Occurrence and Identification of Root-Knot Nematodes on Red Dragon Fruit (Hylocereus polyrhizus) in Hainan, China

Haibo Long 1, Yuan Chen 1,*, Yueling Pei 1, Huadong Li 2, Yanfang Sun 1 and Tuizi Feng 1

Abstract: The genus Meloidogyne is one of the most economically damaging plant parasitic nematodes on a worldwide basis. To date, only the dragon fruits in Brazil were reported infected by M. javanica or M. incognita in previous surveys. From 2019–2021, we conducted our investigation in 62 orchards from 10 counties of Hainan, which are the main producing areas of dragon fruits in China. The collected roots were visually scored by gall index to evaluate the distribution and severity of diseases caused by Meloidogyne. Then, the isolated nematodes were further confirmed by a combination of morphological and molecular analysis. This survey revealed that the root-knot nematodes were widely distributed in red dragon fruit planting areas in Hainan and caused severe symptoms including stunting, wilting, and yellowing of the stems. The nematode populations were identified as M. enterolobii and M. javanica, among which, M. enterolobii accounted for a larger proportion, 93.9%. To our knowledge, this is the first report of M. enterolobii causing diseases on red dragon fruit. The results obtained from this survey will provide an insight into the presence of root-knot nematodes in dragon fruit plants. Also, these results will raise attention for developing effective management strategies to prevent further spread of root-knot nematodes on dragon fruit in Hainan.

Keywords: occurrence; root-knot nematode; Meloidogyne enterolobii; Meloidogyne javanica; dragon fruit

1. Introduction

The dragon fruit (Hylocereus spp.), also known as pitaya or pitahaya, belonging to the family Cactaceae, is the most important tropical fruit crop due to its attractive color, pleasant taste and high antioxidant content [1,2]. This plant is native to the tropical areas of North, Central and South America, and is now extensively grown throughout tropical and subtropical regions worldwide, especially in Asian countries, such as Malaysia, Vietnam, Thailand, Philippines and southern China [3,4]. There are more than 20 species in the genus Hylocereus, with similar stems and flowers in most species but different fruit patterns [5]. However, only four species, such as H. undatus, H. polyrhizus, H. costaricensis and H. megalanthus, are mostly cultivated in different parts of the world [6]. Among these, the red dragon fruit H. polyrhizus is the most popular and widely cultivated in southern China.

Root-knot nematodes (Meloidogyne spp.) are global pests comprised of more than 100 nominal species and they attack a large number of host plants [7,8]. Approximately 90% of the horticultural-crop-producing areas are infested with root-knot nematodes [9]. The major species of root-knot nematodes identified in the tropics and subtropics are M. incognita, M. javanica, M. arenaria and M. enterolobii [10]. These nematodes have been reported to result in yearly losses to the tune of 22% in tropical regions [11]. Nascimento et al. first reported M. javanica infected yellow dragon fruit plants H. megalanthus in Brazil [12]. Then, Souza et al. reported M. incognita infected white dragon fruit plants H. undatus in Brazil [13]. These findings bring an alert about the occurrence of Meloidogyne...
species in dragon fruit crops. So far, there have been no relevant reports about root-knot nematodes infecting dragon fruits in other parts of the world.

Hainan, a tropical island province located at the southernmost tip of mainland China, is an important producer of vegetables and tropical fruits such as guava, banana, mango and dragon fruits. In tropical Hainan, the warm and humid environment, sandy soil texture and long-term monocultures are conducive to the establishment and spread of root-knot nematodes [14]. Over the last decades, numerous surveys have shown that root-knot nematodes commonly occurred on vegetables in Hainan, causing yield losses of 30 to 45%, with severe cases reaching over 70% in some fields [14–16]. *M. incognita*, *M. javanica* and *M. enterolobii* were the major existing species [17–20]. However, root-knot nematodes on fruit crops have received little attention compared to vegetables, and the epidemiological information regarding root-knot nematodes attacking dragon fruits in Hainan is lacking. During 2019–2021, we conducted a detailed survey of root-knot nematodes on dragon fruits in Hainan, China. The objectives of this study were to determine the occurrence and species of root-knot nematodes infecting dragon fruits. The outcome of this study will help developing control strategies for root-knot nematodes on dragon fruit plants.

2. Materials and Methods

2.1. Survey and Sample Collection

The root-knot nematode survey was conducted in 10 major red dragon fruit planting counties of Hainan during 2019–2021 (Table 1). A total of 62 orchards were inspected, and at least five plants in each orchard were randomly selected and sampled. Root samples collected within an orchard were pooled as one sample. Infested roots showing gall symptoms were brought to the laboratory and thoroughly examined for the presence of galls. The number of galls in each root were counted. The galling index (GI) was quantified using the following scale: 0 = no gall on roots, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, 5 = more than 100 galls per root [21]. The frequency of occurrence of root-knot nematodes in each county was calculated as followed:

\[
\text{Frequency (\%)} = \frac{\text{Number of infected orchards}}{\text{Total number of inspected orchards}} \times 100
\]

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequences (5′-3′)</th>
<th>Species</th>
<th>Fragment Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeF</td>
<td>AACTTTTGTGAAATGCGGACTG</td>
<td><em>M. enterolobii</em></td>
<td>293</td>
</tr>
<tr>
<td>MeR</td>
<td>TCAGTCTAGGAGGATCAACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MjF</td>
<td>ACGCTAGAATTCCGACCTGG</td>
<td><em>M. javanica</em></td>
<td>517</td>
</tr>
<tr>
<td>MjR</td>
<td>GTACCAGAAGCAGCCATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MiF</td>
<td>ACGAGGAACCATTTCTCCGTCC</td>
<td><em>M. incognita</em></td>
<td>955</td>
</tr>
<tr>
<td>MiR</td>
<td>ACGAGGAACCATTTCTCCGTCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2. Histological Observation

Nematode-infected root slices of dragon fruit plants were prepared as described by Iberkleid et al. with minor modifications [22]. Galled roots were fixed for 1 week using 2% glutaraldehyde in 50 mM PBS buffer (pH 7.2), dehydrated using an ethanol dilution series (vol/vol; 10, 30, 50, 70, 90, and 100%), and then embedded in Technovit 7100 (Kulzer, Wehrheim, Germany) as recommended by the manufacturer. The embedded roots and gall tissues were sectioned (5 μm) using an ultramicrotome (Leica, Wetzlar, Germany) and stained in 0.05% toluidine blue and mounted in Depex (Sigma, St. Louis, MO, USA). Microscopic observations were performed using bright-field optics, and the images were collected using a digital camera (Nikon, Tokyo, Japan).
2.3. Morphological Identification

Initial identification of the root-knot nematode species was performed by observing the perineal patterns of adult females. Mature females were dissected out from galls on the dragon fruit plant roots. Perineal patterns slides were prepared according to a method mentioned by Eisenback et al., through which perineal patterns of five females were prepared for each sample [23]. Perineal patterns were observed under a microscope to study their characteristics, followed by species identification according to Eisenback et al. [24].

2.4. Molecular Identification

To confirm the species identity, molecular identification was performed. DNA from each nematode population was extracted from ten individual females according to the method used by Holterman et al. [25] The primer sequences of MeF/MeR, MjF/MjR and MiF/MiR used for identification are provided in Table 1 [26,27]. All amplification reactions were performed in a final volume of 50 µL, containing 1 µL of template DNA, 25 µL of 2X Taq PCR Master Mix (Takara, Shiga, Japan), 1 µL of 10 µM each primer, and 22 µL of ddH2O. The program of PCR cycling consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing temperature for 45 s and at 72 °C for 1 min, with a final extension step for 10 min at 72 °C. The annealing temperatures were set up at 55 °C for primers MeF/MeR, 58 °C for primers MjF/MjR, and 62 °C for primers MiF/MiR. After PCR amplification, 10 µL of each PCR product was mixed with 2 µL of 6X loading buffer and loaded on a 1% standard TAE buffered agarose gel. After electrophoresis (100 V for 40 min), the gel was stained with ethidium bromide (0.1 µg/mL) for 20 min, visualized and photographed under UV illumination.

3. Results

3.1. Disease Symptoms Caused by Root-Knot Nematodes

Observation of dragon fruit plants infected by root-knot nematodes in the field were found to be in decline, including stunting, wilting and yellowing of the stems (Figure 1A). Roots of infected plants had moderate to small galls (1–3 mm long) with protruding egg masses on the root surface (Figure 1B–D). The heavily infected roots turned dark, and the epidermis decayed, cracked and was easy to peel off, thereby a number of white females embedded in the vascular cylinder could be seen with the naked eye (Figure 1E,F). Histological observation of root sections revealed the presence of swollen nematodes and multiple nuclear giant cells (Figure 1G–I). These symptoms are typical of root-knot nematode infestation.

3.2. Frequency and Severity of Root-Knot Nematode Diseases

The results of the survey indicated that root-knot nematode disease on dragon fruit plants were present in all surveyed counties of Hainan (Table 2). Out of the 62 inspected orchards, 49 were found infested with root-knot nematodes. Variation in the frequency of the disease were found across the counties sampled. The highest frequency (100%) was found in Dongfang, Ledong and Changjiang, followed by Danzhou (80%), Qionghai (80%) and Sanya (75%) counties. Lingao, Chengmai and Wenchang counties had an equal frequency (60%) of the disease. The lowest frequency (40%) was found in Haikou (Table 2).
Figure 1. Symptoms induced by root-knot nematodes in red dragon fruit plants. (A) Infested dragon fruit plant; (B–D) Infected plant roots showing galls and egg masses; (E,F) Decayed root and pear-shaped females. (G–I) Root slices showing swollen nematodes (n) and giant cells (*). The scale bars indicate 100 μm.

Table 2. Frequency and severity of root-knot nematodes on red dragon fruit plants in different counties in Hainan.

<table>
<thead>
<tr>
<th>Location (County)</th>
<th>Total Number of Orchards Inspected</th>
<th>Number of Orchards Infected</th>
<th>Frequency (%)</th>
<th>Gall Index (Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ledong</td>
<td>9</td>
<td>9</td>
<td>100.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Dongfang</td>
<td>8</td>
<td>8</td>
<td>100.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sanya</td>
<td>8</td>
<td>6</td>
<td>75.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Changjiang</td>
<td>7</td>
<td>7</td>
<td>100.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Danzhou</td>
<td>5</td>
<td>4</td>
<td>80.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Qionghai</td>
<td>5</td>
<td>4</td>
<td>80.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Lingao</td>
<td>5</td>
<td>3</td>
<td>60.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Chengmai</td>
<td>5</td>
<td>3</td>
<td>60.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Wenchang</td>
<td>5</td>
<td>2</td>
<td>40.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Haikou</td>
<td>5</td>
<td>2</td>
<td>40.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The severity of the disease on dragon fruit plants was high in general, based on the results of the average gall index. The gall indices varied from 3.5 to 5.0 (Table 2), being highest in Dongfang (5.0) and Ledong (5.0), followed by Sanya (4.7), Changjiang (4.6) and Danzhou (4.5). The gall indices were the same (4.0) in Chengmai, Qionghai, Lingao and Wenchang counties. The lowest index (3.5) was noticed in Haikou, the county in which the frequency of occurrence was also the lowest.
3.3. Identification of Root-Knot Nematode Species

Two species of root-knot nematodes, *M. enterolobii* and *M. javanica*, were identified from the 49 infested samples using the perineal pattern morphology and molecular SCAR (Sequence-characterized amplified region)-PCR analysis (Figures 2 and 3). The perineal patterns of the mature females of *M. enterolobii* were characterized by the presence of a moderately high to high dorsal arch and mostly lacking lateral lines, but when present, the lines were not conspicuous (Figure 2A,B). *M. javanica* females were characterized by two lateral lines that separate very clearly the dorsal from the ventrally curved (Figure 2C,D).

![Figure 2](image-url) Perineal patterns of *M. enterolobii* (A,B) and *M. javanica* (C,D), obtained from females extracted from collected roots of dragon fruit plants (scale bar = 20 µm).

![Figure 3](image-url) PCR products obtained from genomic DNA of representative root-knot nematode populations isolated from the infested dragon fruit roots. (A) Amplification product (293 bp) with the specific primer pair MeF/MeR. M = 2 kb DNA marker; Lanes 1–7 = populations from galled roots collected in Ledong; Lane 8 = negative control. (B) Amplification product (517 bp) with the specific primer pair MjF/MjR. Lanes 1–5 = populations from galled dragon fruit roots collected in Ledong (1) and Qionghai (2–5); Lane 6 = negative control. (C) No amplification product with the specific primer pair MiF/MiR. Lanes 1–6 = populations from galled roots collected in Ledong; Lane 7 = negative control.

PCR amplification was positive for all the individual female samples analyzed by at least one set of the species–specific primers, Me-F/Me-R and Mj-F/Mj-R, used in this study that produced a 293-bp and 517-bp fragment for *M. enterolobii* and *M. javanica* (Figure 3A,B), respectively. In all cases, a single fragment was observed. No PCR products were obtained from the negative control lacking nematode DNA template. The PCR amplification was negative in all 49 samples analyzed with the specific primers for *M. incognita* (Figure 3C).
3.4. Distribution of Root-Knot Nematode Species

Out of the 49 root-knot-positive samples, *M. enterolobii* and *M. javanica* occurred in 46 and three samples, representing 93.9% and 6.1% of the total populations respectively (Table 3), indicating *M. enterolobii* was the dominant species on dragon fruit plants. *M. enterolobii* was also the most widely spread species associated with dragon fruit plants in Hainan, as this species was detected in all 10 surveyed counties (Table 3). Whereas, *M. javanica* was detected in only three counties, including Dongfang, Ledong and Qionghai (Table 3). *M. incognita* was not found to be infecting dragon fruits in this study.

Table 3. Distribution of root-knot nematode species on red dragon fruit plants in different counties in Hainan.

<table>
<thead>
<tr>
<th>Locations (County)</th>
<th>Samples Analyzed</th>
<th>M. enterolobii</th>
<th>M. javanica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Percentage (%)</td>
<td>Infected</td>
</tr>
<tr>
<td>Ledong</td>
<td>9</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>Dongfang</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>Sanya</td>
<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>Changjiang</td>
<td>7</td>
<td>7</td>
<td>100.0</td>
</tr>
<tr>
<td>Danzhou</td>
<td>4</td>
<td>4</td>
<td>100.0</td>
</tr>
<tr>
<td>Qionghai</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td>Lingao</td>
<td>3</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Chengmai</td>
<td>3</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Wenchang</td>
<td>3</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Haikou</td>
<td>2</td>
<td>2</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>46</td>
<td>93.9</td>
</tr>
</tbody>
</table>

4. Discussion

This survey, conducted in Hainan, China, to assess the occurrence of root-knot nematode disease on red dragon fruit plants *H. polyrhizus*, showed that root-knot nematode disease was quite frequent, as it was observed in all the counties included in this survey. The frequency of occurrence of *Meloidogyne* in general was high and, therefore, so was the intensity of this disease. It seems that red dragon fruit cultivation in the area is suffering infection with root-knot nematodes, as most of the orchards in this area exhibited root gall symptoms. Previous studies reported that root-knot nematodes infected white dragon fruit plants *H. undatus* and yellow dragon fruit plants *H. megalanthus* in Brazil [12,13]. These findings revealed dragon fruit crops were susceptible to root-knot nematode infection.

In the present study, the root-knot nematode species affecting red dragon fruit plants were unequivocally identified using the perineal pattern morphology and molecular SCAR marker methods. Perineal patterns of *M. enterolobii* were oval rounded, with a moderately high to high dorsal arch and lacking obvious lateral lines, which were useful to discriminate between *Meloidogyne* species [28]. Perineal patterns showed two distinct lateral lines, which is typical of *M. javanica*, and usually sets it apart from *M. incognita* and *M. arenaria* [23]. In addition to the morphology, species-specific primers for PCR have been developed to complement the traditional identification of *M. enterolobii* [26] and *M. javanica*. [27].

When using the species-specific primer developed for *M. enterolobii* and *M. javanica*, we obtained the characteristic bands of 293 bp and 517 bp, respectively, confirming their morphological identification results. The same primer set was successfully used in other research [17,29,30].

Our findings revealed that *M. enterolobii* is the overwhelming predominant species on red dragon fruit plants. It was detected in all surveyed counties and accounted for 93.9% of the total populations, while *M. javanica* was only responsible for 6.1%. No mixed infections were found in this study. During the last 20 years, *M. enterolobii* has become one of the most threatening pathogenic nematodes in both tropical and subtropical regions of the world, due to its wide host range, high aggressiveness, increasing geographical distribution, and ability to develop and reproduce on several crops carrying resistance genes toward
tropical root-knot nematodes [31,32]. In Hainan, M. enterolobii has been reported to be the dominant root-knot nematode species in vegetables, followed by M. incognita and M. javanica [18,20], and it also infects several fruit crops such as guava [33], jujube [34], jackfruit [35] and mulberry [36]. The predominance of M. enterolobii might be due to conducive environmental conditions and host suitability favoring M. enterolobii over the other root-knot nematode species.

This study, to the best of our knowledge, is the first report regarding the occurrence of root-knot nematode on dragon fruit crops in China. This is also the first report of M. enterolobii and M. javanica infections in red dragon fruit plants H. polyrhizus. As a perennial crop, dragon fruit stays in the field for many years, subjected to root-knot nematode infection from the seedling stage throughout the economic life of the plantation. Knowledge generated in this study regarding the frequency, identification and distribution of root-knot nematodes in Hainan aids in predicting the potential spread in the future and provides a better understanding of the epidemiology of these important nematodes. In addition, this finding is a valuable resource for developing effective control and management strategies for root-knot nematode on dragon fruit plants in Hainan.

5. Conclusions

From 3-year surveys in dragon fruit orchards in Hainan, we found the dragon fruits were widely infected by root-knot nematodes, which resulted in stem yellowing, wilting or stunting. By morphological and molecular analysis, we also determined that the predominately infected species in Hainan were M. enterolobii, whereas M. javanica was only identified in three counties. As mentioned above, this study will lead dragon fruit growers to pay more attention to root-knot nematode diseases and adopt appropriate controlling strategies.

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Conflicts of Interest: The authors declare no conflict of interest.

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