



DOI:10.22144/ctujoisd.2024.286

## Resistance of Citrus species (*Citrus* spp.) to the root lesion nematode *Pratylenchus* sp.

Le Thi Tu Anh<sup>1</sup>, Nguyen Quoc Si<sup>1</sup>, La Hoang Chau<sup>2</sup>, and Nguyen Ba Phu<sup>1\*</sup>

<sup>1</sup>College of Agriculture, Can Tho University, Viet Nam

<sup>2</sup>ATREM Science and Technology Co. Ltd, Viet Nam

\*Corresponding author (nbphu@ctu.edu.vn)

### Article info.

Received 11 Sep 2023

Revised 10 Jan 2024

Accepted 30 Jan 2024

### Keywords

*Citrus*, *nematode*,  
*Pratylenchus* sp., *resistance*,  
*susceptible*

### ABSTRACT

The study was carried out to evaluate the resistance of various domestic citrus species to the root lesion nematode *Pratylenchus* sp. for further research in rootstock selection as well as nematode management. The experiment was accomplished in a completely randomized design with 19 popular citrus species as 19 treatments, with thirty repeats and one plant for each experimental unit. The population of the nematode after 15 days of inoculation was calculated from growing media and roots for classifying the resistance capability of *Citrus* species. As a result, referred to the statistical analysis results and the Reproductive Index of the target nematode, they were suggestively classified into 5 distinctive groups: (i) resistant species: *C. grandis* var. *Duong La Cam*, *C. maxima* var. *Tam Quy*, *L. littoralis*, *C. nobilis* var. *typica*; (ii) moderately resistant specie: *C. nobilis* var. *Sanh*; (iii) moderately susceptible species: *C. latifolia*, *C. macroptera*, *C. sinensis* cv. *Soan*, *C. microcarpa*, and *C. reticulata* var. *Duong*; (iv) susceptible species: *C. maxima* var. *Da Xanh*, *C. maxima* var. *Long Co Co*, *C. maxima* var. *Nam Roi*, *C. sinensis* var. *Mat*, and *C. nobilis* var. *chrysocarpa*; (v) highly susceptible species: *C. limonia*, *C. hystrix*, *C. volkameriana* and *P. trifoliatius*. Therefore, *C. grandis* var. *Duong La Cam*, *C. maxima* var. *Tam Quy*, *L. littoralis*, and *C. nobilis* var. *typica* could be further researched to select appropriate rootstocks for citrus cultivation in the nematode-infected regions.

## 1. INTRODUCTION

Citrus is one of the most appealing fruits that has been cultivated in many countries worldwide (Dat, 2000). Nhuong (2004) found that in Viet Nam, over 80 species of citrus pests and approximately 40 species of citrus diseases have been identified. Among these, nematodes pose a significant threat to plant growth. Many nematode species such as *Tylenchulus*, *Radopholus*, *Pratylenchus*, and *Meloidogyne* are serious citrus pests (Duncan, 2009). Besides *Tylenchulus semipenetrans*, acknowledged as the most prevalent root parasitic nematode in nearly all citrus-growing regions

worldwide, *Pratylenchus* species also pose a significant threat. They can cause crop yield losses of up to 85% (Nicol et al., 2011) and may lead to even greater losses when interacting with certain soil-borne pathogens (Jones & Fosú-NyNyarko, 2014). In the Vietnamese Mekong Delta, the nematode *Pratylenchus* species has been observed to cause significant damage to citrus, such as hairy root rot, even with as few as 10 individuals per pot under net house conditions (Linh et al., 2012)

In addition to chemical and biological methods, implementing appropriate farming practices significantly contributes to effective nematode

management. One notable measure is the use of nematode-resistant species as rootstock. However, currently there is no study on resistance and susceptibility rootstocks toward *Pratylenchus* sp. (Shokoohi & Duncan, 2018). Since this study initiated the research topic, we considered the resistant and susceptible rootstocks toward *T. semipenetrans*, namely *Poncirus trifoliatus* and *Citrus volkameriana* (Campos & Barbosa Ferraz, 1979), as the resistant and susceptible controls toward *Pratylenchus* sp., respectively. Moreover, this study also referred to the resistance scale to *T. semipenetrans* from Javed et al. (2008) to the target nematode.

## 2. MATERIALS AND METHODS

### 2.1. Period and location

This study was conducted from December 2021 to August 2022 at the Agricultural Research and Experimentation Camp of Can Tho University.

### 2.2. Materials and instruments

Plants were propagated in three ways, seeding (approximately 4 months old), marcotting, and cutting (Table 1). The detail of propagation methods is described by Ve and Phong (2011). Then, those with similar morphological characteristics (strong root systems, bright yellow and evenly distributed root hairs, without pests or diseases) were chosen for nematode treating.

Growing media: using a renovated mixture of river sand and coco peat in the ratio of 7:3 (v/v) (Verdejo-Lucas et al., 2000).

Plastic black cultivating pots, each with a capacity of 1 liter of growing media, measured 14 cm in top diameter, 10 cm in bottom diameter, and 12 cm in height, and were perforated at the bottom for drainage.

Nematodes collection: as the study of Phu et al. (2023), the roots from the citrus gardens infected with *Pratylenchus* sp. were collected. The nematode extraction protocol was applied as Speijer and De Waele (1997). Based on the morphology as described by Phu et al. (2023), *Pratylenchus* sp. was separated.

Nematode isolation instruments: Toshiba blender (MX-60T) made in China, static sieve with 7 cm in diameter - 1 mm in pore size.

Observation instruments: Olympus CX23 microscope, micropipette 1,000-5,000  $\mu$ l made in Japan, counting glass slides.

## 2.3. Methods

### 2.3.1. Experimental design

Arranged in a completely randomized design, this experiment included 19 treatments (19 domestic and nonnative citrus species as mentioned in Table 1), with 30 repetitions in a net house. Each experimental unit consisted of an individual plant cultivated in a single pot.

### 2.3.2. Experimental Implement

Preparation of growing media: The growing media was immersed three times (once per day) in a solution of transparent lime water ( $\text{Ca(OH)}_2$  0.2%, pH 12) to remove weeds, pests, pathogens, and excess components. Subsequently, to replicate the growing conditions described by Phu et al. (2023), intermediate and micronutrients were added (2 g/pot) (15-17% melted  $\text{P}_2\text{O}_5$ ; 28-34%  $\text{CaO}$ ; 16-20%  $\text{MgO}$ ; 25-30%  $\text{SiO}_2$ ; Fe, Mn, B, Zn, Mo), followed by  $\text{Ca(OH)}_2$  to adjust the pH of the growing medium to a range of 6-7. Finally, 1 liter of the adjusted growing media was evenly divided into each clean pot. Planting: selected plants were rinsed under flowing tap water. Their roots were shortened about 10 cm long before soaking in lime water (0.2%  $\text{Ca(OH)}_2$ , pH = 12) for 30 minutes. Afterward, the plants were chopped down to a uniform height before being planted in the prepared pots, in which their root necks were horizontal with the inserted media surface. Adequate moisturization to the growing media (relative humidity of roughly 80%) was maintained equivalently. Plates were laid under the pots to collect the excessive water. After 7 days of planting, weekly irrigated the pots with NPK with a ratio of 30:20:10 at the concentration of 0.2%. After 15 days of planting, when the plants had been stable, the nematodes were inoculated into the pot. Referred to Toto and Nadel (2011), approximately 200 nematode individuals were inoculated around each plant via 4 holes (5 cm deep). Then, the holes were covered with soil and watered to keep moist.

### 2.3.3. Nematode extraction:

Sixty days after nematode inoculation, the density of nematodes in 1 liter of the growing media (individuals per liter) was counted and averaged under a microscope, following extraction using the method revised by the Department of Plant Protection (2016). Briefly, the growing media was strongly stirred with 10 L of tap water prior to collecting the supernatant. We allowed the water to settle down for a minute before pouring it through a 0.05-mm-spore-size sieve. Collect all material on

the sieve into a beaker for natural precipitation. Repeat these steps 3 times. The collected materials were gently poured onto a filter paper located on a

1-mm-spore-size sieve, which sit on a petri dish and remain it stable for a day. Collect the stagnated liquid from the petri dish to observe the nematode.

**Table 1. Name, species, scientific names, and propagation method of each treatment in this study**

No.	Local name	Global name	Scientific name	Propagation methods
1	Buoi Da Xanh	-	<i>Citrus maxima</i> var. Da Xanh	Marcotting
2	Buoi Duong La Cam	-	<i>Citrus grandis</i> var. Duong La Cam	Marcotting
3	Buoi Long Co Co	-	<i>Citrus maxima</i> var. Long Co Co	Marcotting
4	Buoi Nam Roi	-	<i>Citrus maxima</i> var. Nam Roi	Marcotting
5	Buoi Tam Quy	-	<i>Citrus maxima</i> var. Tam Quy	Marcotting
6	Chanh Khong Hat	Tahiti Lime	<i>Citrus latifolia</i>	Marcotting
7	Cam Duong/Da Tu Bien	-	<i>Limnocitrus littoralis</i> (Miq.) Sw.	Seeding
8	Cam Mat	-	<i>Citrus sinensis</i> (L.) Osb. var. <i>Mat</i>	Seeding
9	Cam Nui	Annam papeda	<i>Citrus macroptera</i> Montr. var. <i>annamensis</i> Tan.	Seeding
10	Cam Sanh	King mandarin	<i>Citrus nobilis</i> var. <i>typica</i> Hassk.	Seeding
11	Cam Soan	-	<i>Citrus sinensis</i> (L.) cv. Soan	Seeding
12	Chanh Tau	-	<i>Citrus limonia</i> Osb.	Seeding
13	Hanh/Tac	Calamondin	<i>Citrofortunella microcarpa</i> (B.) Wi.	Seeding
14	Quy Duong	-	<i>Citrus reticulata</i> Blanco var. Duong	Seeding
15	Quy Hong/Quy Tieu	-	<i>Citrus nobilis</i> var. <i>chrysoarpa</i> Lamk.	Seeding
16	Sanh	-	<i>Citrus nobilis</i> var. Sanh	Seeding
17	Truc	Kaffir lime	<i>Citrus hystrix</i> DC.	Seeding
18	Chanh Volka* (susceptible control)	Volkamer lime	<i>Citrus volkameriana</i>	Cutting
19	Cam Ba La* (resistant control)	Trifoliate orange	<i>Poncirus trifoliatus</i> (L.) Raf.	Seeding

Note: scientific names were referred from Ho (2003); \*: nonnative species

Simultaneously, the density of nematodes in 1 g of roots (individuals/1g root) was calculated after being extracted by the renovated grinding method (Speijer & De Waele, 1997) and counted under the microscope as follows: rinse the root samples under strong running tap water. Absorb the excess water with absorbent paper. Observe and selectively cut both brown root hairs and swelling root tips to obtain the weight of 1g, and split them into 0.5-to-1-cm segments. Mix well and put all in a small blender jar primarily containing 40 ml of water. A 2-blade knife of the blender Toshiba MX-60T was utilized to grind the root 3 times (Level 1 - the slowest speed), with 10 seconds/time and a 5-second gap between each grinding time. Pour the blended mixture into a small plastic container before slowly introducing it into a static sieve lined with absorbent paper. Lay the static sieve containing the remained nematodes into a clean plastic box. Add enough distilled water to immerse the absorbent paper, label and place it at room temperature. After 48 hours, the stagnated solution under the sieve was collected in a glass tube, settled for 30 minutes, and then discarded the supernatant to obtain 10 ml/ tube for counting.

### 2.3.4. Collecting data

We separately counted the total of nematodes at all life stages extracted from growing media and infected roots 3 times with the microscope and counting glass slide, then calculated the average value. Since there is currently no published resistance scale for *Pratylenchus* sp. on Citrus, the resistance of citrus species was evaluated using both the scale of Javed et al. (2008) (Table 2) and the reproduction index (RI) based on the total individuals of collectable nematode.

**Table 2. Suggested scale for assessing resistability or susceptibility in citrus to *Pratylenchus* sp. referred from Javed et al. (2008)**

Scale	Response	Nematode density (individuals/g root)
1	Resistant	< 100
3	Moderately resistant	100-200
5	Moderately susceptible	201-300
7	Susceptible	301-500
9	Highly susceptible	> 500

The Reproduction Index (RI) was calculated based on the total collected nematode individuals as the following formation of Noe (1985):

$$RI = \frac{N1}{N0}$$

Wherein:

*N1*: The number of nematodes at the evaluation time

*N0*: The initially quantity of nematodes inoculated

RI < 1: Resistant, RI > 1: Susceptible

**2.4. Data analysis**

Microsoft Excel software was utilized to process data and analyze correlation and regression. Analysis of variance (ANOVA), T-Student test was applied using SPSS 22.0 software. Compare differences between treatments using the Duncan Post-hoc test at the significance of 5%.

**3. RESULTS AND DISCUSSION**

**3.1. Density of nematode *Pratylenchus* sp.**

*3.1.1. Density of Pratylenchus sp. nematodes in the growing media*

Table 3 depicts the density of *Pratylenchus* sp. in 1

L of growing media after 60 days of inoculation. In the growing media of *C. limonia*, *C. volkameriana*, and *P. trifoliatus*, the density of nematodes *Pratylenchus* sp. was the highest, ranging from 451-603 individuals/L media. Secondly, the nematode population in the growing medium of *C. hystrix* was 131 individuals/L media. Thirdly, their density in the growing media of *C. maxima* var. Da Xanh, *C. maxima* var. Nam Roi, and *C. sinensis* var. Mat was 61-74 individuals/L media. The lowest densities of *Pratylenchus* sp. were recorded in the media growing *C. grandis* var. Duong La Cam, *C. maxima* var. Tam Quy, *L. littoralis*, *C. macroptera*, *C. nobilis* var. *typica*, *C. sinensis* cv. Soan, *C. microcarpa*, *C. reticulata* var. Duong, *C. nobilis* var. *chrysocarpa*, and *C. nobilis* var. Sanh, namely 1-23 individuals/L media.

**Table 3. Density of *Pratylenchus* sp. in media growing citrus species after 60 days of inoculation**

Citrus species	Average (individuals per L media)	Sd <sup>+</sup>	Citrus species	Average (individuals per L media)	Sd <sup>+</sup>
<i>C. maxima</i> var. Da Xanh	61 <sup>c</sup>	131	<i>C. latifolia</i>	16 <sup>de</sup>	35
<i>C. grandis</i> var. Duong La Cam	1 <sup>e</sup>	6	<i>C. limonia</i>	564 <sup>a</sup>	655
<i>C. maxima</i> var. Long Co Co	34 <sup>c</sup>	48	<i>C. microcarpa</i>	17 <sup>de</sup>	35
<i>C. maxima</i> var. Nam Roi	74 <sup>c</sup>	119	<i>C. reticulata</i> var. Duong	12 <sup>de</sup>	27
<i>C. maxima</i> var. Tam Quy	4 <sup>e</sup>	18	<i>C. nobilis</i> var. <i>chrysocarpa</i>	16 <sup>de</sup>	30
<i>L. littoralis</i>	3 <sup>e</sup>	9	<i>C. nobilis</i> var. Sanh	23 <sup>d</sup>	44
<i>C. sinensis</i> var. Mat	62 <sup>c</sup>	110	<i>C. hystrix</i>	131 <sup>b</sup>	124
<i>C. macroptera</i>	12 <sup>de</sup>	30	<i>C. volkameriana</i>	451 <sup>a</sup>	536
<i>C. nobilis</i> var. <i>typica</i>	3 <sup>e</sup>	8	<i>P. trifoliatus</i>	603 <sup>a</sup>	994
<i>C. sinensis</i> cv. Soan	14 <sup>de</sup>	30			
Level of significance				1%	
CV (%)				80.2	

*Sd*: standard deviation

<sup>+</sup>: data was converted into log(*X*+1)

In a same column, data with the same alphabet are not different as Duncan test at the significant level of 5%

*3.1.2. Density of the nematode Pratylenchus sp. at the roots*

Table 4 illustrates the density of *Pratylenchus* sp. parasite in the root of citrus species after 60 days of inoculation. The highest density of the nematodes was observed in the roots of *C. limonia* and *C. hystrix*, 2,370 and 2,678 individuals/g root, respectively. Subsequently, the densities of the nematodes were ranked as follows: *C. volkameriana* roots (1,505 individuals/g root), *P. trifoliatus* (1,509 individuals/g root), and *C. sinensis* var. Mat (916 individuals/g root). The number of *Pratylenchus* sp. in the roots of *C. maxima* var. Long Co Co, *C. maxima* var. Da Xanh, *C. maxima* var. Nam Roi, *C.*

*latifolia*, *C. macroptera*, and *C. nobilis* var. *chrysocarpa* were relatively high, fluctuating between 428 and 759 individuals/g root. The data of *C. sinensis* cv. Soan, *C. macrocarpa*, and *C. reticulata* var. Duong were quite high, ranging from 339 to 388 individuals/g root. Then, the nematode density in the roots of *C. nobilis* var. Sanh was low (82 individuals/g root). The population of *Pratylenchus* sp. at the roots of *C. grandis* var. Duong La Cam, *L. littoralis*, and *C. nobilis* var. *typica* were extremely low, namely 6, 10, and 36 individuals/g root, respectively. In particular, no nematode was observed in *C. maxima* var. Tam Quy's roots.

Because of the lack of resistance scale for *Pratylenchus* sp. on Citrus rootstocks, this study referred to both the resistance scale by Javed et al. (2008) and the results of the Duncan post-hoc test for the density of *Pratylenchus* sp. in growing media and root, respectively (Table 3 and Table 4), the citrus species could be divided into groups as the following suggestion:

- Group of resistant species to *Pratylenchus* sp. includes *C. grandis* var. Duong La Cam, *C. maxima* var. Tam Quy, *L. littoralis*, and *C. nobilis* var. *typica* (the density of *Pratylenchus* sp. ranged from 0 to 36 individuals/g root).
- Group of moderately resistant species to *Pratylenchus* sp. includes *C. nobilis* var. Sanh (nematode density was 82 individuals/g root).

- Group of moderately susceptible species to *Pratylenchus* sp. includes *C. maxima* var. Da Xanh, *C. maxima* var. Long Co Co, *C. maxima* var. Nam Roi, *C. latifolia*, *C. macroptera*, *C. sinensis* cv. Soan, *C. microcarpa*, *C. reticulata* var. Duong, and *C. nobilis* var. *chrysocarpa* (*Pratylenchus* sp. density fluctuated 339-759 individuals/g root).
- Group of susceptible species to *Pratylenchus* sp. includes *C. sinensis* var. Mat, *C. volkameriana*, and *P. trifoliatum* (*Pratylenchus* sp. density varied 916-1,509 individuals/g root).
- Group of highly susceptible species to *Pratylenchus* sp. includes *C. limonia* and *C. hystrix* (*Pratylenchus* sp. density spanned 2,370-2,678/g root).

**Table 4. The density of *Pratylenchus* sp. in roots of citrus species after 60 days of inoculation**

Citrus species	Average (individuals per g root)	Sd <sup>+</sup>	Citrus species	Average (individuals per g root)	Sd <sup>+</sup>
<i>C. maxima</i> var. Da Xanh	664 <sup>cd</sup> e	849	<i>C. latifolia</i>	428 <sup>cd</sup> e	496
<i>C. grandis</i> var. Duong La Cam	6 <sup>hi</sup>	29	<i>C. limonia</i>	2,370 <sup>a</sup>	2,179
<i>C. maxima</i> var. Long Co Co	759 <sup>cd</sup>	1.230	<i>C. microcarpa</i>	353 <sup>de</sup>	432
<i>C. maxima</i> var. Nam Roi	671 <sup>cd</sup> e	1.094	<i>C. reticulata</i> var. Duong	339 <sup>e</sup>	477
<i>C. maxima</i> var. Tam Quy	0 <sup>i</sup>	0	<i>C. nobilis</i> var. <i>chrysocarpa</i>	702 <sup>cd</sup> e	772
<i>L. littoralis</i>	10 <sup>gh</sup>	21	<i>C. nobilis</i> var. Sanh	82 <sup>f</sup>	123
<i>C. sinensis</i> var. Mat	916 <sup>bc</sup>	1.122	<i>C. hystrix</i>	2,678 <sup>a</sup>	2,663
<i>C. macroptera</i>	553 <sup>cd</sup> e	800	<i>C. volkameriana</i>	1,505 <sup>ab</sup>	1,338
<i>C. nobilis</i> var. <i>typica</i>	36 <sup>g</sup>	84	<i>P. trifoliatum</i>	1,509 <sup>ab</sup>	1,649
<i>C. sinensis</i> cv. Soan	388 <sup>de</sup>	435			
Level of significance				1%	
CV (%)				29.9	

Sd: standard deviation

+: data was converted into log(X+1)

In a same column, data with the same alphabet are not different as Duncan test at the significant level of 5%

As a result, it showed that *P. trifoliatum* was a susceptible species to the nematode *Pratylenchus* sp. (1,509 individuals/g of roots). However, *P. trifoliatum* was recognized as a resistant species to the nematode *T. semipenetrans* in the world. According to Kaplan (1990) (cited by Verdejo-Lucas et al., 2000), *Poncirus trifoliata* (Trifoliate orange, *P. trifoliatus*) was the only citrus rootstock resistant to the nematode *T. semipenetrans*. Some hybrids with *P. trifoliata* were also resistant to nematodes. “Swingle” citrumelo rootstock (*Citrus paradisi* Macf. x *P. trifoliata*) was highly resistant to *T. semipenetrans* nematodes in Florida (Kaplan & O'Bannon, 1981, cited by Verdejo-Lucas et al., 2000), Italy (Lo Giudice & Inserra, 1980, cited by Verdejo-Lucas et al., 2000) and Venezuela (Crozzoli & Funes, 1992, cited by Verdejo-Lucas et

al., 2000). However, to date, there has been no study on commercial citrus rootstocks that are resistant to the nematode *Pratylenchus* sp. (Shokoohi & Duncan, 2018). Resultantly, this is a remarkable discovery that *P. trifoliatum* is resistant to *T. semipenetrans* but susceptible to *Pratylenchus* species, both of which are root lesion nematodes

### 3.1.3. Correlation between the *Pratylenchus* sp. densities in the growing media and the roots

Figure 1 shows the density of *Pratylenchus* sp. in the growing media and that in roots are positively and strongly correlated with each other at the 1% significance level with the correlation coefficient  $r = 0.509$ . In resistant species, the population of *Pratylenchus* sp. in the growing media and roots are both low, whereas *Pratylenchus* sp. density in the

medium and the roots of susceptible species are both high. The regression result (Figure 3) shows that the density of *Pratylenchus* sp. in roots is determined by the density of *Pratylenchus* sp. in the growing media with the regression equation:

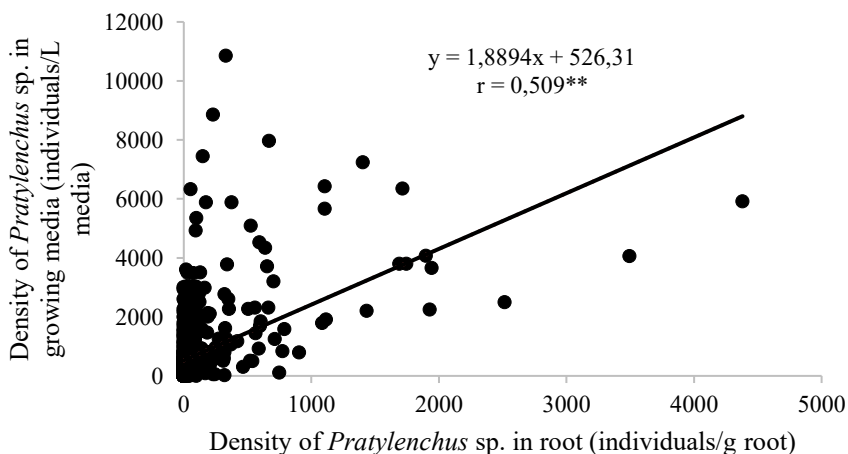
$$Y = 2.84X + 421.44$$

wherein:

Y: density of *Pratylenchus* sp. nematodes in the roots (individuals/g root);

X: density of *Pratylenchus* sp. in growing media (individuals/L media)).

The results show that the density of *Pratylenchus* sp. in the roots is much higher than their density in the media. This is because *Pratylenchus* sp. is an endoparasitic nematode in roots, mainly causing damage inside root hairs (Shokohi & Duncan, 2018). This lesion can lead to the infection of fungal pathogens that rot the root system; only then will the nematodes move out of the soil (Chau, 2003). Therefore, the population of nematodes *Pratylenchus* sp. is often very high in the roots but much lower in the growing media.



**Figure 1. Correlation between the densities of *Pratylenchus* sp. in growing media and roots of citrus species after 60 days of inoculation**

**3.2. Reproductive index of *Pratylenchus* sp. in growing media and roots of citrus varieties**

Table 5 shows the RI of *Pratylenchus* sp. in the growing media and the roots of citrus species. The RI index of *C. limonia*, *C. hystrix*, *C. volkameriana*, and *P. trifoliatus* were the highest, namely 4.67, 14.04, 9.78, and 10.56, respectively. Subsequently, the RI of *Pratylenchus* sp. in *C. maxima* var. Da Xanh, *C. maxima* var. Long Co Co, *C. maxima* var. Nam Roi, *C. sinensis* var. Mat, and *C. nobilis* var. *chrysocarpa* was relatively high, namely 3.63, 3.96, 3.72, 4.89, 3.59, respectively. That of *C. latifolia*, *C. macroptera*, *C. sinensis* cv. Soan, *C. microcarpa*, and *C. reticulata* var. Duong were average, at 2.22 2.82; 2.01; 1.85; and 1.75, respectively. That in *C. nobilis* var. Sanh was low (0.53). The others had the extremely low R index of *Pratylenchus* sp., in detail *C. grandis* var. Duong La Cam (0.04), *C. maxima* var. Tam Quy (0.02), *L. littoralis* (0.07), and *C. nobilis* var. *typica* (0.19).

As the lack of study on the reproductive index of *Pratylenchus* sp. to Citrus species, this study referred to the resistant scale Noe (1985) and the categorization results from the Duncan Post-hoc tests (Figure 4) to divided into groups as following suggestion:

- The group of resistant species to *Pratylenchus* sp. includes *C. grandis* var. Duong La Cam, *C. maxima* var. Tam Quy, *L. littoralis*, and *C. nobilis* var. *typica* (RI = 0.02-0.19).
- Group of moderately resistant species to *Pratylenchus* sp. includes *C. nobilis* var. Sanh (RI = 0.53).
- Group of moderately susceptible species to *Pratylenchus* sp. includes *C. latifolia*, *C. macroptera*, *C. sinensis* cv. Soan, *C. microcarpa*, and *C. reticulata* var. Duong (RI = 1.75-2.82).
- Group of susceptible species to *Pratylenchus* sp. includes *C. maxima* var. Da Xanh, *C. maxima* var. Long Co Co, *C. maxima* var. Nam Roi, *C. sinensis*

var. *Mat*, and *C. nobilis* var. *chrysocarpa* (RI = 3.59-4.89).

– Group of highly susceptible species to *Pratylenchus* sp. includes *C. limonia*, *C. hystrix*, *C. volkameriana*, and *P. trifoliatius* (RI = 9.78-14.67).

**3.3. Evaluation of citrus species in resistance level to the nematode *Pratylenchus* sp.**

According to results of *Pratylenchus* sp. density and RI in treatments, the resistance of Citrus species to the nematode. is concluded in Table 6. Therefore,

the resistance level of citrus species to the root lesion *Pratylenchus* sp. is mainly referred to as the RI, which can be classified as follows:

– Group of resistant species includes *C. grandis* var. Duong La Cam, *C. maxima* var. Tam Quy, *L. littoralis*, and *C. nobilis* var. *typica* (density range 0-36 individuals/g root and RI = 0.02-0.19).

– Group of moderately resistant species includes *C. nobilis* var. Sanh (density range 82 individuals/g root and RI = 0.53).

**Table 5. Reproductive index of *Pratylenchus* sp. after 60 days of inoculation**

Citrus species	RI	Sd <sup>+</sup>	Citrus species	RI	Sd <sup>+</sup>
<i>C. maxima</i> var. Da Xanh	3.63 <sup>b</sup>	4.72	<i>C. latifolia</i>	2.22 <sup>c</sup>	2.47
<i>C. grandis</i> var. Duong La Cam	0.04 <sup>ef</sup>	0.15	<i>C. limonia</i>	14.67 <sup>a</sup>	12.77
<i>C. maxima</i> var. Long Co Co	3.96 <sup>b</sup>	6.26	<i>C. microcarpa</i>	1.85 <sup>c</sup>	2.29
<i>C. maxima</i> var. Nam Roi	3.72 <sup>b</sup>	5.71	<i>C. reticulata</i> var. Duong	1.75 <sup>c</sup>	2.43
<i>C. maxima</i> var. Tam Quy	0.02 <sup>f</sup>	0.09	<i>C. nobilis</i> var. <i>chrysocarpa</i>	3.59 <sup>c</sup>	3.13
<i>L. littoralis</i>	0.07 <sup>ef</sup>	0.12	<i>C. nobilis</i> var. Sanh	0.53 <sup>d</sup>	0.81
<i>C. sinensis</i> var. Mat	4.89 <sup>b</sup>	5.95	<i>C. hystrix</i>	14.04 <sup>a</sup>	13.66
<i>C. macroptera</i>	2.82 <sup>c</sup>	4.10	<i>C. volkameriana</i>	9.78 <sup>a</sup>	8.18
<i>C. nobilis</i> var. <i>typica</i>	0.19 <sup>c</sup>	0.44	<i>P. trifoliatius</i>	10.56 <sup>a</sup>	12.47
<i>C. sinensis</i> cv. Soan	2.01 <sup>c</sup>	2.28			
Level of significance				1%	
CV (%)				38.6	

Sd: standard deviation

+: data was converted into log(X+1)

In a same column, data with the same alphabet are not different as Duncan test at the significant level of 5%.

**Table 6. Categorization of the resistance of citrus species to the nematode *Pratylenchus* sp.**

No.	Local name	Density-based categorization	RI-based categorization	Conclusion
1	<i>C. maxima</i> var. Da Xanh	S	MS	MS
2	<i>C. grandis</i> var. Duong La Cam	R	R	R
3	<i>C. maxima</i> var. Long Co Co	S	MS	MS
4	<i>C. maxima</i> var. Nam Roi	S	MS	MS
5	<i>C. maxima</i> var. Tam Quy	R	R	R
6	<i>C. latifolia</i>	S	S	S
7	<i>L. littoralis</i>	R	R	R
8	<i>C. sinensis</i> var. Mat	MS	MS	MS
9	<i>C. macroptera</i>	S	S	S
10	<i>C. nobilis</i> var. <i>typica</i>	R	R	R
11	<i>C. sinensis</i> cv. Soan	S	S	S
12	<i>C. limonia</i>	HS	HS	HS
13	<i>C. microcarpa</i>	S	S	S
14	<i>C. reticulata</i> var. Duong	S	S	S
15	<i>C. nobilis</i> var. <i>chrysocarpa</i>	S	MS	MS
16	<i>C. nobilis</i> var. Sanh	MR	MR	MR
17	<i>C. hystrix</i>	HS	HS	HS
18	<i>C. volkameriana</i>	MS	HS	HS
19	<i>P. trifoliatius</i>	MS	HS	HS

R: resistant; MR: moderately resistant; MS: susceptible; S: moderately susceptible; HS: highly susceptible

– Group of moderately susceptible species includes *C. latifolia*, *C. macroptera*, *C. sinensis* cv. Soan, *C. macrocarpa*, and *C. reticulata* var. Duong (density range 339-553 individuals/g root and RI = 1.75-2.82).

– Group of susceptible species includes *C. maxima* var. Da Xanh, *C. maxima* var. Long Co Co, *C. maxima* var. Nam Roi, *C. sinensis* var. Mat, and *C. nobilis* var. *chrysocharpa* (density range 664-916 individuals/g root and RI = 3.59-4.89).

– Group of highly susceptible species includes *C. limonia*, *C. hystrix*, *C. volkameriana*, and *P. trifoliatum* (density range 1,505-2,678 individuals/g root and RI = 9.78-14.67)

#### 4. CONCLUSIONS

Based on our results, the resistance of citrus species to the root lesion nematode *Pratylenchus* species is suggested to be classified into five groups as follows:

Resistant species: *C. grandis* var. Duong La Cam, *C. maxima* var. Tam Quy, *L. littoralis*, and *C. nobilis*

#### REFERENCES

- Campos, D. V., & Barbosa Ferraz, L. C. C. (1979). Susceptibility of nine citrus rootstocks to *Tylenchulus semipenetrans*. *Trabalhos apresentados a IV Reuniao Brasileira de Nematologia* (pp. 85-96). Sao Paulo Publisher.
- Chau, N. N. (2003). *Plant nematodes and management basis*. Hanoi, Science and Technology Publisher.
- Dat, D. H. (2000). *Gardening: Develop fruits in our nation, tropical edible fruits with limited tolerance*. Hanoi National Culture publisher.
- Department of Plant Protection (2016). *Practical curriculum in the agricultural nematode*. College of Agriculture and Applied Biology, Can Tho University.
- Duncan, L. W. (2009). Managing nematodes in citrus orchards. In A. Ciancio, K. G. Mukerji (Eds.), *Integrated Management of Fruit Crops and Forest Nematodes* (pp. 135-174), Springer Science Business Media B.V.
- Javed, N., Javed, M., Ilyas, M. B., Khan, M. M., & Inam-Ul-Haq, M. (2008). The reaction of various citrus rootstocks (germplasm) against citrus root nematode (*Tylenchulus semipenetrans* Cobb.). *Pakistan Journal of Botany*, 40(6), 2693-2696.
- Jones, M., & Fosu-Nyarko, J. (2014). Molecular biology of root lesion nematodes (*Pratylenchus* sp.) and their interaction with host plants. *Annals of Applied Biology*, 164, 163-181.
- Linh, D. T., Tuong, L. T., Man, V. M., Cuong, N. H., & Hoa, N.V. (2012). *Study nematode Pratylenchus sp.*

var. *typica*, which can be further researched to select appropriate rootstocks for citrus cultivation in nematode-infected regions.

Moderately resistant species: *C. nobilis* var. *Sanh*.

Moderately susceptible species: *C. latifolia*, *C. macroptera*, *C. sinensis* cv. Soan, *C. microcarpa* and *C. reticulata* var. Duong.

The susceptible species: *C. maxima* var. *Da Xanh*, *C. maxima* var. Long Co Co, *C. maxima* var. Nam Roi, *C. sinensis* var. Mat, and *C. nobilis* var. *chrysocharpa*.

The highly susceptible species: *C. limonia*, *C. hystrix*, *C. volkameriana*, and *P. trifoliatum*.

We highly recommend employing those resistant citrus species as rootstock in soil infected with *Pratylenchus* sp.. Furthermore, it is necessary to pursue the species identification of *Pratylenchus* species and establish a resistance scale for this nematode to enable precise management.

*causing root rot in Cam Sanh* [Conference presentation]. Conference in National Plant Pathogens in Vietnam, Southern Horticultural Research Institute, Tien Giang

Nhuong, V. K. (2004). *Identification and prevention of several citrus pests and pathogens*. Agriculture publisher.

Nicol, J., Turner, S., Coyne, D., Den, N. L., Hockland, S., & Maafi, Z. T. (2011). Current nematode threats to world agriculture. In J. Jones., G. Gheysen & C. Fenoll (Eds), *Genomics and molecular genetics of plant-nematode interactions* (pp. 21-43). The Netherlands Springer.

Noe, J. P. (1985). Analysis and interpretation of data from the nematological experiment. In Barker K. R., Carter C. C., & Sasser N. J. (eds), *An advanced treatise on Meloidogyne, Volume II. Methodology* (pp. 187-196). North Carolina State University Graphics, Raleigh, USA.

Ho, P. H. (2003). *An illustrated flora of Vietnam*. Volume II. Youth publisher.

Phu, N. B., Duyen, D. T. H., Si, N. Q., & Anh, L. T. T. (2023). Survey on the composition of citrus nematodes in the Mekong Delta. *Can Tho University Journal of Science*, 59(5), 139-148. <https://doi.org/10.22144/ctujs.2023.196>

Shokoohi, E. & Duncan, L. W. (2018). Nematode parasites of citrus. In R. A. Sikora, D. Coyne, J. Hallmann, & P. Timper, (Eds), *Plant-parasitic*



- nematodes in subtropical and tropical agriculture* (pp. 446-476). CAB Intern.
- Speijer, P. R., & De Waele, D. (1997). *Screening of Musa Germplasm for resistance and tolerance to nematodes*. International Plant Genetic Resources Institute.
- Toto, S., & Sadeli, N. (2011). Resistance level of some citrus cultivars to the citrus nematode (*Tylenchulus semipenetrans* Cobb) in West Java, Indonesia. *Lucrări Științifice*, 54(1), 11-14.
- Ve, N. B., & Phong, L. T. (2011). *Cirriculum of Edible fruit*. Can Tho University Publishing House.
- Verdejo-Lucas, S., Sorribas, F. J., Forner, J. B. & Alcaide, A. (2000). Resistance of Hybrid Citrus Rootstocks to a Mediterranean Biotype of *Tylenchulus semipenetrans* Cobb. *Hortscience*, 35(2), 269-273.