[Research Note]

Reproduction of tobacco cyst nematode *Globodera tabacum* on several Japanese tomato cultivars

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Tobacco cyst nematode (TCN) *Globodera tabacum* is able to develop and reproduce on tomato plants and eggplants. As a consequence, plants with a heavy infestation show stunted growth. The most effective and environmentally benign method for managing plant nematodes is through exploitation of host resistance. However, comparatively little information is available on the relative rates of reproduction of TCN on different tomato cultivars and resistance to TCN has not been studied in detail. In the present study, we compared the response of several tomato cultivars to inoculation with TCN in order to identify those with greatest resistance. As a result, five new TCN-resistant tomato cultivars, ‘Doctor-K’, ‘Sugar-Lamp’ ‘Carol-10’, ‘Carol-Queen’, and ‘Chelsea-mini’, were identified. Nematol. Res. 48(1), 27–30 (2018).

Key words: resistance, *Solanum lycopersicum*

**INTRODUCTION**

The occurrence of tobacco cyst nematode (TCN) *Globodera tabacum* was recorded for the first time in Japan in 1998, in a greenhouse of eggplants in Kochi Prefecture (Shimomoto et al., 2000; Sumiya et al., 2002). Following this discovery, the infected area was subjected to control programs (Shimomoto et al., 2000) and as a consequence, TCN is thought to have been exterminated in Kochi prefecture (Shimomoto, personal communication, 2016). The TCN in Kochi prefecture were able to develop on tomato plants and eggplants but not peppers (Momota, 2000; Sumiya et al., 2002). Heavily infected plants showed stunted growth. TCN infection in tomato is also associated with increased damage by *Verticillium* and *Fusarium* wilts (Miller, 1975). Currently, the most effective and environmentally benign method for managing plant nematodes is through exploitation of host resistance (Roberts, 2002). A TCN-resistant tomato cultivar, ‘Mini-Carol’, has been identified in Japan (Momota et al., 2003). However, to the best of our knowledge, TCN-resistant tomato cultivars have not been isolated in other countries. Analysis of the tomato cultivar ‘Mini-Carol’ also showed that it is resistant to potato cyst nematode (PCN) *G. rostochiensis* (Uehara et al., 2008). We therefore hypothesized that a resistant tomato cultivar will show resistance to both TCN and PCN. To test this hypothesis, we inoculated PCN resistant and susceptible tomato cultivars with TCN to identify resistant cultivars and to investigate their characteristics.

**MATERIALS AND METHODS**

Nematodes, plant materials, and inoculation method:

The *G. tabacum* used in this study was derived from specimens originally collected in Kochi prefecture in 1998. The nematodes were propagated on greenhouse-grown *Solanum integriflorum* Akanausu (Hiranasu) in 1/5,000a Wagner pots (159 mm diameter, 190 mm deep; ca. 2.5 L soil). TCN cysts were isolated from the soil using the dry flotation method. To isolate cyst nematode eggs, we disrupted TCN cysts in a homogenizer, and the eggs were collected and rinsed with water on a 38 µm sieve. Hatching of TCN eggs was stimulated by a tomato root diffusate at 25°C for 7 days. Second-stage juveniles (J2) were allowed to crawl through laboratory tissue and were suspended in tap water.

Six PCN-resistant tomato cultivars ‘Carol-10’, ‘Carol-Queen’, ‘Chelsea-Mini’, ‘Doctor-K’, ‘Mini-Carol’, and ‘Sugar-Lamp’, and five PCN-susceptible cultivars ‘Carol-7’, ‘Kyouryoku-Beijyu’, ‘Momotaro’, ‘Puchi’, and ‘Taiyou-Shinkoulo’ were used in this study (Uehara et al., 2008). The ‘Doctor-K’ cultivar is heterozygous for the PCN phenotype (Uehara et al., 2010). Moreover, ‘Doctor-K’ is used as a root-stock cultivar with *Verticillium* and *Fusarium* wilt-resistance (Matsunaga, 2015). Grafting is an important method in integrated pest management strategies against soil borne pathogens such as plant parasitic nematodes. Seeds of ‘Doctor-K’ were obtained from self-pollinated plants grown in a greenhouse and were used in this study. Tomato 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 (22–28°C) for approximately 1 month in a greenhouse. Plants were inoculated at about 30 days after germination with 1,000 or 3,000 J2 *G. tabacum* per plant, and then cultivated for 90 days. Three 50 g soil samples were taken from each pot, and cysts were isolated from

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the soil using the dry flotation method. Cyst numbers were counted using a dissecting microscope.

Statistical analysis:
Cyst numbers in soil samples from the different samples were compared using Tukey’s HSD test. Statistical analysis was performed using JMP 8 software (for Windows; SAS Institute). Chi-square analysis was used to examine the fit of the observed segregation to the expected ratio for a single, dominant gene (number of resistant plants to number of susceptible plants = 3:1).

RESULTS AND DISCUSSION

Tomato is the common host of *G. tabacum* (TCN) and *G. rostochiensis* (PCN). The resistance of tomato cultivars to PCN has been investigated in many previous studies (Cabrera Poch et al., 2006; Ellis, 1968; Ellis and Maxon Smith, 1971; Ernst et al., 2002; Ganal et al., 1995; Sobczak et al., 2005; Trifonova et al., 1995; Uehara et al., 2008; 2010; 2011; 2015), whereas that to TCN has been relatively little studied in tomato (Momota, 2000; Suzuki et al., 2003). Here, we inoculated two PCN-resistant cultivars, ‘Doctor-K’ and ‘Mini-Carol’, and a PCN-susceptible cultivar, ‘Kyouryoku-Beijyu’, with 3,000 J2 stage TCN. Collection of cysts from soil after 90 days showed that TCN did not reproduce on the ‘Doctor-K’ and ‘Mini-Carol’ cultivars (Table 1). These results on ‘Mini-Carol’ confirm our previous study showing this is a TCN-resistant cultivar (Momota et al., 2003). Our analysis here shows that ‘Doctor-K’ is also a TCN-resistant cultivar and that ‘Kyouryoku-Beijyu’ is a TCN-susceptible cultivar.

Next, we inoculated the PCN-resistant cultivars ‘Doctor-K’ and ‘Sugar-Lamp’ and the PCN-susceptible cultivars ‘Carol-7’, ‘Puchi’, and ‘Momotaro’ with 1,000 J2 stage TCN. Analysis of cyst numbers in soil after 90 days showed that TCN reproduced on ‘Carol-7’, ‘Puchi’, and ‘Momotaro’ but not ‘Doctor-K’ or ‘Sugar-Lamp’ (Table 2). ‘Carol-7’, ‘Puchi’, and ‘Momotaro’ are therefore susceptible to both PCN and TCN. A previous study likewise indicated that ‘Momotaro’ is susceptible to TCN (Suzuki et al., 2003).

In a third experiment, we inoculated the PCN-resistant cultivars ‘Carol-10’, ‘Carol-Queen’, and ‘Chelsea-mini’, and the PCN-susceptible cultivars ‘Carol-7’ and ‘Puchi’ with 1,000 J2 stage TCN. Analyses of cyst numbers in soil after 90 days showed that ‘Carol-10’, ‘Carol-Queen’, and ‘Chelsea-mini’ were TCN resistant, and that ‘Carol-7’ and ‘Puchi’ were susceptible cultivars (Table 3). As a result of these experiments, we have identified five new TCN-resistant tomato cultivars, namely, ‘Doctor-K’, ‘Sugar-Lamp’ ‘Carol-10’, ‘Carol-Queen’, and ‘Chelsea-mini’; these cultivars also show resistance to PCN.

The identification and characterization of resistance genes in tomato to TCN infection will be of benefit to breeding programs. It will be important to determine whether resistance is conferred by a single major gene or has a polygenic pattern of inheritance. Therefore, we performed a segregation analysis using ‘Doctor-K’. Selfed progeny of ‘Doctor-K’ were inoculated with 3,000 J2 stage TCN and tested for resistance. We found 20 resistant and 5 susceptible plants in the tested progeny. This ratio of resistant to susceptible plants did not differ significantly from a 3:1 ratio ($\chi^2$-value = 0.33, $P = 0.565$).

Table 1. Reproduction of *G. tabacum* on three tomato cultivars.

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>New cysts/50 g dried soil</th>
<th>PCN phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyouryoku-Beijyu</td>
<td>39.3 ± 8.8 a²</td>
<td>S</td>
</tr>
<tr>
<td>Mini-Carol</td>
<td>0 ± 0 b</td>
<td>R</td>
</tr>
<tr>
<td>Doctor-K</td>
<td>0 ± 0 b</td>
<td>R</td>
</tr>
</tbody>
</table>

1Mean ± S.E. of new cysts / 50 g dried soil from pots inoculated with 3,000 J2 (n = 6)
2Different letters denote significant differences at $P < 0.05$ (Tukey’s HSD test)
3S: Susceptible, R: Resistant

Table 2. Reproduction of *G. tabacum* on five tomato cultivars.

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>New cysts/50 g dried soil</th>
<th>PCN phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puchi</td>
<td>38.3 ± 1.5 a²</td>
<td>S</td>
</tr>
<tr>
<td>Momotaro</td>
<td>16.3 ± 0.9 b</td>
<td>S</td>
</tr>
<tr>
<td>Carol-7</td>
<td>10.7 ± 0.9 c</td>
<td>S</td>
</tr>
<tr>
<td>Doctor-K</td>
<td>0 ± 0 d</td>
<td>R</td>
</tr>
<tr>
<td>Sugar-lamp</td>
<td>0 ± 0 d</td>
<td>R</td>
</tr>
</tbody>
</table>

1Mean ± S.E. of new cysts / 50 g dried soil from pots inoculated with 1,000 J2 (n = 3)
2Different letters denote significant differences at $P < 0.05$ (Tukey’s HSD test)
3S: Susceptible, R: Resistant

Table 3. Reproduction of *G. tabacum* on five tomato cultivars.

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>New cysts/50 g dried soil</th>
<th>PCN phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carol-7</td>
<td>10.2 ± 1.4 a²</td>
<td>S</td>
</tr>
<tr>
<td>Puchi</td>
<td>10.4 ± 0.7 a</td>
<td>S</td>
</tr>
<tr>
<td>Carol-Queen</td>
<td>0 ± 0 b</td>
<td>R</td>
</tr>
<tr>
<td>Chelsea-mini</td>
<td>0 ± 0 b</td>
<td>R</td>
</tr>
<tr>
<td>Carol-10</td>
<td>0 ± 0 b</td>
<td>R</td>
</tr>
</tbody>
</table>

1Mean ± S.E. of new cysts / 50 g dried soil from pots inoculated with 1,000 J2 (n = 4)
2Different letters denote significant differences at $P < 0.05$ (Tukey’s HSD test)
3S: Susceptible, R: Resistant
(Table 4). Thus, resistance to TCN might be controlled genetically by a single dominant gene. As a control in this experiment, we inoculated three plants of ‘Doctor-K’ and ‘Taibyou-Shinkou’ cultivars with the J2 TCN. As expected, no cysts were isolated from the soil of the ‘Doctor-K’ plants but were present in the ‘Taibyou-Shinkou’ soil (Table 4).

Table 4. Reproduction of G. tabacum on progeny from selfing of Doctor-K

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>New cysts/50 g dried soil*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible 5 plants</td>
<td>38.0 ± 4.6</td>
</tr>
<tr>
<td>Resistant 20 plants</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Doctor-K (n = 3)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Taibyou-Shinkou (n = 3)</td>
<td>36.8 ± 5.2</td>
</tr>
</tbody>
</table>

*Mean ± S.E. of new cysts / 50 g dried soil from pots inoculated with 3,000 J2

The use of resistant cultivars is considered an effective method for reducing nematode-induced crop loss (Roberts, 2002). In this study, we identified new five TCN-resistant tomato cultivars. We suggest that these tomato cultivars and rootstocks will be useful for the management of TCN populations in areas where the nematodes are prevalent.

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