DRAGON FRUIT (PITAYA) DISEASES IN THAILAND: INCIDENCE AND MANAGEMENT STRATEGIES

Pornpimon Athipunyakom, Suneerat Seemadua, and Chanintorn Doungsa-ard
Plant Protection Research and Development Office, Department of Agriculture,
Bangkok 10900, Thailand
E-mail: pathipunyakom@gmail.com

ABSTRACT

An important aspect of dragon fruit production is the outbreak of diseases, especially in the tropical and subtropical regions. This study was conducted to investigate the distribution and identification of dragon fruit diseases in various locations in Thailand. Diseased plants were collected during October 2012 to September 2014 to observe the prevalence of dragon fruit diseases in Chanthaburi, Rayong, Ratchaburi and Nakhon Ratchasima. Through the survey, three diseases (anthracnose, fruit rot and stem canker) with their respective incidences were identified and recorded. The highest disease incidence in anthracnose on stem, anthracnose on fruit, fruit rot and stem canker were recorded 23.4%, 22.5%, 13.5% and 58.2%, respectively. Identification was based on morphology and molecular characteristics and pathogenicity test. The different symptoms of diseases were isolated on potato dextrose agar (PDA) and the causal agents were identified as Bipolaris cactivora which causes fruit rot, Colletotrichum gloeosporioides and C. truncatum causes anthracnose on stem and fruit as well as Neoscytalidium dimidiatum causes stem canker. The stem canker disease, which is considered a new disease, was found in dragon fruit plantations. This is the first report of dragon fruit diseases caused by C. truncatum and N. dimidiatum in Thailand.

Keywords: dragon fruit disease, pest incidence, plant management

INTRODUCTION

Dragon fruit (Hylocereus undatus) is known by many names: pitaya, pitahaya and strawberry pear. This plant is native to South and Central America. Dragon fruit is now cultivated in the tropical and sub-tropical regions around the world. Currently most dragon fruits are commercially grown in Vietnam, Thailand, and south China. In 1981, Dr. Surapong Kosiyajinda, a fruit and vegetable scientist, conducted a study on dragon fruit and suggested that Thai people be acquainted with this fruit that is widely cultivated in Thailand particularly in the provinces of Chantaburi, Chumphon, Chiang Mai, Chiang Rai, Nakhon Pathom, Nakhon Ratchasima, Pathum Thani, Ratchaburi, Rayong, Samut Sakhon, Samut Songkhram. However, an important problem in producing dragon fruit in Thailand are diseases. They are often the most important hindrance in the production of dragon fruit. In Thailand, a few fungal diseases have been reported to infect the stems and fruit of dragon fruits such as anthracnose which has been reported to infect the stems and fruit caused by Colletotrichum gloeosporioides and C. truncatum (Athipunyakom and Likhitekaraj 2010; Athipunyakom et al. 2012), fruit rot caused by Bipolaris cactivora (Athipunyakom et al. 2009). Anthracnose disease is one of the important diseases in dragon fruit production. The fungi can damage the stems, flowers and fruits of dragon fruit in various locations in Thailand such as Kanchanaburi, Bangkok, Chumphon, Chiang Mai, Chiang Rai,
Chanthaburi, Nakhon Pathom, Pathum Thani, Ratchaburi, Rayong, Samut Sakhon, Samut Songkhram provinces. The pathogens were identified as *Colletotrichum gloeosporioides* and *C. truncatum*. Fruit rot of dragon fruit caused by *Bipolaris cactivora* was found in Pathum Thani, Samut Sakhon, Samut Prakarn, Ratchaburi, Nakorn Pathom, Ratchaburi, Rayong, Chanthaburi, Chiang Mai and Chiang Rai. The symptom included depressed water-soaks lesions with olive to black powdery spot coalescing into soft rot (Athipunyakom et al. 2009).

The aim of this research was to determine the occurrence and distribution of dragon fruit diseases in Thailand and to identify the pathogens using morphological and molecular characteristics.

**MATERIALS AND METHODS**

**Field surveys**
The survey was conducted in the dragon fruit plantations of Chanthaburi, Rayong, Ratchaburi and Nakhon Ratchasima during October 2012 to September 2014. The incidences of different diseases were recorded. Evaluations were conducted on 20 orchards in each province. Data were expressed as percentage.

**Isolation and identification of the pathogens**
Between October 2012 and September 2014, samples were collected from dragon fruit plantations showing symptoms of anthracnose, fruit rot and stem canker in Chanthaburi, Rayong, Ratchaburi and Nakhon Ratchasima. The specimens were aseptically excised from the edges of infected tissue, dissected into 2x2 mm 50 pieces per disease, immersed in 1% sodium hypochlorite for 1 minute, rinsed in sterile water three times and air-dried on a clean bench. Surface-sterilized lesion tissues were then placed on Potato Dextrose Agar (PDA). The plates were incubated at room temperature (28-30°C) and examined daily. Mycelia that grew from the tissue were transferred to PDA plates for further growth and sporulation (Tuite 1972). Identification was based on morphological characteristics as observed under a light microscope. Pure cultures were maintained on PDA slant and liquid paraffin at the Culture Collection, Department of Agriculture, Thailand.

**Molecular identification**

**Culture selection**
Fungal cultures were grown on PDA for 4-16 weeks at 25°C. Actively growing mycelium was scraped off the surface of a culture and transferred to 2 ml of microcentrifuge tubes and the biomass lyophilized at -80°C for 24 hours.

**DNA extraction**
Extraction buffer (1% CTAB, 0.7 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, pH8) was added to fungal samples. The samples were ground in a 2 ml microcentrifugetube and the volume adjusted by adding 700 μl extraction buffer and mixed by inverting the tubes and incubated at 65°C for 1 hour. Samples were centrifuged at 12,000×g for 10 min at 25°C. The aqueous supernatant was transferred into a new microcentrifuge tube with phenol-chloroform-isoamyl alcohol by mixing gently and by centrifugation at 12,000×g for 10 min at 25°C. The upper liquid phase was transferred to a new microcentrifuge tube containing 7.5 M of ammonium acetate. The DNA was precipitated by ethanol (−20°C overnight) by centrifugation at 12,000×g for 10 min at 15°C. The DNA-pellet was
washed with ice-cold 70% ethanol and dried at 25°C. The pellet was redissolved in 50 μl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA pH 8.0).

**PCR amplification**
Primers used for PCR amplification and for sequencing of the internal transcribed spacer region (ITS) were ITS4 and ITS5 (White et al. 1990; Bunyard et al. 1994; Landvik 1996). Amplification was performed in a 50 μl reaction mix: 10 mM of each dNTP (1 μl), 10 μM of each primer (1 μl), 10% of dilution buffer (5 μl), 25 mM of Mg (5 μl), 4 M of enhancer (5 μl) and 60-62% of sterile distilled water (30.8 μl), 0.2 μl of Taq DNA polymerase kit from FERMENTAS and 10-50 ng of genomic DNA template (1 μl) carried out using a PCR Model MJ Research DYAD ALD in 200 μl reaction tubes. (95°C, 0.5 min; 52°C, 1 min; 72°C, 1.5 min; 35 cycles). PCR products (7 μl aliquots) were checked by electrophoresis in 1% agarose gels with 0.003% ethidium bromide in 0.5×TBE buffer (0.044 M boric acid, 1.1 mM EDTA, 0.045 M Tris, pH 8) for purity.

**DNA purification and sequencing**
PCR products were purified using NucleoSpin® Extract Kit (Macherey-Nagel, Germany). The PCR products were sequenced by Macrogen Inc. in Korea with the same primers as those in the PCR amplification.

**Phylogenetic analyses**
Each sequence was checked for ambiguous bases. They were refined visually and assembled using BioEdit 7.0.9.1 (Hall 1999). The consensus sequences for each DNA region were multiple aligned by Clustal W 1.6 (Thompson et al. 1994) and checked manually with all sequences derived from the GenBank database and the accession numbers that are included in the phylogenetic trees. The alignment included the most similar sequence identified through BLAST search.

**Pathogenicity test**
The pathogenicity of each isolates was tested using Koch’s postulate. A 9 mm-diameter young mycelia PDA agar disc was used as inoculum placed on the surface of asymptomatic fruits and stems, either unwound and wound with a sterile needle. The inoculate stems and fruits were kept at room temperature (28-30°C) in dark.

**RESULTS AND DISCUSSION**

**Field surveys**
Survey was carried out in October 2012–September 2014 in the dragon fruit plantations of Chanthaburi, Rayong, Ratchaburi, Nakhon Ratchasima. Three diseases were found in dragon fruit orchard in Thailand such as anthracnose on stem and fruit (Figure 1A, B), fruit rot (Figure 1C) and stem canker (Figure 1D). The highest disease incidence in anthracnose on stem, anthracnose on fruit, fruit rot, and stem canker were recorded 23.4%, 22.5%, 13.5% and 58.2%, respectively (Table 1). The highest anthracnose disease incidence on stem (23.4%) was recorded in Chanthaburi province (Table 1). The highest anthracnose disease incidence on fruit (22.5%) was recorded in Rayong province (Table 1). The highest fruit rot disease incidence (13.5%) was recorded in Rayong province (Table 1). The highest stem canker disease incidence (58.2%) was recorded in Chanthaburi province (Table 1).
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Figure 1. Diseases of dragon fruit in Thailand: A) Anthracnose on stem; B) Anthracnose on fruits; C) Fruit rot; D) Stem canker

Table 1. Disease incidence of dragon fruit plantation surveyed in various locations.

<table>
<thead>
<tr>
<th>Locations</th>
<th>Anthracnose (C. gloeosporioides)</th>
<th>Anthracnose (C. truncatum)</th>
<th>Fruit rot (B. cactivora)</th>
<th>Stem canker (N. dimidiatum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stem</td>
<td>fruit</td>
<td>stem</td>
<td>fruit</td>
</tr>
<tr>
<td>Chanthaburi</td>
<td>23.4</td>
<td>17.1</td>
<td>8.7</td>
<td>20.4</td>
</tr>
<tr>
<td>Rayong</td>
<td>15.0</td>
<td>17.7</td>
<td>8.9</td>
<td>22.5</td>
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<tr>
<td>Ratchaburi</td>
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<td>9.6</td>
<td>4.5</td>
<td>16.8</td>
</tr>
<tr>
<td>Nakhon Ratchasima</td>
<td>9.2</td>
<td>7.6</td>
<td>4.2</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Identification of the pathogens and pathogenicity
Three diseases were found in dragon fruit orchard in the provinces of Chanthaburi, Rayong, Ratchaburi and Nakhon Ratchasima. These were anthracnose, fruit rot and stem canker.
**Anthracnose**

The different symptoms of anthracnose diseases were isolated on PDA and the causal agents were identified as *Colletotrichum gloeosporioides* and *C. truncatum*. The first symptoms of infected stems and fruits showed reddish brown lesions with yellow haloes. The center of a lesion showed brown and coalesced into the highest of anthracnose disease incidence on stem. It was also found that *C. gloeosporioides* and *C. truncatum* were detected in 23.4% and 8.9% at Chanthaburi and Rayong, respectively, whereas *C. truncatum* and *C. gloeosporioides* were detected in 22.5% and 9.6% of disease incidences on fruits at Rayong and Ratchaburi, respectively (Table 1). In this survey, we found the anthracnose disease on stem caused by *C. gloeosporioides* more than *C. truncatum* while the anthracnose disease on fruit caused by *C. truncatum* more than *C. gloeosporioides*.

The tissue transplanting method was used to isolate plant pathogenic fungi from infected stems (Figure 2A and 2B) and fruits (Figure 3A, B) on PDA and incubated at room temperature. Colonies on PDA at first white, became gray with concentric rings of salmon-colored spore mass. Identification was based on morphological characteristics. The pathogen was identified as *C. gloeosporioides* (Figure 2C, D). The second symptoms on stems and fruits are sunken circular lesions. The center of the lesions became tan in color and were dotted with many dark fruiting bodies of the fungus. Colonies grown on PDA changed from grayish to dark grey with an average colony diameter of 80.5 mm after 7 days, conidia mass honey. Conidia were falcate and 18.2 to 20.8 × 2.55 to 3.28 μm (Figure 3D). Sclerotia and setae are abundant (Figure 3D). Identification was based on morphological and cultural characteristics, the causal agent was identified as *C. truncatum* (Figure 3C, D).

Figure 2. Anthracnose disease on stem caused by *Colletotrichum gloeosporioides*: A, B) Symptom on stem; C) conidia on conidiophores; D) conidid with oil-droplet-like body
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Figure 3. Anthracnose disease on fruit caused by *Colletotrichum truncatum*: A,B) Symptoms on fruits; C) Acervulus on fruit; D) Acervuli with setae and falcate conidia.

Morphological characters of this isolate, which was isolated from the symptom on dragon fruit, agreed with the original description of *C. truncatum* in that conidia were falcate, sclerotia and setae are abundant. In addition, the molecular data of this isolate based on the internal transcribed spacer (ITS) region was 100% identical to the ITS sequence of an epitype (Damm et al. 2009). Therefore, the fungus was identified as *C. truncatum*.

Pathogenicity tests were conducted on stems and fruits inoculated with mycelium and conidia of *C. gloeosporioides* and *C. truncatum* was used as inoculum. The fungus was reisolated with symptomatic stem and fruit tissues. The pathogenicity test showed that *C. gloeosporioides* and *C. truncatum* were the cause of anthracnose on dragon fruits.

In this study, the isolates of *C. truncatum* which isolated from dragon fruit were previously identified as *C. capsici*. These isolates are morphologically similar to *C. capsici* from anthracnose of chilli (Than et al. 2008). Molecular diagnostics are recommended to confirm the identity of these isolates which was identified as *C. truncatum*. It is similar to anthracnose of dragon fruit disease caused by *C. truncatum* in China (Guo et al. 2014) and in Malaysia (Vijaya et al. 2015).

**Fruit rot**

The symptom included depressed water-soaks lesion with olive to black powdery spot coalescing into soft rot on fruit (Figure 4A) and flower. In addition, the pathogens also destroyed flowers (Figure 4B). The pathogens form black colonies, and hair on fruit (Figure 4C). Conidiophores were pale to light brown, caespitose (Figure 4D), straight or flexuous, and often swollen at the apex and at the base. Conidia with a basal hilum were straight, ellipsoidal, fusiform or obclavate, 2-4 pseudosepta, plae light brown to golden brown and were 20 - 54 x 6 -11 (ave. 34.75 x 7.28) µm (Figure 4 E, F). The causal fungus was identified as *Bipolaris cactivora* (Petrak) Alcorn. Pathogenicity tests were conducted on fruits inoculated with mycelium and conidia was used as inoculum.
The fungus was reisolated with symptomatic fruit tissues. It is similar to fruit rot of strawberry pear (pitaya) caused by *B. cactivora* in Okinawa Prefecture, Japan (Taba et al. 2007) and in Florida (Tarnowski et al. 2010). Tarnowski et al. (2010) indicated that *B. cactivora* causes flower and fruit rot on pitahaya, but does not seriously affect mature plant stems. The flower rot does not appear to significantly increase the incidence but may provide inoculum for the fruit rot. The high incidence of fruit rot affecting commercial operations in Miami-Dade County over the past two years requires an effective disease management strategy. Athipunyakom et al. (2009) reported that fruit rot of dragon fruit (*Hylocreus undatus* Haw.) caused by *B. cactivora* was found at Pathum Thani, Samut Sakhon, Samut Prakarn, Ratchaburi, Nakorn Pathom, Ratchaburi, Rayong, Chanthaburi, Chiang Mai and Chiang Rai.

![Figure 4. Flower and fruit rot of Dragon fruit caused by *Bipolaris cactivora*: A) Early brown spot lesion on fruit; B) Flower rot; C) Black colonies, hairy on fruit; D) Cespitose conidiophores and irregularly at the apex; E, F) Conidia.](image-url)
Stem Canker
In 2012, a new dragon fruit disease called stem canker, was found in Chanthaburi, Rayong, Ratchaburi and Nakhon Ratchasima. It is characterized by many small, circular, reddish brown spot on the diseased stems (Figure 5A). The spots continuously expanded, and formed large areas of canker on stems (Figure 5B). Some lesions developed near the ribs of stem (Figure 5C, D). Yellowing of tissues was followed by softening and the rotten stench of tissues. Black pycnidia fruited on the stem which was subsequently rotten (Figure 5E, F and 6A, B). There was also the fungus conidia aseptate, hyaline substance, thick-walled, smooth, subcylindrical to oblong-elliptical, sometimes slightly curved, with rounded ends. There was hyaline after discharge from pycnidia (Figure 6 C, D).

Figure 5. Stem canker symptom caused by Neoscytalidium dimidiatum: A) Early brown spot lesion on stem; B) Sunken and brown lesion symptoms; C, D) Early infection from the rib; E, F) Black pycnidia on surface fruit.
For identification, cultures were grown on PDA at room temperature. After three days, colonies were seen with dark grey to black aerial mycelium form. The colonies produced abundant conidia that occurred in arthric chains in aerial mycelium. Mycelium were branched, septate, hyline to brown which constricted in to spore chains and disarticulated into arthroconidia. The arthroconidia were cylindrical truncate, orbicular to doliform, dark brown, 0-1 septate. The teleomorph was never observed in PDA culture. It is similar to stem canker of dragon fruit disease caused by *Neoscytalidium dimidiatum* in Taiwan (Chuang et al. 2012) and China (Yi et al. 2013; 2015).

Morphological characters of this isolate, which was isolated from the symptom on dragon fruit, agreed with the original description of *Neoscytalidium dimidiatum* in that the arthroconidia were cylindrical truncate, orbicular to doliform, dark brown. The DNA sequence containing 679 characters of the internal transcribed spacer (ITS) region was 100 % identical to *N. dimidiatum*, using BLASTN 2.2.32 (Zheng et al. 2000). The ITS sequence of this isolate also had similarity to the type sequence of *N. novaehollandiae* (NR 111260), but was only at 99 % of identity. Based on morphological characters as well as the similarity of the ITS sequence, the fungus was identified as *N. dimidiatum*.

In this study, the isolates of *N. dimidiatum* which were isolated from dragon fruit were previously identified as *Dothiorella* sp. These isolates are morphologically similar to
Botryospheria dothidea from stem spot on Hylocereus undatus in Mexico (Valencia-Biotin, et al. 2003). Molecular diagnostics are recommended to confirm the identity of these isolates, which were identified as *N. dimidiatum*. It is similar to stem canker of dragon fruit disease caused by *Neoscytalidium dimidiatum* in Taiwan (Chuang et al. 2012) and China (Yi et al. 2013; 2015).

*Neoscytalidium dimidiatum* has a wide geographical and host range such as *Albizia lebbeck*, *Delonix regia*, *Ficus carica*, *Ficus* spp., *Peltophorum pterocarpum* and *Thespesia populena* in Oman; on *Arbutus*, *Castanea*, *Citrus*, *Ficus*, *Juglans*, *Musa*, *Populus*, *Prunus*, *Rhus*, *Sequoiaadendron* in the USA; and on *Mangifera indica* in Niger (Ray et al. 2010). Stress factors such as water stress enhance the severity of disease caused by this fungus and symptoms include branch wilt dieback, canker, gummosis and tree death (Punithalingam and Waterson 1970). This pathogen can cause dragon fruit canker and spot on the stem or fruit ((Lan et al. 2012; Chuang et al. 2012; Yi et al. 2013; Mohd et al. 2013), internal black rot in fruit (Ezra et al. 2013), stem canker (Ni et al. 2013) fruit internal brown rot in Guangdong Province, China (Yi et al. 2015). This is the first reported causing stem canker on dragon fruit in Thailand.

**Management strategies**

Dragon fruit diseases can have significant constraints in production, especially when they occur in environments with high rainfall and uniform, warm temperatures. The successful management of plant disease utilizes several principles and practices, regardless of the host and environment in which it is grown. These include the avoidance, exclusion and eradication of the causal agents. The removal of infested debris and host materials is another common eradication strategy but it is the most important to control the diseases especially in dragon fruit production in Thailand.

**REFERENCES**


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