported that temperatures above 28°C make the Mi-gene ineffective. The Mi-gene for resistance to root knot nematodes is same in all cultivars. The repeated cultivation of Mi-resistant gene cultivars may lead to selection of virulent nematode populations that overcome the nematode resistance genes.

Effect of infestation with M. javanica on the growth of the different tomato cultivars

Percentage reductions in growth were observed between inoculated and un-inoculated plants. It was evident that cultivars with higher nematode

infestations had in general greater reduction in growth parameters compared to least susceptible cultivars. The cultivars Roma VFN and Prostar F1 that had high galling indices had highest dry shoot reductions of inoculated compared to un-inoculated at 30.18% and 23.22% respectively. Cultivar Cal J had the greatest reduction in the number of flowers at 52%, followed by Roma VFN (36.3%), Rambo F1 (32.3%) and Prostar F1 (31.93%). The cultivar Kilele F1 with a low galling index (2) had a percentage reduction of 10% in the number of flowers, while the cultivar Oxly with a galling index of 1.2 had a slight increase in the number of flowers when un-inoculated plants were compared with inoculated ones. Many authors have reported reduction in growth due to infestation with root-knot nematodes (Esfahani and Pour 2006; Okporie *et al*., 2014; Hussain *et al*., 2015). Severe root galling and arrested root development interferes with absorption of mineral salts and water (Clark *et al*., 2003) and this reduces growth in plants.

Conclusion

Tomato plants infestation with root-knot nematodes brings about reduction in plant growth due to inability of galled roots to absorb water and mineral salts. Therefore, there is need to control root-knot nematodes in order to increase on growth and tomato production. There were different levels of susceptibility to root knot nematodes in the different tomato cultivars and this could be due to differences in the genotypes of the tomato cultivars. The best tomato cultivars to use are those that are less susceptible to RKN. However this would be simplistic approach to choosing the best tomato cultivars as there are many other factors that affect tomato production. For instance, the quality and shelf life of a tomato cultivar is very important for marketing purposes. There are other diseases and emerging pests that are already a big challenge to the farmers. A case in point is *Tutor absoluta* that was first reported in 2014 that could literally wipe out a whole crop of tomato.

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