[Research Note]

Reproduction of *Meloidogyne incognita* on eggplant rootstock cultivars and effect of eggplant rootstock cultivation on nematode population density

Taketo Uehara[1,*], Masami Sakurai[2], Keita Oonaka[3], Yasushi Tateishi[1], Takayuki Mizukubo[1,4] and Kazuhiro Nakaho[1]

The southern root-knot nematode *Meloidogyne incognita* is an important pathogen of solanaceous plants worldwide. To assess resistance and suppressiveness to the nematode of the Japanese cultivars of eggplant rootstocks, we conducted greenhouse experiments. Numbers of nematode egg masses were significantly lower on *Solanum torvum* rootstock cultivars, Tonashimu, Torero, and Torvum vigor, than the cultivars of the other *Solanum* species after 45 days of cultivation. Next, we examined the effect of Tonashimu on the population density of the nematode in soil. After 116 days of cultivation, the second-stage juvenile density of the nematode in soil was reduced, and was significantly lower in pots of Tonashimu, than in those of *Solanum melongena* cultivars. These results suggest that the Japanese rootstock cultivars of *S. torvum* are resistant to the nematode, and could suppress nematode density in soil. Nematol. Res. 46(2), 87–90 (2016).

Key words: grafting, resistance, root-knot nematode, *Solanum torvum*

INTRODUCTION

Eggplant (*Solanum melongena*) is cultivated worldwide and is especially popular in Asian countries such as China and India. Eggplant was introduced into Japan during the Nara period (AD 710 to 794) and today is an important domestically produced vegetable (Saito, 2004). Many crops, including eggplant, are attacked by the southern root-knot nematode *Meloidogyne incognita*, resulting in yield losses. In eggplant, 20–29% of total yield is estimated to be lost because of *Melodogyne* spp. (*M. incognita* is dominant) in the tropics (Sasser, 1979) and the economic threshold level of eggplant is reported to be 5.4 *M. incognita* second-stage juveniles (J2) per 100 g soil (Netcher and Sikora, 1990). Recently, Watanabe *et al.* (2013) reported that soil densities of two J2 per 20 g soil or greater with the Baermann technique have been shown to significantly reduce eggplant yield (cultivar Senryo 2go) at 50 days after planting.

Most plant nematode control methods rely on chemical agents. Although soil fumigants such as 1,3-dichloropropene are commonly used, the use of such chemicals is likely to be subject to increasing restriction owing to environmental and safety concerns. The exploitation of resistant cultivars is the most effective and environmentally benign method to reduce nematode-induced crop loss. However, no eggplant cultivar resistant to *M. incognita* has been found.

Eggplant cultivars grafted onto resistant rootstocks are used to prevent various diseases, including bacterial wilt (caused by *Ralstonia solanacearum*), and Fusarium wilt (caused by *Fusarium oxysporum*) (Yoshida, 2004). *Solanum torvum* has a poor germination rate and slow seedling development but is employed as rootstock for eggplant cultivation because of its vigor and resistance to serious soil-borne diseases as described above (Gousset *et al.*, 2005; Miceli *et al.*, 2014). Moreover, *S. torvum* has been found to exhibit resistance to *M. incognita* in several studies conducted in Japan and abroad (Ali *et al.*, 1992; Daunay and Dalmasso, 1985; Dhivya *et al.*, 2014; Hara *et al.*, 1983; Shetty and Reddy, 1985). To date, however, no studies have compared the degree of *M. incognita* resistance among inoculated Japanese eggplant rootstock cultivars. In this study, we inoculated a number of different Japanese eggplant rootstock cultivars with *M. incognita* to determine their relative resistances. Additionally, we cultivated eggplant and eggplant rootstocks to examine the effects on nematode population densities in a greenhouse.

MATERIALS AND METHODS

Resistant evaluation based on root gall index and egg mass count:

Two experiments were conducted; in the first, *M. incognita* (Chiba population) isolated from a tomato (*Solanum lycopersicum*) field in Tōgane, Chiba, Japan, was propagated on the tomato cultivar Momotaro carrying the root-knot nematode resistance gene *Mi*. *Mi*-virulent populations of *M. incognita* have been

[1] Central Region Agricultural Research Center, NARO, 2-1-18 Kannonndai, Tsukuba, Ibaraki 305-8666, Japan
[2] Gunma Agricultural Technology Center, 493 Nishiobokata, Isesaki, Gunma 379-2224, Japan
[3] Mie Prefecture Agricultural Research Institute, 530 Ureshinokawagita-cho, Matsusaka, Mie 515-2316, Japan
[4] Present address: Maruwa Biochemical Co. Ltd, Ami Development Center, 1 Hoshinosato, Ami, Ibaraki 305-0326, Japan
*Corresponding author, e-mail: uehara@affrc.go.jp

reported in many regions of Japan, which is not uncommon with greenhouse production systems. The inoculation experiment included one eggplant cultivar Senryo 2go, and eight eggplant rootstock cultivars (Table 1), grown using seeds purchased from a local supplier. Eggplant rootstock and eggplant cultivar seeds were planted in 9-cm-diameter black polyethylene pots (7.6 cm deep, *ca*. 0.36 L soil; Tokai Kasei. Co., Ltd. Gifu, Japan) filled with horticultural soil (Nihon Hiryo Co., Ltd, Tokyo, Japan) and maintained at a mean temperature of 25°C for approximately 1 month. A glass pipette was used to inoculate six pots of each cultivar at a rate of 1,000 J2 per pot, after which the pots were maintained at a mean temperature of 25°C from February 15 to May 7 (83 d) in 2013. After removing the plants from the pots and rinsing the roots with water, the gall index was evaluated using a subjective scale from 0 to 4 (Hara *et al*., 1983).

In the second experiment, *M. incognita* (Niigata population) isolated from a tomato field in Kitakanbara, Kajikawa, Niigata, Japan, was propagated on Momotaro tomato plants. *Meloidogyne incognita* (Niigata population) is also a *Mi*-virulent population. We used two *M. incognita* populations to determine whether resistance differed with different nematode populations. This inoculation experiment included the eggplant cultivar Senryo 2go, six eggplant rootstock cultivars, one tomato cultivar and two tomato rootstock cultivars (Table 2). As eggplant and tomato are both members of the genus *Solanum*, it is possible to graft them together (Matsuzoe *et al*., 1993; Yoshida, 2004). These seeds were also purchased from a local supplier. Eggplant rootstock, eggplant, tomato rootstock, and tomato cultivar seeds were planted in 9-cm-diameter black polyethylene pots filled with horticultural soil and maintained at a mean temperature of 25°C for approximately 1 month. A glass pipette was used to inoculate four pots of each rootstock and eggplant cultivar at a rate of 500 J2 per pot, after which the pots were maintained at a mean temperature of 25°C from June 27 to August 10 (45 d) in 2014. After removing the plants from the pots and rinsing the roots with water, the roots were stained with 0.02% phloxine B, and the number of egg masses was counted per root system.

Effect of eggplant rootstock cultivation on nematode population densities:

For this experiment, infested soil containing 578.5 ± 30.0 (mean ± SE) nematodes per 20 g soil, evaluated using the Baermann technique, was prepared by propagating *M. incognita* (Niigata population) on the tomato cultivar Momotaro grown in 1/5,000 a Wagner pots (159 mm in diameter and 190 mm deep, *ca*. 2.5 L soil). The infested soil was packed into 1/10,000 a pots (113 mm in diameter and 140 mm deep, *ca*. 1.0 L soil) used in the experiment. One-month-old seedlings of Senryo 2go, Daitaro, and Tonashimu cultivars grown in 6-cm-diameter black polyethylene pots (5.5 cm deep; Tokai Kasei. Co., Ltd.) were transferred to these pots. Senryo 2go is the most popular eggplant cultivar, whereas Daitaro is a popular rootstock cultivar with higher bacterial wilt resistance than Tonashimu. Of the rootstock cultivars Tonashimu, Torero, and Torvum vigor, Tonashimu was selected because it tended to have fewer root knots and egg masses than the other two cultivars (Tables 1 and 2) and was considered easy to handle owing to its lack of thorns. After transplanting, the plants were cultivated in a greenhouse at a mean temperature of 25°C from July 8 to October 31 (116 d) in 2013. At the end of the cultivation period, nematodes were extracted from soil using the Baermann technique and J2 were counted using a biological microscope.

Statistical analyses:

To determine whether the different cultivars had significantly different *M. incognita* resistance potential we used the Steel-Dwass' test for gall index and Tukey's test for egg mass count and J2 density. Statistical analysis was performed using the software BellCurve for Excel (Social Survey Research Information Co., Ltd. Tokyo, Japan).

RESULTS AND DISCUSSION

The root-knot gall index for cultivars Tonashimu and Torero was zero (Table 1), namely no galls were observed on Tonashimu and Torero. Although a small number of galls were observed on Torvum vigor, the number was lower than that observed on other rootstock cultivars (Table 1). Conversely, large numbers of root galls were observed on rootstock cultivars Akanasu, Meet, and Senryo 2go. While a small number were present, significantly fewer egg masses were observed on the Tonashimu, Torero, and Torvum vigor rootstock cultivars (Table 2). In our assessment of tomato rootstock resistance to nematodes, we observed greater numbers of egg masses on Doctor K, Momotaro, and Green-guard than on eggplant cultivars and eggplant rootstock cultivars (Table 2). Several studies in Japan and abroad have found that *S. torvum* is resistant to *M. incognita* (Ali *et al*., 1992; Daunay and Dalmasso, 1985; Dhivya *et al*., 2014; Shetty and Reddy, 1985). Tonashimu, Torero, and Torvum vigor are all *S. torvum*, suggesting that the Japanese cultivars of *S. torvum* are resistant to *M. incognita*, as well as foreign cultivars of this plant species.

In the pot experiments with Senryo 2go and Daitaro, nematode population densities increased 2.6- and 1.7-fold relative to initial population densities, respectively; in contrast, in pots planted with Tonashimu, the nematode population density decreased (Table 3).

In our study, we demonstrated that Tonashimu, Torero, and Torvum vigor exhibit substantially greater *M. incognita* resistance than other eggplant rootstocks. As substantially increasing *M. incognita* population densities with eggplant would inevitably affect the growth of any subsequent crop, we also demonstrated that Tonashimu is unlikely to cause sudden increases in nematode population density. We suggest that using a resistant rootstock is an eco-friendly method to suppress nematode population densities that can be combined with other control methods including agricultural chemicals, solarization, and reductive soil disinfestation to achieve integrated management of *M. incognita*.

Table 1. Root-knot gall formation as indicated by gall index on eggplant rootstock cultivars infected with *Meloidogyne incognita* (Chiba population).

Cultivar	Plant species	Gall index[a]	
		Mean[b]	Range
Tonashimu	*Solanum torvum*	0 c	0
Torero	*S. torvum*	0 c	0
Torvum vigor	*S. torvum*	0.5 bc	0–1
Karehen	*S. sanitwongsei*	1.5 b	1–2
Daitaro	*S. melongena*	2.4 b	2–3
Taibyo-VF	*S. gradifoliumt*×*S. melongena*	3.7 ab	3–4
Senryo 2go	*S. melongena*	4.0 a	4
Akanasu	*S. integrifolium*	4.0 a	4
Meet	*S. integrifolium*×*S. melongena*	4.0 a	4

[a]Gall index scored by root gall produced on root systems inoculated with 1,000 second-stage juveniles (J2) per pot ($n = 6$) as 0 to 4.
[b]Means within a column followed by the same letters are not significantly different at $P < 0.05$ (Steel-Dwass' test).

Table 2. Comparison of *Meloidogyne incognita* (Niigata population) reproduction on eggplant rootstock cultivars.

Cultivar	Plant species	Egg mass[a]
		Mean ± SE[b]
Tonashimu	*Solanum torvum*	0.3 ± 0.3 f
Torero	*S. torvum*	0.8 ± 0.3 f
Torvum vigor	*S. torvum*	0.8 ± 0.5 f
Daitaro	*S. melongena*	106.5 ± 34.3 e
Hikyaku	*Solanum* sp.	123.3 ± 19.8 de
Senryo 2go	*S. melongena*	171.8 ± 23.0 cde
Nasuno-inochi	*Solanum* sp.	211.3 ± 11.1 bcd
Doctor-K	*S. lycopersicum*	268.3 ± 10.9 abc
Momotaro	*S. lycopersicum*	272.8 ± 34.5 ab
Green-guard	*S. lycopersicum*	318.8 ± 24.3 a

[a]Mean number of egg masses produced on root systems inoculated with 500 second-stage juveniles per pot ($n = 4$).
[b]Means ± SE within a column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test).

Table 3. Population changes of *Meloidogyne incognita* (Niigata population) second-stage juveniles (J2) with cropping eggplant or eggplant rootstocks by pots experiment.

Cultivar	Initial (Jul. 8)	Final (Oct. 31)	*Pf*/*Pi*
	Mean ± SE[a]	Mean ± SE[a,b]	
Senryo 2go	578.5 ± 30.0	1482.0 ± 119.1 a	2.6
Daitaro	578.5 ± 30.0	1002.3 ± 38.8 b	1.7
Tonashimu	578.5 ± 30.0	62.7 ± 11.0 c	0.1

[a]Mean numbers of J2 extracted from 20 g fresh soil samples by the Baermann funnel technique ($n = 4$).
[b]Means ± SE within a column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test).

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