RESEARCH NOTE/NOTA DE INVESTIGACIÓN

REACTION OF TOMATO (*SOLANUM LYCOPERSICUM*) CULTIVARS TOWARDS ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) AND BACTERIAL WILT (*RALSTONIA SOLANACEARUM)*

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ABSTRACT

Kidane, E., A. Seid, and M. Kebede. 2019. Reaction of tomato (*Solanum lycopersicum*) cultivars towards root-knot nematode (*Meloidogyne incognita*) and bacterial wilt (*Ralstonia solanacearum)*. Nematropica 49:246-253.

Tomato (*Solanum lycopersicum*) is one of the most popular vegetable crops grown worldwide. However, tomato production is constrained by root-knot nematode (*Meloidogyne incognita*) and bacterial wilt (*Ralstonia solanacearum*) either singly or as a disease complex. Thus, the reaction of tomato cultivars Assila, Cochoro, Marmande, and Moneymaker was evaluated in a glasshouse experiment. Disease resistance against a single isolate of *M. incognita* and *R. solanacearum* alone or as a co-infestation was tested. Six inoculation sequences (simultaneous, *M. incognita* inoculation 10 days prior to *R. solanacearum*, and *R. solanacearum* inoculation 10 days prior to *M. incognita*) were considered in the study. The experiment was factorially arranged in a completely randomized design with four replications. Responses of tomato genotypes to inoculation sequences were made based on tomato biomass and growth and disease development parameters. Inoculation sequence significantly ($P \leq 0.05$) influenced plant and disease parameters. Inoculation of *M. incognita* 10 days prior to *R. solanacearum* led to maximum (3.75) wilt severity at the final wilt assessment. This same sequence reduced plant height, fresh root weight, and shoot dry weight by 24.99, 55.93, and 51.88%, respectively. Inoculation with *M. incognita* alone increased all nematode-associated parameters. Severe nematode and bacterial diseases were recorded on Marmande. Conversely, Assila performed well against the nematode and bacteria compared to the other cultivars tested. The overall results of this study demonstrated that inoculation sequence greatly influenced the response of tomato genotypes to *M. incognita* and *R. solanacearum* and their complex. However, studies of this kind should be further executed with many isolates of pathogens, inoculum levels, and tomato genotypes under actual farmer's conditions to verify the validity of the results.

Key words: Disease complex, disease parameter, inoculum, plant parameter, synergistic interaction

RESUMEN

Kidane, E., A. Seid, and M. Kebede. 2019. Reacción de cultivares de tomate (*Solanum lycopersicum*) hacia nematodos de nudo de raíz (*Meloidogyne incognita*) y marchitez bacteriana (*Ralstonia solanacearum*) Nematropica 49:246-253.

El tomate (*Solanum lycopersicum*) esuno de loscultivos de vegetalesmáspopularesentodo el mundo. Sin embargo, la producción de tomateestálimitadapor el nematododelnudo de la raíz (*Meloidogyne incognita*) y el marchitamientobacteriano (*Ralstonia solanacearum*) individualmente o como un complejo de enfermedad. Por lo tanto, la reacción de loscultivaresde tomate Assila, Cochoro, Marmande, y Moneymaker se evaluóenunexperimento de invernadero. Se probarondiferentesniveles de resistencia a la enfermedad contra un solo aislado de *M. incognita* y *R. solanacearum* co-infestación y orden de inoculación. Se consideraronseisesquemas de inoculaciónen el estudio. El experimento se organizófactorialmenteenundiseñocompletamente al azar con cuatrorepeticiones. Las respuestas de losgenotiposdeltomate a losesquemas de inoculación se hicieronen base a la biomasa del tomate y losparámetros de crecimiento y desarrollo de la enfermedad. Los horarios de inoculacióninfluyeronsignificativamente (P <0.05) enlosparámetros de plantas y enfermedades. La inoculación de *M. incognita* 10 días antes de *R. solanacearum*condujo a unagravedadmáxima de marchitamiento (3.75) en la evaluación final de marchitamiento. Dichoprograma de inoculaciónredujo la altura de la planta, el peso fresco de la raíz y el peso secodelbroteen 24.99, 55.93 y 51.88%, respectivamente. La inoculación con nematodos de nudo de raíz solo incrementótodoslosparámetrosasociados a nematodos. Se registraron nematodos graves y enfermedadesbacterianas con Marmande. A la inversa, Assila se desempeñóbien contra las enfermedadesencomparación con otroscultivares. Los resultados generales de esteestudiodemostraron que losprogramas de inoculacióninfluyeronen gran medidaen la respuesta de losgenotipos de tomate a *M. incognita* y *R. solanacearum* y sucomplejo. Sin embargo, estudios de estetipodebenrealizarse con muchosaislamientos de patógenos, niveles de inóculo y genotipos de tomateencondicionesreales de losagricultores para verificar la validez de losresultados.

Palabras clave: Complejo de enfermedad, inóculo, interacciónsinérgica, parámetro de enfermedad, parámetro de planta

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Commercial tomato (*Solanum lycopersicum*) cultivation in Ethiopia dates from 1980 with a production area of 80 ha in the upper Awash by Merti Agro industry. Tomatoes were grown for domestic and export markets (Lemma, 2006). By 1993, the total tomato production area had increased to 833 ha with cultivation expanding to other parts of Ethiopia. Tomato acreage increased to 7,257 ha with 393,730 t of production by 2014 (FAOSTAT, 2014). Tomatoes constitute a major farming activity in Ethiopia due to the favorable ecological conditions, importance of tomato in the daily diet, and proximity to the export markets of the Republic of Djibouti and other Middle East countries (Tadele and Mengistu, 2000; Mekete *et al*., 2003; Joosten *et al*., 2011). However, tomato yield in Ethiopia is low (8 t/ha) compared to neighboring countries like Kenya (21 t/ha) and to yields in America, Europe, and Asia (54, 42, and 35 t/ha, respectively) (FAO, 2013). This yield difference is attributed to biotic and abiotic factors.

Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the most damaging biotrophic threats to tomato production (Sakhuja and Jain, 2001; Coyne *et al*., 2009; Seid *et al*., 2015). According to Stewart and Dangachew (1967), *M. incognita, M. javanica,* and *M. ethiopica* have been reported in Ethiopian tomato production fields. Recently, Seid *et al*. (2019) added the occurrence of *M. arenaria* and *M. hapla*. Seid *et al*. (2019) found *M. incognita* and *M. javanica* widely distributed in the major tomato-growing areas of Ethiopia with *M. incognita* being the major threat to tomato production (Tadele and Mengistu, 2000; Wondirad and Mekete, 2002; Gemechis *et al*., 2012; Abebe *et al*., 2015; Seid *et al*., 2019).

The magnitude of damage caused by RKN increases in combination with other soil borne pathogens (Sikora and Fernande, 2005). Bacterial wilt of tomato, caused by *Ralstonia solanacearum,* increased when RKN were present (Back *et al*., 2002). Valdez (1976) reported that damage to the root system caused by RKN was responsible for increases in the intensity of bacterial wilt. Combinations of *Meloidogyne* spp. with bacterial plant pathogens caused greater damage than either pathogen alone (Pitcher, 1965; Siddiqui *et al*., 2012). Insufficient information is available on the interaction between *M. incognita* and *R. solanacearum* on tomato crops in Ethiopia.

Understanding of the disease complex or interaction between *M. incognita* and *R. solanacearum* will help in designing appropriate management options for commercial and small-scale farmers. Therefore, this study was initiated to evaluate the reaction of selected tomato cultivars to *M. incognita* and *R. solanacearum* co-infection and order of inoculation on tomato growth and disease development.

The response of four tomato genotypes with different levels of resistance to *M. incognita* and *R. solanacearum* were assessed in a glasshouse at the Raree Glasshouse Facility, Haramaya University, Ethiopia. The tomato cultivars tested were Assila (resistant to RKN), Cochoro (moderately resistant to RKN), Moneymaker (susceptible to RKN), and Marmande (susceptible to RKN). Both Moneymaker and Cochoro seeds were obtained from Melkasa Agricultural Research Center, whereas Marmande and Assila seeds were obtained from a seed company. Soil was collected from a field at Raree, Haramaya University and passed through a 0.5-mm pore sieve. Sand, manure, and clay were mixed in the ratio of 3:1:1. The mix was autoclaved at 110°C for 2 hr. Two kilograms of the sterilized soil was placed into 15-cm-diam. plastic pots. Seeds of the tomato cultivars were surface sterilized using a 0.1% NaOCl solution for 2 min and rinsed three times with sterile distilled water. Sterilized seeds were sown in pots filed with autoclaved soil mix.

The population of *M. incognita* was collected from farms in the Babile area of eastern Hararghe, Ethiopia. The RKN isolate was identified molecularly at Ghent University, Belgium (Seid *et al*., 2019). A pure culture of the population was maintained on Marmande for inoculum. RKN-infected tomato was uprooted and the roots washed gently with tap water to remove adhering soil particles. The washed roots were submerged in Phloxine B (0.15 g/L) for 15 min to observe the egg masses and facilitate counting (Holbrook *et al*., 1983). Second-stage juveniles (J2) were extracted from the roots using a modified Baermann funnel technique (Hooper *et al*., 2005). Tomato seedlings at the four-leaf stage were inoculated with 3,000 *M. incognita* J2 (Sundaresh *et al*., 2017).

The isolate of *R. solanacearum* was collected from wilted tomato plants, identified at the Plant Protection Laboratory, Haramaya University, and confirmed by reference strains preserved at the laboratory. The identified pure culture isolate of *R. solanacearum* was preserved on freshly prepared yeast extract dextrose carbonate (YDC) slants and stored at 4°C until used for experiments. Inoculum of *R. solanacearum* was prepared by growing cultures on petri plates of YDC for 48 hr at 28-30°C. The plates were flooded with sterile distilled water and the media surface scrapped with a sterile glass rod. The inoculum concentration was adjusted to 10^8 cfu/ml using a spectrophotometer to an optical density of 0.01 at 600 nm (Sundaresh *et al*., 2017). Ten milliliters of bacterial suspension was inoculated into each pot.

For inoculation of *M. incognita* and *R. solanacearum*, soil was carefully removed from a 4-cm-diam. area around the tomato root along the sides of the pot to a depth of 2-3 cm avoiding damage to the roots. The inoculum suspensions were poured around the roots, and the soil was returned to the pot. For the controls, distilled water was added in equal volume to the suspension of nematodes and bacteria. After inoculation, the plants were lightly watered and then maintained in the glasshouse for 8 wk.

The reaction of the four tomato cultivars when challenged with the two pathogens alone and in combination was assessed (Table 1). Twenty-four treatments including controls (4 cultivars x 6 inoculation sequences) were arranged in a completely randomized design with 4 replications on a bench in the glasshouse.

Wilt development was evaluated weekly by visual inspection of bacterial wilt symptoms starting 2 weeks after inoculation and continuing up to 8 weeks. Wilting was recorded and rated using a 0 to 4 scoring index (Roberts *et al*., 1998) where: 0 = no leaves wilted, 1 = 25% of leaves wilted, 2 = 26 to 50% of leaves wilted, 3 = 51 to 75% of leaves wilted, and 4 = 76 to 100% of leaves wilted. Based on wilt scores, area under disease progress curve (AUDPC) was calculate according to the formula (Madden *et al*., 1995):

$$\text{AUDPC} = \sum_{i=0}^{n-1} (0.5)(Xi + 1 + Xi)(ti + 1 - ti)$$

Where, Xi = wilt score at the i^{th} assessment; ti = the i^{th} wilt score assessment time in days; and n = total number of observations.

Eight weeks after inoculation, data were collected on plant growth and nematode infection.

Table 1. Treatment combinations of *Meloidogyne incognita* (*MI), Ralstonia solanacearum* (*RS*) alone or in inoculation sequences on four tomato cultivars evaluated in a glasshouse study.

S/N	Treatment combinations in the inoculation procedures[z]
1	Assila + RS alone
2	Assila + MI alone
3	Assila + RS+MI simultaneously
4	Assila + MI 10 days prior to RS inoculation
5	Assila + RS 10 days prior to MI inoculation
6	Assila Uninoculated control)
7	Cochoro + RS alone
8	Cochoro + MI alone
9	Cochoro + RS+MI simultaneously
10	Cochoro + MI 10 days prior to RS inoculation
11	Cochoro + RS 10 days prior to MI inoculation
12	Cochoro (Uninoculated control)
13	Moneymaker + RS alone
14	Moneymaker + MI alone
15	Moneymaker + RS+MI simultaneously
16	Moneymaker + MI 10 days prior to RS inoculation
17	Moneymaker + RS 10 days prior to MI inoculation
18	Moneymaker (Uninoculated control)
19	Marmande + RS alone
20	Marmande + MI alone
21	Marmande + RS+MI simultaneously
22	Marmande + MI 10 days prior to RS inoculation
23	Marmande + RS 10 days prior to MI inoculation
24	Marmande (Uninoculated control)

[z]RS = *R. solanacearum* alone; MI = *M. incognita* alone; MI + RS = Simultaneous inoculation of *M. incognita* and *R. solanacearum*;

The height of the plant was measured starting from the soil line to the tip of the stem. After removing the shoots, the roots were freed from adhering soil by gently washing with tap water and blotting to remove excess water. The number of egg masses per plant was counted after staining the roots with Phloxine B. The final nematode population (*Pf*) was calculated by counting J2 and eggs in a 100 cm^3 sub-sample of soil extracted using a modified Baermann funnel technique (Van Bezooijen, 2006). The number of J2 and eggs in roots were determined by extracting nematodes from a subsample of 5 g roots (Hussey and Barker, 1973). The nematode reproduction factor (RF) was determined by dividing final population density (*Pf)* by the initial inoculation density (*Pi*). Bacterial population densities (CFU) were determined at experiment termination. Approximately 50 mm long stem tissue segments above the root base were collected from wilted plants and ground with a mortar and pestle. The original solution and ten-fold serial dilutions of the homogenate were spread onto NA and KB medium (Elsharkawy *et al*., 2015). The colonies were counted after 2 to 3 days of incubation at 28-30°C. Presumptive colonies of *R. solanacearum* were confirmed by an immune fluorescent colony staining (IFCS) test (Van Vuurde, 1990).The number of leaves and flowers per plant, days to 50% flowering, fresh shoot and root weights, dry shoot weight, and galls per plant were also recorded but not reported here.

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) to determine the influence of RKN and bacteria inoculation on response of tomato cultivars. All analyses were performed using SAS GLM procedure (SAS Institute, 2001), and treatment mean separations were made using LSD at 0.05 probability level when found significant.

The cultivar, inoculation sequence, and the interaction significantly ($P \leq 0.05$) influenced plant height, AUDPC, egg mass/plant, RF, and CFU (Tables 2 and 3). Plant heights differed among the cultivars (Table 2). The tallest plants were the

uninoculated controls compared to other treatments. Assila plants were the tallest and Marmande plants the smallest. RKN inoculation 10 days prior to *R. solanacearum* had the greatest effect on plant height. The combined inoculation of pathogens reduced plant height irrespective of the inoculation sequence. The greatest number of egg masses per plant was obtained from the susceptible Marmande when inoculated with RKN alone and on susceptible Moneymaker when RKN inoculation was 10 days prior to the bacterium (Table 3). The lowest number of egg masses developed on all cultivars when *R. solanacearum* was inoculated 10 days prior to RKN inoculation. Simultaneous inoculation tended to not affect the number of egg masses/plant except on Marmande (Table 3). RKN RF was greatest on Marmande, regardless of inoculation with *R. solanacearum*. RF on the resistant cultivars Assila and Cochoro was not greatly affected by the presence of the bacteria regardless of inoculation sequence (Table 3). Typical wilt symptoms appeared 15 days after bacterial inoculation. Cultivar and inoculation sequence significantly ($P \leq 0.05$) effected AUDPC (Table 2). Wilt increased with time. The lowest bacterial wilt severity was observed on Assila followed by Cochoro at all assessment dates. The lowest AUDPC was recorded on Assila (Table 2). RKN inoculation 10 days before *R. solanacearum* resulted in the highest AUDPC values. Cultivar, inoculation sequence, and their interaction had significant ($P \leq 0.05$) effects on bacterial colonization (Table 3). The highest bacterial populations were found in Marmande (Table 2). Assila had consistently high bacterial populations compared to Cochoro and Moneymaker (Table 2).

The interaction among *M. incognita, R. solanacearum*, and tomato cultivars highlights the

Table 2. Effect of inoculation sequence of *Meloidogyne incognita* and *Ralstonia solanacearum* on tomato plant height and area under the disease progress curve (AUDPC) of four tomato genotypes under glasshouse conditions.

Treatment[z]	Plant height (cm)	AUDPC (score-days)
Cultivar		
Assila	74.92 a	12.50 b
Marmande	53.17 c	14.69 a
Moneymaker	71.29 ab	15.63 a
Cochoro	66.58 b	14.69 a
Mean	66.49	14.38
LSD (0.05)	***	**
Inoculation sequence		
Control	77.50 a	0.00 c
MI	65.00 bc	0.00 c
MI + RS	64.44 bc	20.63 b
MI, RS	58.13 c	22.97 a
RS, MI	67.38 b	22.03 ab
RS	66.50 b	20.63 b
LSD (0.05)	***	***
CV (%)	14.74 (1.17)	19.44(1.29)

[z]MI = *M. incognita* alone; MI + RS = Simultaneous inoculation of *M. incognita* and *R. solanacearum*; MI, RS = *M. incognita* inoculation 10 days prior to *R. solanacearum*; RS, MI = *R. solanacearum* inoculation 10 days prior to *M. incognita;* and RS = *R. solanacearum* alone. RGPP = root gall per plant; AUDPC = Area under disease progress curve and DAI = Days after inoculation. CV (%) = Coefficient variation (%) and number in bracket refers to log transformed CV value. ** = Highly significant at 0.01. *** = Very high significant at 0.001.

Table 3. Effect of inoculation sequence of *Meloidogyne incognita* and *Ralstonia solanacearum* on bacterial colony forming units (CFU), egg mass/plant, and nematode reproduction (RF = *Pf*/*Pi*) on four tomato cultivars under glasshouse conditions[z].

	Tomato cultivar											
	Assila			Cochoro			Marmande			Moneymaker		
Treatment	EM	RF	CFU	EM	RF	CFU	EM	RF	CFU	EM	RF	CFU
Uninoculated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MI	62.0	10.3	0.0	17.0	10.1	0.0	115.0	81.3	0.0	115.0	81.3	0.0
MI + RS	47.0	16.7	5.4	33.0	17.8	1.7	48.0	119.1	7.2	48.0	119.1	7.2
MI, RS	34.0	11.3	2.5	40.0	16.5	2.6	96.0	91.9	4.6	96.0	91.9	4.6
RS, MI	16.0	11.6	5.5	13.0	19.5	3.4	8.0	109.9	6.3	8.0	109.9	6.3
RS	0.0	0.0	5.1	0.0	0.0	5.1	0.0	0.0	6.1	0.0	0.0	6.1
Cul. x Inoc.	***	***	**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Genotype	***	***	***	17.0	10.1	0.0	115.0	81.3	0.0	115.0	81.3	0.0
Inoc.	***	***	***	33.0	17.8	1.7	48.0	119.1	7.2	48.0	119.1	7.2
CV (%)	63.42 (1.80)	95.73 (1.98)	12.27 (1.09)	40.0	16.5	2.6	96.0	91.9	4.6	96.0	91.9	4.6

[z]CFU = Colony forming units x 10^2; EM = Egg mass/plant; RF = Reproduction factor *(*final nematode population density/initial nematode population density); MI = *M. incognita* alone; MI + RS = Simultaneous inoculation of *M. incognita* and *R. solanacearum*; MI, RS = *M. incognita* inoculation 10 days prior to *R. solanacearum* inoculation; RS, MI = *R. solanacearum* inoculation 10 days prior to *M. incognita* inoculation; RS = *R. solanacearum* alone; Cul. = Cultivar; and Inoc. = Inoculation sequence. CV(%) = Coefficient of variation in % with log transformed CV value in brackets. *** = Significant at 0.001.

challenges in management of this disease complex. In general, infection by both pathogens resulted in reduced plant growth. Swain (1989) reported that the combined infection of *M. incognita* and *R. solanacearum* on a resistant brinjal cultivar was synergistic towards the development of wilt symptoms and affected plant growth. Bacterial wilt was greater in plants previously infected with RKN than when infection occured simultaneously or RKN infection followed bacterial infection. Both Haider *et al.* (1989) and Sundaresh *et al*. (2017) demonstrated a similar response between *M. incognita* and *R. solanacearum.* Whereas Khan (1993) found that wilt caused by *R. solanacearum* was more severe in resistant cultivars of tomato and eggplant in the presence of *M. incognita,* the RKN-resistant Assila included in our study did not behave in such a manner. Assila may have some resistance to bacterial wilt whereas the RKN-resistant Cochoro does not.

Because the impacts from the two pathogens can be more severe in combination, it is important to consider management of both pathogens. It is clear that prior infection by RKN increases the severity of bacterial wilt in tomato, therefore, reducing RKN infection is important. RKN infection can be managed by transplanting clean seedlings into the field. The use of RKN-resistant Assila can also help to manage bacterial wilt and losses associated with nematodes. Future work should evaluate RKN resistant tomato cultivars with pedigrees similar to Assila at a range of *R. solanacearum* inoculum levels under field conditions to determine if this provides sufficient protection against losses to nematodes and bacterial wilt.

ACKNOWLEDGMENTS

This research was financially supported by Ministry of Education of the Federal Democratic Republic of Ethiopia and technically by Haramaya University, Ethiopia.

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Received: 30/XI/2018 *Accepted for publication:* 6/VI/2019

Recibido: *Aceptado para publicación:*