RESEARCH/INVESTIGACIÓN

GRAFTING FOR MANAGEMENT OF ROOT-KNOT NEMATODES, *MELOIDOGYNE INCOGNITA*, IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

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ABSTRACT

Owusu, S. B., C. K. Kwoseh, J. L. Starr, and F. T. Davies. 2016. Grafting for management of root-knot nematodes, *Meloidogyne incognita*, in tomato (*Solanum lycopersicum* L.). Nematropica 46:14-21.

The basic role of grafting vegetables worldwide has been to provide resistance to soil-borne pathogens and improve yield. The root-knot nematode-resistant tomato cultivars 'Celebrity', 'Big Beef', and 'Jetsetter' roots were grafted with scions of the susceptible cultivars 'Tropimech' and 'Power' and tested in plant-house and field experiments for their ability to increase yield of the susceptible tomato cultivars in the presence of *Meloidogyne incognita*. Grafting reduced nematode population levels in the plant-house. In an untreated field, nematode population levels were lower in Power that had been grafted on Celebrity, Jetsetter, and Big Beef rootstocks than Power that was either self-grafted or ungrafted. Fruit yield, including the number and weight of fruit, was higher with the resistant cultivars as rootstocks in a field that had not been treated with a nematicide, but no significant differences were seen in a treated field. The use of rootstocks with nematode resistance can be effective for management of root-knot nematodes on susceptible tomato cultivars.

Key words: grafting, *Meloidogyne incognita*, resistance, tomato.

RESUMEN

Owusu, S. B., C. K. Kwoseh, J. L. Starr, and F. T. Davies. 2016. Injertos para el manejo de nematodos formadores de agallas en las raíces, *Meloidogyne incognita*, en tomate (*Solanum lycopersicum* L.). Nematropica 46:14-21.

El objetivo básico del injerto de hortalizas en todo el mundo ha sido proporcionar resistencia a los patógenos del suelo e incrementar la producción. Vástagos de los cultivares susceptibles 'Tropimech' y 'Power' fueron injertados sobre raíces de los cultivares resistentes a los nematodos formadores de agallas 'Celebrity', 'Big Beef', y 'Jetsetter' y ensayados en invernadero y campo en relación a su habilidad para incrementar la producción de los cultivares de tomate susceptibles en presencia de *Meloidogyne incognita*. El injerto redujo los niveles de población del nematodo en invernadero. En un campo no tratado los niveles de población del nematodo fueron menores en Power injertado en los patrones Celebrity, Jetsetter, y Big Beef que en Power sin injertar o que había sido injertado en sí mismo. La producción de frutos, tanto en número como en peso de los frutos, fue mayor con los cultivares resistentes usados como patrones en un campo que no había sido tratado con nematicidas, pero no se encontraron diferencias significativas en campos tratados. El uso de patrones con resistencia a nematodos puede ser efectivo para el manejo de nematodos formadores de agallas en las raíces en cultivares de tomate susceptibles.

Palabras clave: injerto*, Meloidogyne incognita,* resistencia, tomate.

INTRODUCTION

Cultivated tomato (*Solanum lycopersicum* L.) is one of the world's most important crops due to the high value of its fruit both for fresh market consumption and in numerous types of processed products (Giovanni *et al*., 2004). Tomato is cultivated in Ghana on over 4,410 ha with an average yield of 7.2 Mt/ha and achievable yield of 15.0 Mt/ha (SRID, 2009). In Ghana, the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, infestations limit tomato production (De Lannoy, 2001), causing severe economic losses by supressing both the quantity and quality of marketable yields (Jaiteh *et al*., 2012). These pests cause galls or root knots on infected plants, restricting root function. Yield reduction on tomatoes as a result of stunting and reduced flowering from root-knot nematode infection ranges between 30% to 65%, depending on the cultivar (Manzanilla-López and Starr, 2009).

Root-knot nematodes have been managed with practices such as host-plant resistance, rotation with non-hosts, prudent use of soil fumigants, sanitation, and destruction of residual crop roots (Whitehead, 1998). The use of resistant varieties remains the most suitable choice, particularly for small-scale farmers with limited resources. Unfortunately, locally preferred tomato cultivars may lack genetic disease resistance. Farmers are interested in these cultivars because of their adaptability to local conditions and may not be willing to give them up for nematode-resistant cultivars from other locales that may be less well adapted. Grafting can be used to enhance soil-borne disease resistance of local susceptible tomato cultivars having desired qualities (Sakata *et al*., 2007). The purpose of this research was to evaluate the ability of nematode-resistant cultivars to support and increase yield of locally adapted, susceptible tomato cultivars when used as rootstocks.

MATERIALS AND METHODS

Three experiments were conducted at the Departments of Crop and Soil Sciences and Horticulture of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Five tomato cultivars were used in these experiments with 'Big Beef', 'Celebrity', and 'Jetsetter' resistant to Verticillium wilt, Fusarium wilt, nematodes, and tobacco mosaic virus (VFNT). Also, these cultivars were used as nematode-resistant rootstocks, and 'Tropimech' (VF) and 'Power' (locally grown nematode-susceptible cultivar) as scions. Seed of the exotic cultivars were obtained from Texas A&M University, College Station, TX, USA. Tropimech was obtained from Agriseed GH. Ltd. (imported from Technisem, France,) and Power from Crop Research Institute of CSIR, Kwadaso, Ghana.

Experiment 1: Response of tomato cultivars to root-knot nematodes in the plant-house

Three-week-old seedlings of tomato cultivars Jetsetter, Big Beef, Celebrity, Tropimech, and Power were planted singly in 1-L plastic pots filled with 0.8 L of steam-sterilized topsoil and river sand in 3:1 ratio. Before planting, soil analysis was carried out and results were 87% sand, 8% silt, 5% clay, pH 7.1. *Meloidogyne incognita* inoculum was collected from the previous experiment conducted by Kwara *et al.* (2014), who confirmed the species based on esterase and malate dehydrogenase isozyme (MDH) phenotypes and species-specific PCR test. The species (*M. incognita*) was maintained on tomato (*Solanum lycopersicum*) cv. Pectomech on steam-sterilized soil placed in plant-house at the Faculty of Agriculture, KNUST. *Meloidogyne incognita* eggs were extracted from galled roots of tomato using the NaOCl method (Hussey and Barker, 1973). Inoculation with 2,000 eggs of *M. incognita* per seedling was done one week after planting. Two holes of 3 cm deep and 3 cm from the base of the plant was made using a stick, and the egg suspension was pipetted into the holes and covered with soil. The plants were fertilized with 25 ml of 0.1 % solution of compound fertilizer (15-15-15, N-P-K) (OLAM Ghana Ltd.) every 2 wk. Eight weeks later, root gall ratings were made for galling severity according to a 0-10 scale (Bridge and Page, 1980), and eggs were extracted from 5 g roots from each pot using the Hussey and Barker (1973) method. The juvenile population density per 100 cm^3 sub-sample of soil from each pot was assessed using the modified Baermann tray method (Whitehead and Hemming, 1965). The experiment was composed of five treatments (cultivars) in a completely randomized design with four replications. Plants were maintained in a plant-house with average temperature and humidity of 26°C and 85%, respectively.

Experiment 2: Assessment of graft combinations for resistance to root-knot nematode

Power and Tropimech were used as scions with Jetsetter, Big Beef, and Celebrity used as nematode-resistant rootstocks. Grafting was done using the grafting tube technique (Rivard and Louws, 2008), and grafts were allowed to heal for 10 d in a modified healing chamber. Healed plants were acclimatized for 1 wk by moving the plants from the plant-house to full sunlight before transplanting singly into pots. Plants were inoculated with 2,000 *M. incognita* eggs in 2-L pots with 1.8 L of the previously described soil and maintained in the plant-house. The number of eggs per 5 g of roots, *M. incognita* juveniles per 100 cm^3 soil, root galling severity, and fresh root weight were assessed as stated above. Nine treatments (Power ungrafted, Power self-grafted, Big Beef, Celebrity, Jetsetter grafted with Power, Tropimech ungrafted, Tropimech self-grafted, and Big Beef and Jetsetter grafted with Tropimech) were arranged in complete randomized design (CRD) with four replications. Celebrity grafted with Power plants were destroyed by rats, therefore, this treatment was excluded from the experiment.

Experiment 3: Assessment of graft combinations for resistance to root-knot nematode and yield in field-tests

Two sites, a field located at the Department of Crop and Soil Sciences that had not been treated with a nematicide and a nematicide-treated field at the Department of Horticulture experimental site, KNUST), were used for the field assessment. The fields were 198.9 m apart and both were previously cropped with tomato. The untreated field had a mean pre-plant nematode density in the top 30 cm of soil of 10.8 ± 12.2 and 17.3 ± 20.9 *M. incognita* juveniles per 100 cm^3 for major and minor season, respectively. This was estimated from 20 core samples each consisting of two sub-samples of 100 cm^3 soil. The untreated field soil had textural properties of 85% sand, 9% silt, 6% clay, and pH of 6.8 and the treated field had 81% sand, 13% silt, and 6% clay texture with pH of 6.9. Nematodes such as *M. javanica*, *M. arenaria*, *Helicotylenchus*, and free-living species were also found but very low in terms of population density. However, *M. incognita* was the most dominant nematode observed. From previous studies at the same field, *M. incognita* was also the most dominant root-knot nematode observed (Kwara *et al*., 2014). This was confirmed by esterase and malate dehydrogenase isozyme (MDH) phenotypes and species specific PCR test after cultivation of tomato.

For the treated field, Carbodan 3% G (carbofuran), (Shenzhen Baocheng Chemical Industrial Company Ltd., China) at 100 g/100 m linear meter was applied in-furrow at planting. Two weeks after nematicide treatment, soil samples were collected as described above to verify the nematicide effect by extraction using the modified Baermann tray method (Whitehead and Hemming, 1965). Both fields were planted at the same time with the same treatments and number of replications. Planting was done in the major (March-June 2014) and minor season (July-October 2014). The average rainfall for March-June received was 176.5 mm with a maximum and minimum temperature of 31.3 and 21.8°C, respectively. July-October had an average rainfall of 144.5 mm and a maximum and minimum temperature of 28.3°C and 21.0°C, respectively. Twenty ridges were constructed by hand using a hoe. The ridges were raised, with each measuring 2.5-m long and 50-cm wide with 1 m between ridges, with a total surface area of 84.5 m^2. The seedlings were planted 50 cm apart on the ridges. Seedlings of ungrafted Power and Power grafted on Big Beef, Jetsetter and Celebrity were planted in both treated and untreated fields. One grafted-seedling was planted per hill, and there were six plants per ridge serving as a plot. Regular hoeing to control weeds was done and Lambda Super 2.5 EC (25 g lambda-cyhalothrin per litre), (Trustchem Company Ltd., China) at 400-800 ml/ha, was sprayed 10- to 14-d interval to manage insect pests. Granular fertilizer, (N-P-K 15-15-15) was applied twice at the rate of 10 g per plant. The first dose was applied 3 wk after transplanting and second dose applied at 40 d after transplanting.

Root weight, gall rating, eggs per 5 g of roots, and juveniles per 5 g roots were assessed as described above. Also, fruit yield, number and weight of fruit were assessed. Fruit weight was recorded by weighing 10 fruits from each plot. Power was selected as the only scion for the field experiment because it is the most widely cultivated by farmers whereas Tropimech is less popular in the area. Also, Power is observed to be highly susceptible to root-knot nematodes. There were four replications per scion-rootstock graft, and the experiment was arranged in a randomized complete block design. All nematode egg and juvenile counts were log $(x + 1)$ transformed and analyzed. Data for all experiments were analyzed using analysis of variance (ANOVA) (Genstat, version 12). Least significant difference test was used to determine significance between pairs of mean. Differences are reported at $P < 0.05$.

RESULTS

Response of tomato cultivars to root-knot nematodes in the plant-house

The root gall rating ranged from 1 to 8. The lowest gall ratings were observed on Big Beef, Jetsetter, and Celebrity and the highest were recorded on Power (Table 1). Root weights, egg counts and *M. incognita* numbers from Jetsetter, Celebrity, and Big Beef were significantly lower than nematode-susceptible Power and Tropimech. While the resistant cultivars

Table 1. Reaction of tomato cultivars inoculated with *Meloidogyne incognita* in pots in the plant-house.

Tomato cultivars	Root gall rating[x]	Root weight (g)	Egg count[y] no./ 5g roots	*M. incognita*[y]/ 100 cm^3 soil
Susceptible				
Power	8.0 a[z]	5.1 a	95,462 a	17,500 a
Tropimech	7.3 a	3.7 b	106,302 a	17,250 a
Resistant				
Jetsetter	1.0 b	1.5 c	3,822 b	1,750 b
Celebrity	1.0 b	2.0 c	1,918 b	2,500 b
Big Beef	1.0 b	1.9 c	1,642 b	2,000 b
CV (%)	14.9	27.1	12.8	9.1

[x]Gall rating scale of 0-10 where, 0 = no galls on root and 10 = root system completely galled.
[y]Back-transformed data shown after data was Log (x + 1) transformed before analysis.
[z]Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

Table 2. Effects of grafting and inoculation with *Meloidogyne incognita* on plant growth, root galling, and root-knot nematode density on tomato cultivars in pots in the plant-house.

Treatment	Root weight (g)	Root gall rating[x]	*M. incognita*/100 cm^3 soil[y]	Egg count no./5g roots[y]
Power				
Non-grafted	12.9 a[z]	7.8 a	17,500 ab	105,018 a
Self-grafted	13.0 a	8.0 a	20,000 ab	117,125 a
Grafted on Jetsetter	2.1 d	1.0 b	2,000 d	6,392 d
Grafted on Big Beef	3.0 cd	1.3 b	5,000 cd	22,922 bc
Grafted on Celebrity	4.4 c	1.0 b	1,750 d	1,852 e
Tropimech				
Non-grafted	10.5 b	8.0 a	23,250 a	106,302 a
Self-grafted	14.0 a	8.0 a	22,750 ab	86,828 a
Grafted on Jetsetter	3.2 cd	1.5 b	12,500 bc	28,475 b
Grafted on Big Beef	3.0 cd	1.8 b	5,250 c	8,578 cd
CV (%)	18.0	15.2	7.5	7.2

[x]Gall rating scale of 0-10 where 0 = no galls on root and 10 = root system completely galled.
[y]Back-transformed data shown after data was Log (x + 1) transformed before analysis.
[z] Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

supported some root-knot nematode reproduction, levels were significantly lower than the susceptible cultivars.

Both Power and Tropimech, ungrafted or self-grafted plants had significantly higher root weight, level of root galling, and number of eggs compared to their grafts on the resistant Jetsetter, Big Beef, and Celebrity. For *M. incognita* juveniles/100 cm^3 soil, there were no significant differences between Tropimech grafted on Jetsetter and ungrafted or self-grafted treatments (Table 2).

Assessment of graft combinations for root-knot nematode-resistance and yield in nematicide-treated and untreated fields - major season

Root galling and weight were highest on ungrafted and self-grafted Power, and lowest on Power scions grafted on Celebrity, Jetsetter, and Big Beef rootstocks (Table 3). Self-grafted or ungrafted

Table 3. Effects of *Meloidogyne incognita* on yield of tomato rootstocks grafted with ‘Power’ as scion for first planting in nematode-infested field.

Treatment	Root gall rating[x]	Root weight (g)	Egg count no./5g roots[y]	*M. incognita*/5g of roots[y]
Non-grafted cv. Power	6.8 a[z]	45.4 a	137, 000 a	5, 450 a
Self-grafted cv. Power	7.0 a	40.9 a	113, 400 a	3, 450 a
Grafted on Celebrity	1.2 b	21.7 b	10, 905 b	970 b
Grafted on Jetsetter	1.2 b	21.4 b	5, 325 b	1, 300 b
Grafted on Big Beef	2.3 c	19.9 b	7, 300 b	1, 650 b
CV (%)	11.3	30.4	5.6	10.6

[x]Gall rating scale of 0-10 where 0 = no galls on root and 10 = root system completely galled.
[y]Back-transformed data shown after data was Log ($x + 1$) transformed before analysis.
[z]Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

Table 4. Effects of *Meloidogyne incognita* on yield of tomato rootstocks grafted with ‘Power’ as scion for first planting in nematicide-treated and untreated fields.

	Fruit yield (t/ha)		Weight/fruit (g)		Fruit number/plot	
Treatment	Untreated[x]	Treated[y]	Untreated	Treated	Untreated	Treated
Non-grafted cv. Power	5.9 c[z]	12.6 a	31.7 b	68.7 a	59.5 a	90.5 a
Self-grafted cv. Power	8.1 c	17.0 a	22.9 b	65.3 a	68.8 a	63.3 b
Grafted on Jetsetter	19.2 a	15.7 a	58.3 a	65.6 a	84.0 a	69.3 b
Grafted on Big Beef	13.4 b	16.5 a	59.7 a	67.4 a	73.0 a	75.0 b
Grafted on Celebrity	13.3 b	15.4 a	51.2 a	57.1 a	83.3 a	64.3 b
CV (%)	25.4	34.5	18.2	18.7	19.2	12.0

[x]Department of Crop and Soil Sciences.
[y]Department of Horticulture.
[z]Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

Power also had the highest number of eggs and *M. incognita* juveniles extracted from 5 g root tissue.

Power scions grafted on Jetsetter, Big Beef, and Celebrity rootstocks had significantly higher fruit yield than ungrafted and self-grafted Power in the infested field. In the treated field, there were no yield differences between treatments (Table 4). Fruit weight in the untreated field ranged from 22.9 to 59.7 g while that of the treated field was from 57.1 to 68.7 g. There were significant differences ($P < 0.05$) among treatment means in the untreated field but no differences were recorded in the treated field. Ungrafted Power had the lowest number of fruit (59.5), while Power grafted on Jetsetter had the highest fruit number in the untreated field. Ungrafted Power had the highest fruit number in the treated field.

Assessment of graft combinations for root-knot nematode resistance and yield - minor season

Root galling was significantly higher in ungrafted and self-grafted Power and lower in Power grafted on Celebrity, Jetsetter, and Big Beef rootstocks (Table 5). The number of eggs per 5 g roots extracted from Power grafted on Celebrity, Jetsetter, and Big Beef was lower compared to ungrafted and self-grafted Power. *Meloidogyne incognita* juvenile numbers per 5 g roots extracted from self-grafted and ungrafted Power and Power grafted on Big Beef were not significantly different. Root weight ranged from 12.7 to 51.8 g with the highest recorded in self-grafted Power and lowest on Power grafted onto Jetsetter.

Fruit yield was significantly higher in Power grafted on Celebrity, Jetsetter, and Big Beef in the

Table 5. Effects of *Meloidogyne incognita* on yield of tomato rootstocks grafted with 'Power' as scion for second planting in nematode infested field.

Treatment	Root gall rating[x]	Root weight (g)	Egg count no./5g roots[y]	*M. incognita*/5g of roots[y]
Non-grafted cv. Power	6.7 a[z]	39.8 b	170, 400 a	7, 000 a
Self-grafted cv. Power	8.0 a	51.8 a	182, 000 a	7, 100 a
Grafted on Celebrity	2.0 b	13.4 d	20, 400 b	1, 800 b
Grafted on Jetsetter	2.3 b	12.7 d	9, 600 b	1, 600 b
Grafted on Big Beef	3.7 b	22.8 c	16, 400 b	2, 900 a
CV (%)	24.9	11.4	5.0	5.3

[x]Gall rating scale of 0-10 where 0 = no galls on root and 10 = root system completely galled.
[y]Back-transformed data shown after data was Log (x + 1) transformed before analysis.
[z]Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

Table 6. Effects of *Meloidogyne incognita* on yield of tomato rootstocks grafted with 'Power' as scion for second planting in nematicide-treated and untreated fields.

	Fruit yield (t/ha)		Weight/fruit (g)		Fruit number	
Treatment	Untreated[x]	Treated[y]	Untreated	Treated	Untreated	Treated
Non-grafted cv. Power	5.8 b[z]	18.3 a	34.4 bc	65.1 a	73.0 a	80.0 a
Self-grafted cv. Power	5.3 b	15.3 a	22.6 c	66.1 a	58.3 a	78.3 a
Grafted on Jetsetter	11.2 a	15.9 a	49.6 ab	66.4 a	70.5 a	74.8 ab
Grafted on Big Beef	12.6 a	14.8 a	62.0 a	60.7 a	67.5 a	70.8 bc
Grafted on Celebrity	11.3 a	10.2 a	29.0 c	51.1 a	73.3 a	67.3 c
CV (%)	32.6	31.4	33.8	16.8	19.5	6.2

[x]Department of Crop and Soil Sciences.
[y]Department of Horticulture.
[z]Data are means of four replicates. Means followed by the same letter within a column do not differ according to LSD ($P < 0.05$).

untreated field. Tomatoes from Power grafted onto Jetsetter and Big Beef were also larger (Table 6). Fruit number per plot in the treated field ranged from 67.3 to 80.0. Fruit number of ungrafted and self-grafted Power were higher than with Power grafted onto Big Beef and Celebrity.

DISCUSSION

Grafting tomato scions with preferred horticultural traits onto cultivars that confer resistance to root-knot nematodes is a viable management tactic for resource-poor tomato growers (Sakata *et al.*, 2007). In our first experiment, the three cultivars, Celebrity, Jetsetter, and Big Beef, which were reported as resistant by Kwara *et al.* (2014), were confirmed to be resistant to the *M. incognita* found in this region. Susceptible host plants allow *M. incognita* juveniles to enter the roots, reproduce, and produce severe root galling (Karssen and Moens, 2006). The resistant cultivars as rootstocks reduced nematode reproduction compared to susceptible cultivars. According to Ornat *et al.* (1997), Colyer *et al.* (1998), and Hanna (2000), nematode-resistant cultivars can be used to enhance nematode-susceptible crops by reducing nematode population levels.

The number of *M. incognita* juveniles per 5 g roots was not different in Big Beef than from the susceptible cultivars in the second planting. This could be due to the nematode population build up from the previous major season planting, as was noted by Ros-Ibáñez *et al.* (2014) and Ros *et al.* (2002), who reported increased nematode incidence and population aggressiveness after using the same rootstocks in the same soil for two successive years.

According to Augustin *et al.* (2002), Besri (2002), and Poffley (2003), the yield advantage of grafted plants is significant when they are grown on untreated soil. In this experiment, fruit yield and weight per fruit were generally higher with Celebrity, Jetsetter, and Big Beef grafted with Power in both seasons in the untreated field. The use of a rootstock with resistance to specific pathogens or other biotic stress may negatively impact yield in the absence of targeted biotic agents (Bletsos *et al.*, 2003; Leonardi and Giuffrida, 2006; Rivard and Louws, 2008). This was shown by the ungrafted and self-grafted Power in the treated field with higher fruit numbers compared to Power grafted on Big Beef and Celebrity. According to Cohen *et al.* (2002, 2007), grafting may have a neutral impact on yield, which was shown in fruit yield and weight per fruit from the treated field in both seasons.

The results of the present study suggest that grafting nematode-susceptible tomato cultivar onto a resistant cultivar can be an effective management method on root-knot infested soils, especially when preferred nematode-resistant cultivars and or nematicides are not available.

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