Detection of *Nigrospora sphaerica* in the Philippines and the susceptibility of three *Hylocereus* species to reddish-brown spot disease

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ABSTRACT

Diseases are among the major problems that negatively affect dragon fruit profitability worldwide. Diseases of dragon fruit in the Philippines are yet to be identified and reported. This study elucidates the causal agent of a disease infecting stems of dragon fruit grown in Los Baños, Laguna, Philippines. The fungus was isolated and identified as *Nigrospora* sp. based on morphological and cultural characteristics in potato dextrose agar medium. Using the DNA sequence of the internal transcribed spacer (ITS) gene region, isolate MBDF0016b was identified as *Nigrospora sphaerica*. The Philippines strain was closely related to the Malaysian strain, which also causes reddish-brown spot in dragon fruit (*H. polyrhizus*), and to other *N. sphaerica* isolates from other host-plant species. *Nigrospora sphaerica* MBDF0016b was pathogenic to *H. megalanthus, H. undatus,* and *H. polyrhizus* in detached stem and glasshouse assays. The same fungus was re-isolated from the inoculated stems and thus, establishing Koch's postulate. This paper is the first confirmed scientific record of a dragon fruit disease in the Philippines and the first report of *N. sphaerica* as a dragon fruit pathogen causing reddish-brown spot disease in *H. megalanthus*.

Keywords: Dragon fruit; ITS gene; *H. megalanthus; H. polyrhizus; H. undatus.*

INTRODUCTION

Plants in the genus *Hylocereus* are unique-looking, vine-climbing plants that bear edible fruits. Dragon fruit, as it is known commercially, belongs to the Cactaceae family that has been consumed by humans for over 9,000 years (Nobel, 2002). Today, dragon fruit is a high-value crop that contributes to the global import/export market (Tel Zur, 2015). The three commonly cultivated species are *H. undatus* (pink-skinned, white-fleshed), *H. polyrhizus* (red-skinned, red-fleshed), and *H. megalanthus* (yellow-skinned, white-fleshed) (Ortiz-Hernandez and Carrillo-Salazar, 2012). The fruits are nutritious (Wichienchot *et al.,* 2010; Tel Zur, 2015) and have medicinal qualities (Le Bellec *et al.*, 2006; Tenore *et al.,* 2012). The fruits are also rich in betalain, which is used as a food colorant (Henry, 1996; Ortiz-Hernandez, 1999; Wynbraniec

et al., 2007), and are sources of income to growers (Balendres and Bengoa, 2019). It was first commercially grown in Vietnam but is now widely grown in Israel (Nerd *et al.,* 2002; Tel Zur, 2015) and in other Southeast Asian countries, with high demands from the food industry in the USA and Europe (Nerd *et al.,* 2002; Balendres and Bengoa, 2019). Production of dragon fruits has increased since the 1970s due to the expansion of the area (Tel Zur, 2015). Dragon fruit production, however, is not immune to pre- and post-harvest issues like short storage life, the non-stable supply of fruits (Mizrahi and Nerd, 1999; Nerd *et al.*, 2002), and the occurrence of destructive diseases (Balendres and Bengoa, 2019).

Twenty-six pathogens cause various diseases in dragon fruit (Balendres and Bengoa, 2019). The most destructive are anthracnose (Masyahit *et al.,* 2013; Meetum *et al.,* 2015), stem canker (Chuang *et al.,* 2012), and viral disease (Guo *et al.,* 2014). Diseases occur in many dragon fruit growing areas, particularly in the Southeast Asian region (Thailand, Malaysia, Vietnam, Taiwan). All three cultivated species are susceptible to many of these diseases (Balendres and Bengoa, 2019). Of note, however, among the dragon fruit growing regions in Southeast Asia, there are no scientific reports of dragon fruit diseases in the Philippines. There are anecdotal claims of stem canker and anthracnose, but they are yet to be confirmed. The lack of information on dragon fruit disease in the country may result in significant yield loss when an outbreak of disease occurs since appropriate disease control measures are yet to be elucidated. In the Philippines, the dragon fruit industry is growing, and dragon fruit has become a highly valued cash crop (Eusebio and Alaban, 2018; Rodeo *et al.,* 2018). Dragon fruit is now planted in 450 hectares (2017), more than doubled the production area in 2012 (182 ha) (Philippine Statistics Authority, 2018). Production volume in 2017 reached 1,463 metric tons, grown mainly from in the Ilocos, Cagayan Valley, Calabarzon, Central Luzon, and Central Visayas regions (Eusebio and Alaban, 2018; PSA, 2018). Dragon fruit is now also grown in Davao, Bukidnon, and South Cotabato (Rodeo *et al.,* 2018).

In July 2019, a disease was observed in several dragon fruit plants grown near the experimental station of the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), Laguna, Philippines. Diseases are a major concern in dragon fruit production. Since dragon fruit is becoming a high-value crop, attention must be given to these diseases that could negatively affect the yield of the plant and reduce the income of the growers. Knowledge of the etiology of plant disease is the first and critical step in the development, selection, and deployment of effective disease control measures. Thus, this study was aimed at identifying the causal agent of the disease in dragon fruit by using a combined morphological, cultural, pathogenicity, and molecular techniques, and the pathogenicity of the causal agent to three cultivated *Hylocereus* species.

MATERIALS AND METHODS

Sample collection and fungal isolation

Freshly cut stems of dragon fruit showing reddish-brown spots were collected near the IPB, CAFS, UPLB experimental station in Los Baños, Laguna, Philippines (14.1699° N, 121.2441° E, 22 meters above sea level). Diseased-samples were brought to the Plant Pathology

Laboratory of IPB. Healthy and infected portions of the plant tissues were cut into small blocks (3 mm^2). Blocks were then surface sterilized using a 10% sodium hypochlorite (NaOCl) solution (v/v. Zonrox, Philippines) for 1 min and rinsed in sterile distilled water. Plant tissues were then air-dried in sterile tissue paper inside a laminar flow hood and then transferred onto potato dextrose agar (PDA) medium. Plates were stored at room temperature (28-30 °C) for three days (with 14 hours light in 24 hours cycle). Fungus growing in the PDA medium was then purified and further characterized.

Morphological and cultural characterization

Five-mm of the fungal mycelial plug, from a seven-day-old culture, was transferred to a new PDA medium and incubated at the same incubation condition mentioned above. After seven days, mycelial form and color were recorded. Radial growth was measured 3 days after because the fungus was fast-growing and has already reached the edge of the Petri plate on the seventh day. Conidia length was measured from 30 randomly selected conidia of the seven-day-old culture (three replicate plates), under a microscope (Olympus CX22, Japan). Images were analyzed using the ImageJ software (Version 1.51s, Wayne Rasband, National Institutes of Health, USA).

PCR assay

Fungal genomic DNA of isolate MBDF0016b was extracted using a cetyltrimethylammonium bromide (CTAB) extraction procedure (Culling, 1992; Doyle and Doyle, 1987). The fungal genomic DNA was used as a template for the succeeding polymerase chain reaction (PCR) assay to amplify the partial sequence of the internal transcribed spacer (ITS) gene region. The PCR assay was performed in MyCycler™ Thermal Cycler (Bio-Rad, USA) in a 15-µL reaction volume. The reaction mix contained 1x PCR Buffer (Invitrogen), 2.0 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTPs (Invitrogen), 0.2 µM each of the forward (ITS4, 5'-TCCTCCGCTTATTGATATGC-3') and reverse (ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') primers (White *et al.,* 1990), one U *Taq* DNA Polymerase (Invitrogen), 2 µL of the fungal genomic DNA, and DEPC-water to volume. The thermal cycling conditions were as follows; initial denaturation at 94 °C for five min, followed by 24 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for one min and a final extension at 72 °C for seven min. The PCR product was resolved by gel electrophoresis [1.5% Agarose (Vivantis) 0.5X Tris-Acetate-EDTA buffer containing 2 µL GelRed solution (Biotium) (PowerPac™ and Sub-Cell GT, (Bio-Rad Laboratories)]. The PCR product was sent to Apical Scientific Sdn. Bhd. (Malaysia) for DNA sequencing.

Molecular characterization and phylogenetic analysis

A consensus DNA sequence was derived from the forward and reverse sequences using the sequence editing software Geneious. An analysis using the BLASTN program (Zhang and Madden, 1997) was performed using the consensus sequence to determine the closest fungal genera of the isolate based on the highest percent similarity, e-value, and highest query cover. Afterward, the verified authentic DNA sequences of the partial sequence of the ITS gene region of 16 *Nigrospora* species (Table 1), according to Wang *et al.* (2017), were used for comparison with the sequence of the MBDF0016b isolate. After identifying the closest *Nigrospora* species,

the DNA sequence of isolate MBDF0016b was further compared with the DNA sequences of other 10 *N. sphaerica* isolates (Table 2) available in the GenBank. In all analysis, *Arthrinium malaysianum* (CBS 102053) was used as the outgroup (Grous and Groenewald, 2013). Sequences were aligned using CLUSTALW, and the phylogenetic trees were inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The heuristic search was obtained automatically by applying the Maximum Parsimony method, and branch support of the trees was assessed by bootstrapping with 1000 replicates (Felsenstein, 1985). Analyses were conducted in MEGA7 software (Kumar *et al.,* 2016).

Pathogenicity test

A detached stem assay was performed to establish pathogenicity and Koch postulates. Tests were performed in healthy 20-cm long stems of *H. undatus, H. polyrhizus,* and *H. megalanthus*, which were obtained from the IPB Field Germplasm. Fifteen (15) µL of the spore suspension (10^6 spores mL^{-1}) of a seven-day-old fungal culture was inoculated in wounded (pin-pricked) and unwounded sites of the surfaced-sterilized (by NaOCl) stems. Three replicate stems were inoculated. Each stem had three inoculated sites. Thus, a total of nine technical replicates. Plants inoculated with water served as a negative control check. Stems were placed in covered containers overlaid with a wet tissue to promote high humidity, and containers were kept at room temperature. Symptom development was recorded at seven and 14-days post-inoculation (dpi).

In vivo pathogenicity test was also performed in the glasshouse on three-week-old rooted cuttings of *H. undatus* and *H. polyrhizus* plants grown in potting soil. A 20-µL of the spore suspension of MBDF0016b was inoculated in wounded sites. To avoid the spore suspension from evaporating, the inoculated area was covered with transparent tape (approximately 2x2 cm). The effectivity of this method was previously tested by comparing the symptoms of the plants inoculated with spore suspension with and without transparent plastic tape placed on the wounded, inoculated site. Symptom development was assessed ten dpi. There were three replicate plants, and each plant had three inoculation sites. Plants inoculated with water served as a negative control check. At seven and ten dpi, respectively, in the detached stem and *in vivo* pathogenicity tests, re-isolation of the fungus from the infected sites were performed to establish Koch's postulate.

Table 1. *Nigrosopora* species and their corresponding isolates, host, and ITS gene sequences that were used in this study.

Species	Isolate	Host	Locality	ITS GenBank Accession	Reference
N. sphaerica	LC2840	*Harpullia longipetala*	China	KX985965	Wang *et al.* (2017)
N. aurantiaca	CGMCC 3.18130*=LC 7302	*Nelumbo sp.* (leaf)	China	KX986064	Wang *et al.* (2017)
N. bambusae	CGMCC 3.18327*=LC 7114	*Bamboo* (leaf)	China	KY385307	Wang *et al.* (2017)
N. camelliae-sinensis	LC 3287	*Camellia sinensis*	China	KX985975	Wang *et al.* (2017)
N. chinensis	CGMCC 3.18127*=LC 4575	*Machilus breviflora*	China	KX986023	Wang *et al.* (2017)
N. gorlenkoana	CBS 480.73*	*Vitis vinifera*	Kazakhstan	KX986048	Wang *et al.* (2017)
N. guilinensis	CGMCC 3.18124*=LC 3481	*Camellia sinensis*	China	KX985983	Wang *et al.* (2017)
N. hainanensis	CGMCC 3.18129*=LC 7030	*Musa paradisiaca* (leaf)	China	KX986091	Wang *et al.* (2017)
N. lacticolonia	CGMCC 3.18123*=LC 3324	*Camellia sinensis*	China	KX985978	Wang *et al.* (2017)
N. musae	CBS 319.34*	*Musa paradisiaca* (fruit)	Australia	KX986076	Wang *et al.* (2017)
N. oryzae	LC 6759	*Oryza sativa*	China	KX986054	Wang *et al.* (2017)
N. osmanthi	CGMCC 3.18126*=LC 4350	*Osmanthus sp.*	China	KX986010	Wang *et al.* (2017)
N. pyriformis	CGMCC 3.18122*=LC 2045	*Citrus sinensis*	China	KX985940	Wang *et al.* (2017)
N. rubi	CGMCC 3.18326*=LC 2698	*Rubus sp.*	China	KX985948	Wang *et al.* (2017)
N. vesicularis	CGMCC 3.18128*=LC 7010	*Musa paradisiaca* (leaf)	China	KX986088	Wang *et al.* (2017)
N. zimmermanii	CBS 290.62*	*Saccharum officinarum* (leaf)	Ecuador	KY385309	Wang *et al.* (2017)
Arthrinium malaysianum	CBS 102053	*Macaranga hullettii*	Malaysia	NR_120273.1	Crous and Groenewald (2013)

CGMCC= China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; CBS= Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI= Culture Collection of CABI Europe UK Centre, Egham, UK; LC= working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. *= ex-type culture. ITS= internal transcribed spacers.

Table 2. *Nigrosopora sphaerica* and their corresponding isolates, host, and ITS gene sequences were used in this study.

Isolate	Host	Locality	ITS GenBank Accession	Reference	Disease
LC7312	*Nelumbo sp.* (leaf)	China	KX985935	Wang *et al.* (2017)	
LC 2705	*Rosa sp.*	China	KX985952	Wang *et al.* (2017)	
LC3477	*Camellia sinensis*	China	KX985982	Wang *et al.* (2017)	
LC2840	*Harpullia longipetala*	China	KX985965	Wang *et al.* (2017)	
LC 2958	*Cleyera japonica*	China	KX985966	Wang *et al.* (2017)	
LC 4174	*Rhododendron arboreum*	China	KX985989	Wang *et al.* (2017)	
JD-TP-5	*C. sinensis*	India	KJ767520	Dutta *et al.* (2015)	*Nigrospora* Leaf Blight
QY-6	*C. sinensis*	China	KP731976	Liu *et al.* (2015)	*Nigrospora* Leaf Blight
BJ28	*Hylocereus undatus*	China	KU196741	Liu *et al.* (2016)	Reddish-brown Spot Disease
PC KS4A1 C R2	*H. polyrhizus*	Malaysia	MK408581	Kee *et al.* (2019)	Reddish-brown Spot Disease

CGMCC= China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; CBS= Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI= Culture Collection of CABI Europe UK Centre, Egham, UK; LC= working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. *= ex-type culture. ITS= internal transcribed spacers.

RESULTS

Disease incidence

Small to large reddish-brown spots were observed in dragon fruit stems (Fig. 1a) collected from the IPB Experimental Station. Severe spots that have coalesced have also been observed. Initial symptom assessment indicates that a fungus caused the disease.

Morphological and cultural characteristics

Although several fungi grew from the different tissue placed in the PDA, only one fungus morphotype was observed. A fungus with white to grayish mycelia was isolated (Fig. 1b) from all infected tissue placed in the PDA medium. The fungus was fast-growing, with mycelial growth reaching the edge of the plate on the 7th-day post-incubation. Dark and round-shaped conidia, measuring an average of 14.93 µm (30 conidia, ranging from 12.18 to 16.73 µm), were observed (Fig. 1c) from the seven-day-old culture. Culture and morphology characteristics resemble those of *Nigrospora* sp. based on previous reports.

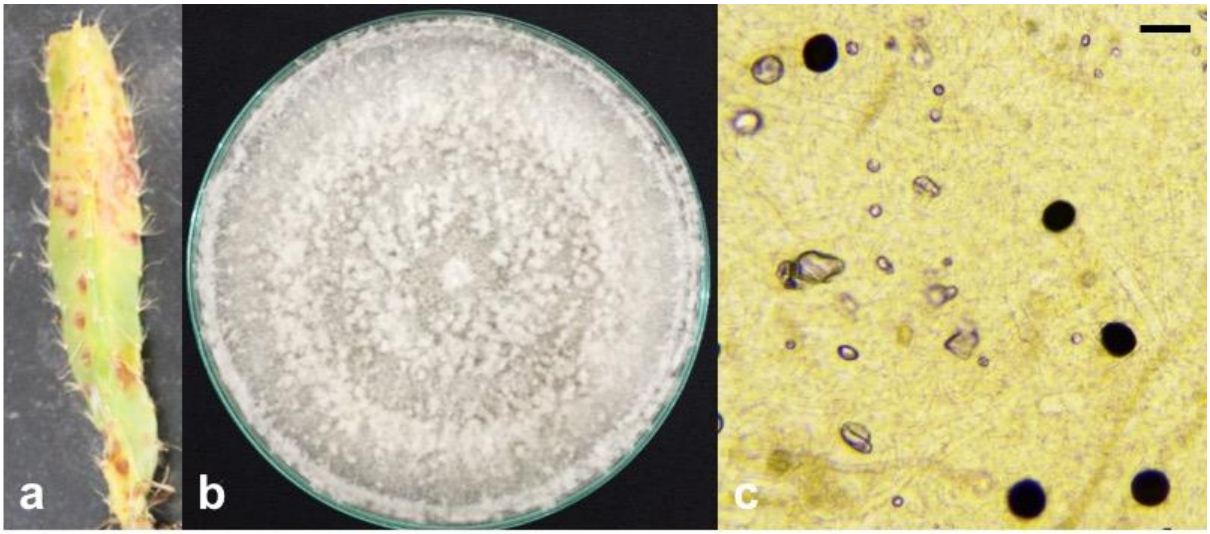

Figure 1. Diseased dragon fruit stem showing reddish-brown spots (a), mycelial growth of the fungus (b), and fungus morphology showing dark conidia in PDA medium at seven days post-incubation (c). The bar at the upper left is 20 µm.

Molecular characteristics and phylogeny

A 585 base pair amplicon, of the isolate MBDF0016b isolate, was corroborated by gel electrophoresis after PCR analysis. Initial analysis of the DNA sequence using the BLASTN software showed a 100% similarity to two *N. sphaerica* accessions submitted to GenBank (MG832554 and MG832530) and 98-99% similarities to more than 20 other *N. sphaerica* accessions. The species of the pathogen was validated as *N. sphaerica* using the DNA sequence (isolate MBDF0016b) of the partial sequence of the ITS gene, which aligned with the DNA sequences of 16 other validated *Nigrospora* species (Table 1, Wang *et al.,* 2017). Results

showed a 99.35% similarity of the MBDF0016b sequence to *N. sphaerica* (LC2840 isolate). A phylogenetic tree was constructed (Fig. 2), further inferring isolate MBDF0016b as *N. sphaerica.*

The DNA sequence of the fungus MBDF0016b also aligned with sequences of other *N. sphaerica* isolates from dragon fruits and other host-plant species. The phylogenetic tree (Fig. 3) revealed a close relationship between the Philippine isolate MBDF0016b and the Malaysian strain PC K4A1 C R2, which was also isolated from dragon fruit. Interestingly, the MBDF0016b isolate (from this study) was more closely related to *N. sphaerica* strains isolated from other plant species than with *N. sphaerica* strain BJ28 from dragon fruit in China.

Pathogenicity test

Fungal isolate MBDF0016b was pathogenic to the three dragon fruit species in the detached stem assay, and in *H. undatus* and *H. polyrhizus* stems in the glasshouse assay. Small, reddish-brown, necrotic lesions were observed in the inoculated wounded stems seven dpi (Fig. 4). No infection was observed in the control treatment. The pathogen was re-isolated from the diseased tissues, and the fungus was identical to the inoculated pathogen, thus establishing Koch's postulate.

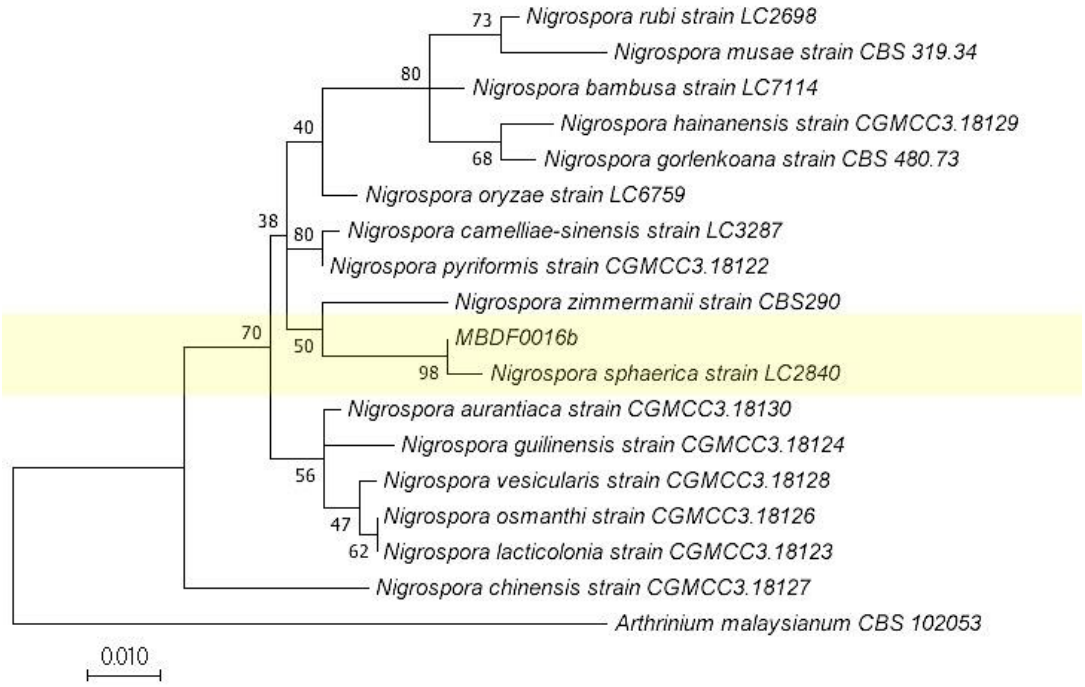

Figure 2. Phylogenetic tree generated by the maximum likelihood analysis of the ITS gene sequences of *N. sphaerica* MBDF0016b (this study) and other *Nigrospora* species as described by Wang *et al.* (2017). *Arthinium malaysianum* CBS 102053 served as an outgroup.

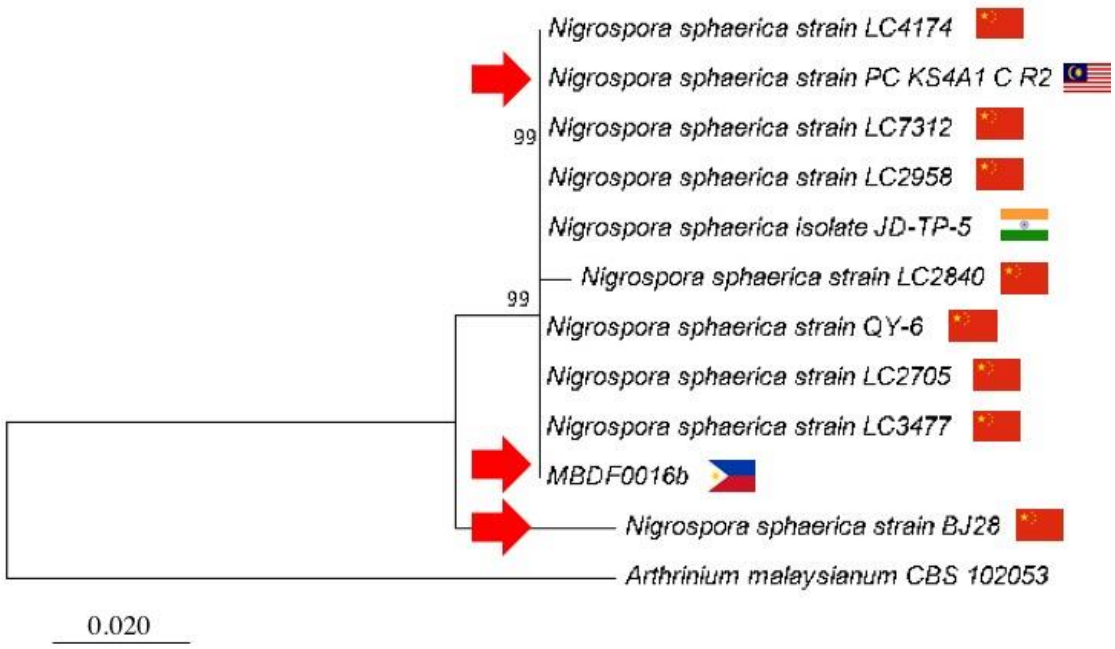

Figure 3. Phylogenetic tree generated by the maximum likelihood analysis of the partial sequence of the ITS gene of *N. sphaerica* MBDF0016b (this study) and other *N. sphaerica* reported in dragon fruit (PC KS4A1 C R2 and BJ28) and other host-plant species. *Arthinium malaysianum* CBS 102053 served as an outgroup.

Figure 4. Pathogenicity of *N. sphaerica* MBDF0016b in wounded (W) *H. megalanthus* at 7 and 14 dpi. No infection was observed in unwounded (UW) parts of the stem, and the control treatment.

DISCUSSION

Diseases are a major concern of dragon fruit growers worldwide. Diseases have significantly reduced the yield and fruit quality in commercial dragon fruit production (Balendres and Bengoa, 2019). One particular disease is the reddish-brown spot caused by *Nigrospora sphaerica* and *N. lactocolonia.* The former has been reported in China (Liu *et al.,* 2016) and Malaysia (Kee *et al.*, 2019), while the latter has only been reported in Malaysia (Kee *et al.,* 2019). The pathogens were found to cause the reddish-brown spot in *Hylocereus undatus* (Liu *et al.*, 2016) and *H. polyrhizus* (Kee *et al.*, 2019). The present study reports the occurrence of the same disease, reddish-brown spot caused by *N. sphaerica*, in dragon fruit stem in the Philippines. This is the first report of a dragon fruit disease caused by *N. sphaerica* in the Philippines. The Bureau of Plant Industry-National Plant Quarantine Services Division has been informed.

Nigrospora sphaerica belongs to the phylum Ascomycota and has a wide host range (Wang et al. 2017). It causes leaf blight in tea (*Camellia sinensis*) (Liu *et al.,* 2015), lotus (*Nelumbo* spp.), and *Musa paradisiaca* in China (Wang *et al.,* 2017). In dragon fruit, *N. sphaerica* causes reddish-brown spots on stems. *Nigrospora sphaerica* BJ28 was first reported as a dragon fruit pathogen in China on the red-skinned, white-fleshed species (*H. undatus*) (Liu *et al.,* 2016). The pathogen was later found infecting the red-fleshed species (*H. polyrhizus*) in Malaysia (Kee *et al.*, 2019). The present study reports the natural infection of *N. sphaerica* MBDF0016b in the yellow-skinned, white-fleshed species (*H. megalanthus*). Isolate MBDF0016b was also pathogenic to both *H. undatus* and *H. polyrhizus* in both detached stem and glasshouse assays. This is the first evidence of cross-pathogenicity of the same *N. sphaerica* species to other *Hylocereus* species performed under a similar (experimental) pathogenicity condition. The result suggests that *Hylocereus* species are likely, in general, susceptible host of *N. sphaerica,* and other *Hylocereus* species may also be susceptible*.* From breeding for disease resistance perspective, resistance genes are likely to be sourced from a wild relative of dragon fruits.

The morphology of *N. sphaerica* from Malaysia (Kee *et al.*, 2019), China (Liu *et al.,* 2016), and the Philippines (this study) were similar. However, analysis of the ITS gene region revealed that the isolate from the Philippines (MBDF0016b) is more closely related to the isolate (PC K4A1 C R2) from Malaysia with a nucleotide identity of >99% than from China. Nucleotide identity between the Philippines and China isolates was 96.56%. Kee *et al.* (2019) reported a nucleotide identity of 96.3% between Malaysia and China isolates. Further, the MBDF0016b (Philippines) and PC K4A1 C R2 (Malaysia) isolates are also closely related to *N. sphaerica* originating from other host-plant species (e.g., *C. sinensis* and *V. vinifera*) (Wang *et al.,* 2017), forming one clade, but separating isolate BJ28. Together, the analysis (of the isolates used) indicates that two genomic strains of *N. sphaerica* are currently present that causes various diseases of dragon fruit and other host-plants, with the China isolate (BJ28) being separated from the rest of the collections.

Nigrospora oryzae and an unknown *Nigrospora* species have been previously associated with diseases in rice and sorghum, respectively, in the Philippines (Tangonan, 1999). The latter has never been further elucidated and identified. The lack of culture impedes the re-identification

of the fungi. Hence, this study reports *N. sphaerica* as the other species from the genus *Nigrospora* that is associated with a plant disease in the country. This study recommends the addition of *N. sphaerica* in the next edition of the Host Index of Plant Diseases in the Philippines. The result from the phylogenetic analysis also suggests that the common strain, from Malaysia and the Philippines, may have a broader host range. Hence, *N. sphaerica* MBDF0016b may cross infect with other plant species. Studies underpinning the cross-infectivity of *N. sphaerica* MBDF0016b to other plant species, including weeds, could provide additional knowledge on the epidemiology and control of this pathogen.

CONCLUSION

The fungal pathogen, *Nigrospora sphaerica,* cause a reddish-brown spot of *H. megalanthus* stem in the Philippines. The fungus was isolated from diseased-stem tissue, and the fungus appears as white to grayish culture, in PDA medium, and has a dark and round-shaped conidium. The *N. sphaerica* from the Philippines was closely related to *N. sphaerica* isolates of dragon fruits from Malaysia and China based on the DNA sequence of the partial sequence of the ITS gene. The Philippine isolate was highly similar (>99%) to the Malaysian isolate than with China. The *N. sphaerica* isolate was pathogenic to the three cultivated *Hylocereus* species (*H. undatus, H. polyrhizus,* and *H. megalanthus*).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

Balendres, M.A.; Bengoa, J.C. 2019. Diseases of dragon fruit (*Hylocereus* species): etiology and current management options. *Crop Protection* 126, 104920. https://doi.org/10.1016/j.cropro.2019.104920.

Chuang, M.F.; Ni, H.F.; Yang, H.R.; Shu, S.L.; Lai, S.Y.; Jiang, Y.L. 2012. First report of stem canker disease of pitaya (*Hylocereus undatus* and *H. polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan. *Plant Disease, 96*, 906-907. https://doi.org/10.1094/PDIS-08-11-0689-PDN https://doi.org/10.1094/PDIS-08-11-0689-PDN.

Cullings, K.W., 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology, 1*, 233-240. https://doi.org/10.1111/j.1365-294X.1992.tb00182.x.

Crous, P.W.; Groenewald, J.Z. 2013. A phylogenetic re-evaluation of *Arthrinium*. *IMA Fungus, 4*, 133–154. https://doi.org/10.5598/imafungus.2013.04.01.13.

Doyle, J.J.; Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin, 19,* 11-15.

Eusebio, J.E.; Alaban, M.C. 2018. Current status of dragon fruit and its prospects in the Philippines. Country report, Dragon Fruit Regional Network Initiation Workshop, April 23-24, 2018, Taipei, Taiwan.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution, 39*, 783-791.

Guo, L.W.; Wu, Y.X.; Ho, H.H.; Su, Y.Y.; Mao, Z.C.; He, P.F.; He, Y.Q. 2014. First report of dragon fruit (*Hylocereus undatus*) anthracnose caused by *Colletotrichum truncatum* in China. *Journal of Phytopathology, 162*, 272-275. https://doi.org/10.1111/jph.12183.

Henry, B.S. 1996. Natural food colours. In: Hendry, G.A.F., Houghton, J.D. (Eds.), Natural Food Colorants. Blackie Chapman & Hall, London, pp. 40-79.

Le Bellec, F.; Vaillant, F.; Imbert Eric, E. 2006. Pitahaya (*Hylocereus* spp.): a New Fruit Crop, a Market with a Future. https://doi.org/10.1051/fruits:2006021.61.10.1051/fruits:2006021.

Liu Y. J.; Tang Q.; Fang, L. 2015. First report of *Nigrospora sphaerica* causing leaf blight on *Camellia sinensis* in China. *Plant Disease*, *100*, 221. https://doi.org/10.1094/PDIS-04-15-0493-PDN.

Liu, F., Wu, J. B., Zhan, R. L., & Ou, X. C. (2016). First report of reddish-brown spot disease on pitaya caused by *Nigrospora sphaerica* in China. *Plant Disease*, *100*(8), 1792. https://doi.org/10.1094/PDIS-01-16-0063-PDN.

Kee, Y. J.; Hafifi, A.B.M.; Huda-Shakirah, A.R.; Wong, K.L.; Jin, X.L., Siti Nordahliawate, M.S.; Zakaria, L.; Mohd, M.H. 2019. First report of reddish brown spot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) caused by *Nigrospora lacticolonia* and *Nigrospora sphaerica* in Malaysia. *Crop Protection, 122,* 165-170. https://doi.org/10.1016/j.cropro.2019.05.006.

Kumar, S.; Stecher, G.; Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution, 33,* 1870-1874. https://doi.org/10.1093/molbev/msw054.

Masyahit, M.; Sijam, K.; Awang, Y.; Ghazali, M. 2013. The occurrence of anthracnose disease caused by *Colletotrichum gloeosporioides* on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia, In: Palupi, E.R., Krisantini, Warrington, I.J. (Eds.), IV International Symposium on Tropical and Subtropical Fruits. *Int Soc Horticultural Science*, Leuven 1, pp. 187-195.

Meetum, P.; Leksomboon, C.; Kanjanamaneesathian, M. 2015. First report of *Colletotrichum aenimigma* and *C. siamense*, the causal agents of anthracnose disease of dragon fruit in Thailand. *Journal of Plant Pathology, 97,* 402-402.

Mizrahi, Y.; Nerd, A. 1999. Climbing and columnar cacti: new arid land fruit crops. In: Janick, J. (Ed.), Perspective on New Crops and New Uses. ASHS Press, Alexandria, USA, pp 358-366.

Mizrahi, Y.; Nerd, A.; Nobel, P. 1997. Cacti as crops. *Hortic. Rev.* 18, 291-320.

Nobel, P.S. 2002. Cacti: Biology and Uses. University of California Press, Los Angeles, California, p. 291.

Ortíz-Hernandez, Y.D. 1999. Pitahaya. Un nuevo cultivo para Mexico. Editorial Limusa, Mexico City.

Ortíz-Hernandez, Y.D.; Carillo-Salazar, J.A., 2012. Pitahaya (*Hylocereus* spp.): a short review. *Communicata Scientiae, 3*, 220-237.

Philippine Statistics Authority 2018. Dragon fruit volume of production in the Philippines per region.

Rodeo, A.J.; Castro, A.; Esguerra, E. 2018. Postharvest handling of dragon fruit (*Hylocereus* spp.) in the Philippines. Dragon Fruit Regional Network Initiation Workshop, pp. 125-131.

Tamura, K.; Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzee. *Molecular Biology and Evolution, 10*, 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

Tangonan, N. 1999. Host index of plant diseases in the Philippines. Nueva Ecija. Department of Agriculture, Philippine Rice Research Institute, Philippines.

Tel Zur, N. 2015. R&D of pitahayas - dragonfruit - vine cacti: limitations and challenges and the current global market. *Acta Hortic*. 1067, 365-370.

Tenore, G.C.; Novellino, E.; Basile, A. 2012. Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts. *Journal of Functional Foods, 4,* 129-136. https://doi.org/10.1016/j.jff.2011.09.003.

Wang, M.; Liu, F.; Crous, P. W.; Cai, L. 2017. Phylogenetic reassessment of *Nigrospora*: ubiquitous endophytes, plant and human pathogens. *Persoonia*, *39,* 118-142. https://doi.org/10.3767/persoonia.2017.39.06.

White, T.J.; Bruns, T.; Lee, S.; Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315-322.

Wichienchot, S.; Jatupornpipat, M.; Rastal, R.A. 2010. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry, 120*, 850-857. https://doi.org/10.1016/j.foodchem.2009.11.026.

Wybraniec, S.; Nowak-Wydra, B.; Mirka, K., Kowalski, P.; Mizrahi, Y. 2007. Minor betalains in fruits of *Hylocereus* species. Phytochemistry 68, 251-259. Nerd, A., Tel-Zur, N., Mizrahi, Y., 2002. Fruits of vine and columnar cacti. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses. University of California Press, Los Angeles, California, pp. 185-198. https://doi.org/10.1016/j.phytochem.2006.10.002.

Zhang, J.; Madden, T.L. 1997. PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation. *Genome Research*, 7:649-656.