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Molecular Characterization and Pathogenicity of *Stagonosporopsis cucurbitacearum* Causing Gummy Stem Blight Disease of Watermelon (*Citrullus lanatus* L.) in Malaysia

(Pencirian Molekul dan Kepatogenan *Stagonosporopsis cucurbitacearum* Penyebab Penyakit Hawar Batang Bergam Tembikai (*Citrullus lanatus* L.) di Malaysia)

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ABSTRACT

Watermelon (*Citrullus lanatus* L.) is a popular fruit crop with high economic value and widely grown in Malaysia. In January 2020, gummy stem blight (GSB) has become a threat for production of watermelon in Malaysia particularly in warm and humid climates, but the causative agent of GSB infecting watermelon is unknown. This disease decreases the fresh fruit marketability. Watermelon plants cultivar Red Rocky showing varied degree (40-90%) of suspected GSB, were collected from two main watermelon growing areas in Malaysia. Initial symptoms appeared as marginal brown necrotic lesions on leaves, while on the stems showed water-soaked, necrotic lesions and exuded reddish-brown gummy exudate on the stems. A total of ten isolates were isolated from lesions on leaves and stems of watermelon plants affected by GSB. All ten fungal isolates were identified as *Stagonosporopsis cucurbitacearum* on the basis of morphological characteristics and phylogenetic analysis of combined sequences of the internal transcribed spacer (ITS) and β-tubulin regions. All isolates were proven to be pathogenic when inoculated on the leaves and stems of the watermelon plant and the the fungal isolates were consistently reisolated from the diseased watermelon plants confirming Koch's postulates. Pathogenicity tests indicated that there were significant differences in virulence among the *S. cucurbitacearum* when inoculated on the leaves and stems of the watermelon plant. Understanding the etiology of the pathogen will help in disease management of gummy stem blight disease in Malaysia.

Keywords: Cucurbits; gummy stem blight; *Stagonosporopsis cucurbitacearum*; watermelon

ABSTRAK

Tembikai (*Citrullus lanatus* L.) ialah tanaman buah-buahan yang popular dengan nilai ekonomi yang tinggi dan ditanam secara meluas di Malaysia. Pada Januari 2020, penyakit hawar batang bergam (GSB) telah menjadi ancaman kepada pengeluaran tembikai di Malaysia terutamanya dalam iklim panas dan lembap, tetapi agen penyebab GSB yang menjangkiti tembikai tidak diketahui. Penyakit ini mengurangkan kebolehpasaran buah segar. Kultivar tanaman tembikai 'Red Rocky' yang menunjukkan pelbagai darjah (40-90%) GSB yang disyaki dikumpulkan dari dua kawasan penanaman tembikai utama di Malaysia. Simptom awal muncul sebagai luka nekrotik coklat marginal pada daun, manakala pada batang menunjukkan luka nekrotik yang direndam air dan eksudat bergetah coklat kemerahan pada batang. Sebanyak sepuluh pencilan telah diasingkan daripada luka pada daun dan batang pokok tembikai yang terjejas oleh GSB. Kesemua sepuluh pencilan kulat telah dikenal pasti sebagai *Stagonosporopsis cucurbitacearum* berdasarkan ciri morfologi dan analisis filogenetik bagi jujukan gabungan kawasan *spacer* transkripsi dalaman (ITS) dan β-tubulin. Semua pencilan telah terbukti bersifat patogen apabila disuntik pada daun dan batang pokok tembikai dan pencilan kulat diasingkan semula secara tekal daripada tumbuhan tembikai berpenyakit yang mengesahkan postulat Koch. Ujian kepatogenan menunjukkan bahawa terdapat perbezaan yang ketara dalam kevirulenan dalam kalangan *S. cucurbitacearum* apabila disuntik pada daun dan batang pokok tembikai. Memahami etiologi patogen akan membantu dalam pengurusan penyakit penyakit hawar batang getah di Malaysia.

Kata kunci: Hawar batang bergam; kukurbit; *Stagonosporopsis cucurbitacearum*; tembikai

Introduction

Watermelon (*Citrullus lanatus* L.) belongs to the family of Cucurbitaceae is a commercial crop widely grown in tropical and subtropical regions and it is being consumed in Malaysia for its edible flesh and nutritional values (Ismail & Abd Razak 2020). Watermelon is cultivated throughout the states with different environmental conditions with high annual rainfall. In 2019, Malaysia production of watermelon was 144,213 tons, with the land areas coverage at 8,308 hectares (Food and Agriculture Organization of the United Nations 2019).

Stagonosporopsis species have been reported as causal agents of watermelon in the United States (Keinath, Farnham & Zitter 1995), pumpkin in China (Zhao et al. 2018) and cucurbit crops (Chiu & Walker 1949). GSB disease caused by *S. cucurbitacearum* is one of the most common fungal diseases in watermelon-growing areas especially in warm and humid seasons and it affects all developmental growth of watermelon plants (Newark et al. 2020; Zhao et al. 2019). Increased cultivation of watermelon and large-scale cultivation of popular varieties in watermelon-producing regions have resulted in an increase in watermelon diseases. *S. cucurbitacearum* is an ascomycete fungus that can survive on seeds, weeds, and plant debris from previously infected cucurbits (Keinath 2002). Conidia of *Stagonosporopsis* spp. serve as the major inoculum causing epidemics of gummy stem blight disease. *Stagonosporopsis* spp. can cause necrotic lesions on leaves, vines and black rot appears on watermelon fruits is the vines are severely infected with GSB. Gummy stem blight is considered one of the most serious foliar diseases on watermelon plants (Keinath 2011). Gummy stem blight resulted in more than 60% of leaves defoliation and fruit rot has been reported (Newark et al. 2020). This disease also poses a threat to watermelon production by causing loss and reducing fruit quality. Gummy stem blight caused of 15-50% yield losses in greenhouses and open fields (Li et al. 2015; Yao et al. 2016) . Reports on occurrence of GSB in watermelon in other countries are available, however there is no report of this disease on watermelon in Malaysia. Previous studies have reported that *S. cucurbitacearum* can cause postharvest damage of cucurbit hosts including watermelon fruits in Turkey (Basım et al. 2016), Taiwan (Huang & Lai 2019), India (Mahapatra et al. 2020), and cantaloupe in Thailand (Nuangmek et al. 2018).

GSB is caused by three different species *S. cucurbitacearum* (syn. *Didymella bryoniae*), *S. citrulli*, and *S. caricae,* but these fungal species were morphologically indistinguishable but genetically distinct species of *Stagonosporopsis* (Stewart, Turner & Brewer 2015). These three species can be separated from each other based on the morphology and the combined DNA sequence data of internal transcribed sequence (ITS) and a partial β-tubulin (*TUB*) gene (Nuangmek et al. 2018). Although watermelon is great importance in Malaysia, nad gummy stem blight has become more destructive, the etiology of this disease has not been systematically investigated. Identification of the species and accurate information of their occurrence, pathogenicity to watermelons is critical for identifying the infectious agents associated with gummy stem blight in Malaysia. However, the causal agent of GSB on watermelon remains unknown and a comprehensive study elucidating the correct identity of this pathogen using molecular techniques has never been conducted. Thus, the objective of this study was to identify, characterize the causal agents of gummy stem blight symptoms on watermelon plants using morphological and molecular characteristics.

Materials and Methods

In January and February 2020, twenty samples of leaves and stems, which showed characteristic symptoms of GSB on watermelon plants were collected from two watermelon fields located in the Seri Kembangan (2º59’05.096”N 101º43’57.229”E) and Serdang districts of Selangor (3º00'14.967”N 101º42’00.581”E). Tissues (5 mm^2) from each symptomatic leaf and stem were excised and surface sterilized using 0.5% NaOCl for two minutes and rinsing twice with sterile distilled water (Ismail & Abd Razak 2020). The tissues were dried on sterilized filter paper, placed onto potato dextrose agar (PDA) plates (Difco, USA) and incubated in a chamber at 28 °C with 12 h photoperiod for 7 days. Single-spore cultures were transferred aseptically to new PDA plates. Pure colonies were obtained after three purifications and stored on PDA slants at 4 °C. The colony characteristics were recorded daily for 10 days. The morphological characteristics of conidia including size, shape and color were recorded. Conidial size (length and width) of each isolate (n=50) were observed and measured with a Olympus CX31 RBSF microscope fitted digital eyepiece camera after 10 days of incubation.

The fungal genomic DNA of each isolate was extracted from fresh mycelia by using a DNeasy Plant Mini Kit (Qiagen, Germany). Polymerase chain reaction (PCR) amplification of the isolated DNA was performed using two pairs of primers, the universal primers ITS4 /

ITS5 (White et al. 1990), and β-tubulin (*TUB*), Bt2a/Bt2b (Stewart, Turner & Brewer 2015). The amplified PCR products were separated on 1% agarose gels containing MIDORI^{Green} Advance nucleic acid stain (Nippon Genetics, Europe) and the DNA fragments were visualized under UV transilluminator (Bio-Rad Laboratories Inc., USA). A 1 kb DNA ladder (BenchTop, Promega) was used as a molecular size standard marker. The PCR products were sequenced using an Applied Biosystems 3730 Genetic Analyzer at the Apical Scientific Sdn. Bhd Company. To identify the isolates obtained in this study, sequences were subjected to BLAST search nucleotide analysis to find similarities with those sequences available in the National Center for Biotechnological Information (NCBI) database. Ten fungal isolates of this study were used for phylogenetic analysis and seven sequences of the *Stagonosporopsis* species that had the greatest sequence identity (>95%) were retrieved from the NCBI GenBank database. Multiple sequence alignments for the aligned sequences were calculated using ClustalW (Tamura et al. 2013). A phylogenetic tree was constructed using the maximum likelihood (ML) method with the combined sequences of ITS and β-tubulin genes using *Heterophoma poolensis* as the outgroup. Phylogeny was constructed using MEGA software version 7.0 (Kumar, Stecher & Tamura 2016).

The pathogenicity of each isolate was tested on healthy leaves and stems of five 22-days-old potted Red Rocky watermelon seedlings that were surfaced-disinfested with 70% ethanol. For each isolate, 5 leaves and stems on watermelon seedlings were inoculated. Conidial suspension was prepared according to the methods described by Li et al. (2015). Conidial suspensions (1×10^6 conidia/mL) were placed at wounded sites on the middle of the watermelon leaves. Wound inoculation involved pinpricking the middle of the leaves (one site per leaf and one site per stem) and inoculating a conidial suspension onto the wound. Watermelon leaves and stems inoculated only with sterile water served as controls. The leaves and stems were covered with moist cotton and sealed with parafilm and the inoculated watermelon seedlings were covered with plastic bags to maintain high relative humidity for 3 days. Inoculated plants were maintained in the greenhouse at 28 °C and 90% relative humidity. All fungal isolates used in the pathogenicity tests were reisolated from the diseased tissues and the identity of fungal isolates was confirmed as described earlier. Disease symptoms were assessed daily for 3-10 days.

Results

In symptomatic watermelon plants, circular, tan to dark-brown necrotic lesions appear on the leaves especially at the margins. The lesions enlarge rapidly until the entire leaf is blighted (Figure 1(A) & 2(B)). The lesions were surrounded by a yellow halo, water-soaked spots or reddish-brown lesions may develop on the infected leaves (Figure 1(C)). In addition to the foliar phase, canker was also developed on the main stem and spreads to the vines of watermelon if the cortical tissues of stems were infected. As the disease progressed, these lesions dried up, cracked and released reddish-brown gummy exudate (Figure 1(D)).

In total, ten isolates of *Stagonosporopsis* spp. were recovered from diseased watermelon plants sampled from watermelon fields. Typical cultural and colony characteristics are shown in Figure 1(E). On PDA media, all isolates first developed whitish to gray zonate colonies, which became olivaceous green as the age the fungal culture increased. Colonies on PDA had covered the entire surface of the plate (9.0 cm in diameter) after 7 days. All of the isolates that sporulated in culture medium showed typical *Stagonosporopsis* morphology. All isolates were provisionally identified as *Stagonosporopsis* sp. on the basis of conidial morphology (Aveskamp et al. 2010). All isolates did not produce pycnidia on PDA. The conidia were hyaline, oblong, monoseptate or non-septate, smooth and the size ranged from 5.0 - 14.0 µm (length) × 3.0 - 5.0 µm (width) (n = 50) (Figure 1(F)).

The PCR products of approximately 530 bp, and 400 bp were generated for ITS and β-tubulin, respectively. The 10 isolates were compared based on the sequences of internal transcribed spacer (ITS) region (GenBank accession numbers MN849174, MN849175, MW757161 to MW757168) and β-tubulin (GenBank accession numbers MT627322, MT627323 to MW760874 to MW760881). The aligned sequences were compared to known sequences in GenBank, sequence homology was greater than 99% for all isolates. The sequences were deposited in GenBank (Table 1).

Virulence of all isolate was assayed using wounded leaves and stems of watermelon seedlings cultivar Red Rocky. For all ten isolates, inoculation of wounded leaves and stems resulted in typical gummy stem blight lesions, which were first observed at 3 - 7 days post inoculation (dpi). Isolates of *S. cucurbitacearum* were highly virulent on watermelon seedlings. The isolates were grouped into two categories based on the lesion

diameter. Isolates M1, M2, M3, M4, M5, M6, and M7 were grouped as highly aggressive isolates as they caused larger lesions on leaves and stems with average diameter 4 - 10 cm at 7 days of inoculation. Isolates F1, L and M8 were grouped as moderately aggressive isolates, which developed smaller lesions on the stem and leaves (1- 4 cm average diameter) at 7 dpi. The results indicated that the isolates differed in virulence on watermelon plants. Isolate M1 showed significant virulence as compared to isolate F1 and was able to cause the largest necrotic

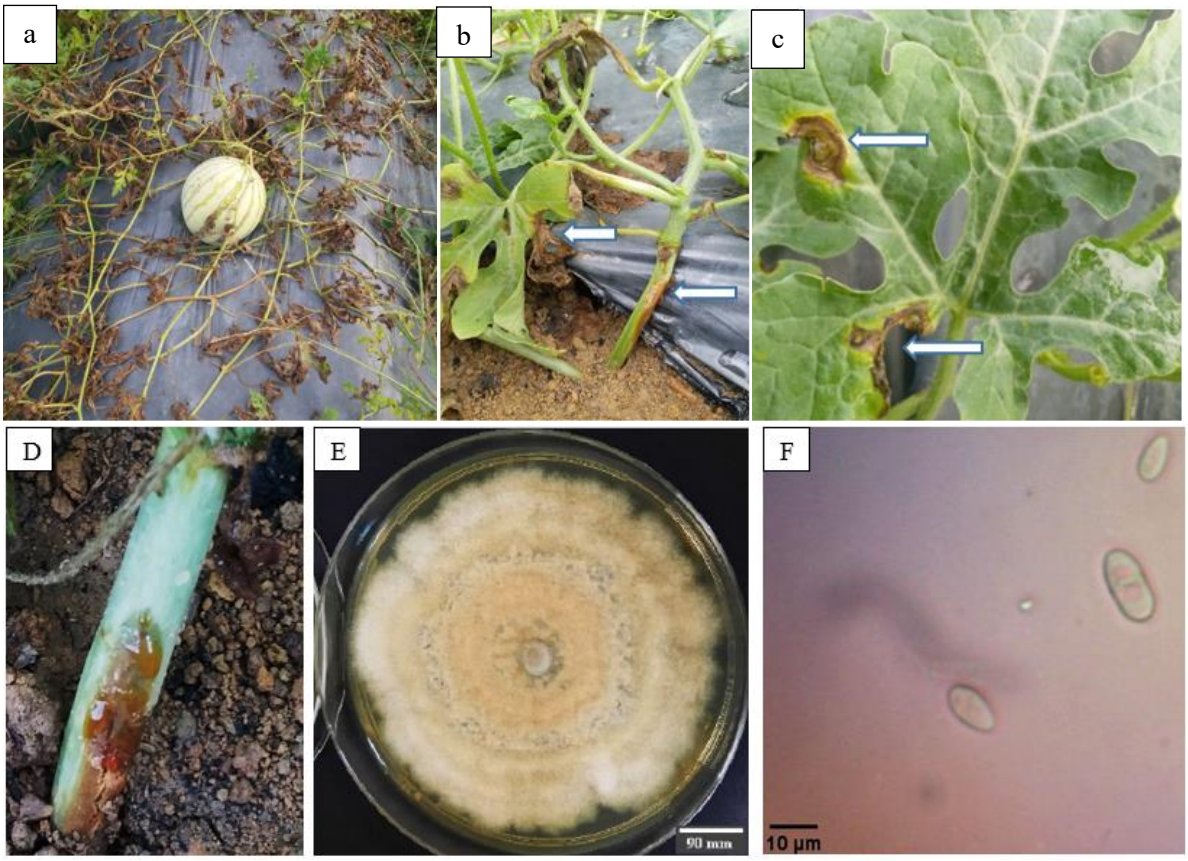

FIGURE 1. a. Natural symptom of gummy stem blight observed in watermelon field associated with *S. cucurbitacearum,* b. The necrotic lesions enlarge rapidly until the entire leaf is blighted, c. Typical symptom of gummy stem blight at the leaf margins of watermelon, d, Canker and reddish-brown gummy exudate on stem of watermelon, e. Colony morphology of *S. cucurbitacearum* grown on PDA after 7 days at 28 °C, f. Conidia of *S. cucurbitacearum* at 400× magnification (Scale bar = 10 µm)

lesions on watermelon seedlings. The necrotic lesions caused by isolates F1, L and M8 were significantly smaller (Table 2). Initial symptoms included circular, tan to brown water-soaked lesions around the inoculation sites that expanded until the entire leaf is blighted. The stems developed water-soaked, dark brown lesions, after 3 days of inoculation and later became dried. The control plants did not develop any gummy stem blight symptom. The pathogen was reisolated from all of the inoculated plants, thus fulfilling Koch's postulates. Morphological characteristics of the reisolated fungi were consistent with the original isolates described in this study.

TABLE 1. Isolates used for the phylogenetic analysis in this study and GenBank accession numbers of *S. cucurbitacearum* sequences for ITS and β-tubulin (*TUB*) genes

Species	Isolates	Location	GenBank accession number	
			ITS[a]	β -tubulin[b]
S. cucurbitacearum	F1	Seri Kembangan, Selangor	MN849175	MT627322
S. cucurbitacearum	L	Seri Kembangan, Selangor	MN849174	MT627323
S. cucurbitacearum	M1	Serdang, Selangor	MW757161	MW760874
S. cucurbitacearum	M1	Serdang, Selangor	MW757161	MW760874
S. cucurbitacearum	M3	Serdang, Selangor	MW757163	MW760876
S. cucurbitacearum	M4	Serdang, Selangor	MW757164	MW760877
S. cucurbitacearum	M5	Serdang, Selangor	MW757165	MW760878
S. cucurbitacearum	M6	Serdang, Selangor	MW757166	MW760879
S. cucurbitacearum	M7	Serdang, Selangor	MW757167	MW760880
S. cucurbitacearum	M8	Serdang, Selangor	MW757168	MW760881

[a]Internal transcribed spacer (ITS) ribosomal DNA (rDNA) region-based primers ITS4 and ITS5 (White et al. 1990). [b] β-tubulin (*TUB*) region-based primers Bt2a and Bt2b (Stewart, Turner & Brewer 2015)yet genetically distinct lineages that occur in overlapping geographic ranges and niches. Using a multilocus sequencing approach we discovered that gummy stem blight of cucurbits is caused by three genetically distinct species: Stagonosporopsis cucurbitacearum (syn. Didymella bryoniae

The combined phylogenetic analysis (ITS and β-tubulin) included 18 taxa, and the alignment of the sequences resulted in a total of 929 characters. The phylogenetic analysis showed all 10 isolates in this study clustered in a large clade with *S. cucurbitacearum* ex-type cultures, CBS 214.65, CBS 133.96, CBS 386.65 with a bootstrap value of 85% (Figure 2).

DISCUSSIONS

Currently, the etiology of gummy stem blight disease in Malaysia was neglected by researchers, even though this disease is a major fungal disease of watermelon. Rapid identification of plant pathogens is currently classified using molecular data based on phylogenetic inference. Several *Stagonosporopsis* spp. have been reported in other countries and regions, however, the identification of causal agent of gummy stem blight disease on watermelon occurred in commercial production fields in Malaysia is not known. Based on morphological characterization, phylogenetic analyses involving two loci (ITS and β-tubulin) and inoculation tests on watermelon seedlings, the present study showed that ten *S. cucurbitacearum* isolates cause gummy stem blight in Malaysia. All isolates were proved to be pathogenic to watermelon leaves and stems. The findings offer precise information about pathogen identity, which is important for screening of resistant varieties and development of effective disease management strategies.

TABLE 2. Aggressiveness of *S. cucurbitacearum* isolates on watermelon

Isolate	Location	Host	Mean lesion diameter (cm)	Aggressiveness
F1	Seri Kembangan, Selangor	Watermelon	2.02 [b]	M
L	Seri Kembangan, Selangor	Watermelon	1.98 [b]	M
M1	Serdang, Selangor	Watermelon	10 [a]	H
M2	Serdang, Selangor	Watermelon	6.3 [a]	H
M3	Serdang, Selangor	Watermelon	7.3 [a]	H
M4	Serdang, Selangor	Watermelon	6.7 [a]	H
M5	Serdang, Selangor	Watermelon	8.01[a]	H
M6	Serdang, Selangor	Watermelon	7.7 [a]	H
M7	Serdang, Selangor	Watermelon	5.05 [ac]	H
M8	Serdang, Selangor	Watermelon	2.55 [b]	M

Means followed by the same letters are not significantly different ($P < 0.05$). Aggressiveness is evaluated based on lesions diameter. Highly aggressive (H) (average diameter 4 -10 cm) and moderate aggressive (M) (average diameter 1- 4 cm)

S. cucurbitacearum was consistently isolated and identified from diseased samples. In this study, *S. cucurbitacearum* were isolated from lesions on leaves and stems at different stage of watermelon development. *Stagonosporopsis* can infect all aboveground plant parts of cucurbits (Keinath 2011). Any residual plant material from previous infected watermelon plants could act as source of inoculum in regions where they occur and contributed to the prevalence of *Stagonosporopsis* occurring on watermelon.

Species of *S. cucurbitacearum* have been differentiated based on morphological and phylogenetic analysis. The most frequently described morphological characteristics include conidial morphology, growth and pigment production. However, these traditional morphology-based characters are not reliable for taxonomic classification at the species level. The conidial characteristics observed in this study concurred with previously reported by Basim et al. (2016), Garampalli et al. (2016), and Nuangmek et al. (2018). The correct species identification of fungal pathogens is essential for crop protection and disease management strategies. Molecular characterization using ITS and β-tubulin gene confirmed the genetic placement of *S. cucurbitacearum* obtained in this study. PCR analysis and the size of the ITS and β-tubulin fragments amplified indicated that the isolates were placed in the proper clade. The *S. cucurbitacearum* isolates obtained in this study clustered in a well-supported clade with the ex-type cultures of *S. cucurbitacearum*.

Koch's postulates were confirmed and all of the isolates were pathogenic to watermelon plants. Wounding

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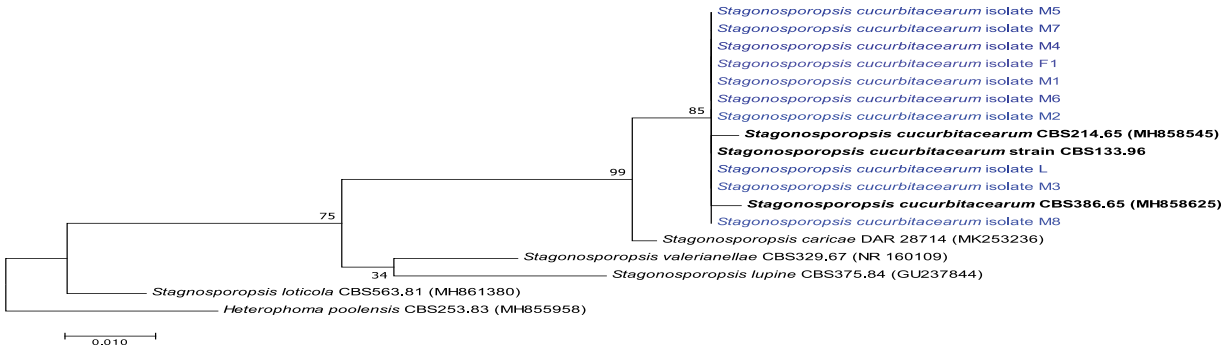

FIGURE 2. Maximum-likelihood phylogram based on the combined sequences of internal transcribed spacer (ITS) and β-tubulin using MEGA 7.0 software using 1,000 replicates. The bootstrap values are indicated on the branches. The isolates obtained in this study are given in blue. Ex-type cultures of *S. cucurbitacearum* are bold. The tree was rooted with *Heterophoma poolensis* CBS253.83 (MH855958)

prior to inoculation was necessary for enhanced disease development on leaves and stems. In the pathogenicity assay, *S. cucurbitacearum* showed similar disease symptoms similar to those observed in watermelon field. The pathogenicity tests on Red Rocky watermelon seedlings confirmed the different level of pathogenicity and aggresiveness among the isolates. The variation among *S. cucurbitacearum* isolates in causing the disease on watermelon was conformed as described earlier by Babu et al. (2015), Chiu and Walker (1949), and Li et al. (2015). Among 10 *Stagonosporopsis* species identified in this study, seven isolates from Serdang area exhibited the highest level of pathogenicity based on the size of the disease lesions developed after artifical inoculation. Similar finding reported by St. Amand and Wehner (1995), who observed a significant difference in the variation in virulence between the two different geographical regions. However, these findings have no agreement with the work of Li et al. (2015) and Newark et al. (2017), who reported that the variation in virulence and aggressiveness of isolates were not significantly related to the location. More research on the genetic and biological characteristics of different *Stagonosporopsis* species from diverse geographical areas will be needed to understand the emergence and spread of gummy stem blight disease. Climate change and high temperature could be one of the factors for expansion of different level of aggresiveness of the isolates.

This study also confirmed *S. cucurbitacearum* occurs in tropical region affecting watermelon in Malaysia. *Stagnosporopsis* could not only infect watermelon but also latently colonize such as cantaloupe, squash and sponge guard. High relative humidity and temperature may favor the development of disease symptom. Information on the susceptibility of other cucurbits in Malaysia need to be conducted and the information can be useful for resistance breeding program. To determine whether weed control is necessary to minimize the primary infection in the field, it is worth investigating whether the *S. cucurbitacearum* identified in this study could colonize the weeds commonly present in and nearby watermelon fields in Malaysia. Artificial inoculation assays will be required to understand the

host range of the *S. cucurbitacearum*. To the best of our knowledge, this is the first study to provide evidence that *S. cucurbitacearum* is the primary causal agent causing gummy stem blight on watermelon in Malaysia. Effective and reliable control strategies must be developed due to its potential to cause yield losses in watermelon.

Conclusions

Stagonsporopsis cucurbitacearum have been reported as the most common serious fungal pathogen that can infect a wide range of cucurbit crops worldwide. The isolation from the diseased watermelon plant samples showed that *S. cucurbitacearum* is the main causative agent that responsible for gummy stem blight (GSB) disease. The results from morphological and molecular studies confirmed that all the ten isolates were found to be *S. cucurbitacearum*. This study found out that the molecular identification using ITS rDNA and *TUB* gene were adequately verify and differentiate the *Stagonosporopsis* spp. at species level. Moreover, these two genes are beneficial to provide information and knowledge on Stagonosporopsis taxonomy.

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References

Aveskamp, M.M., de Gruyter, J., Woudenberg, J.H.C., Verkley, G.J.M. & Crous, P.W. 2010. Highlights of the Didymellaceae: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycolology* 65: 1-60.

Babu, B., Kefialew, Y.W., Li, P.F., Yang, X.P., George, S., Newberry, E. & Paret, M.L. 2015. Genetic characterization of *Didymella bryoniae* isolates infecting watermelon and other cucurbits in Florida and Georgia. *Plant Disease* 99: 1488-1499.

Basım, E., Basım, H., Abdulai, M., Baki, D. & Oztürk, N. 2016. Identification and characterization of *Didymella bryoniae* causing gummy stem blight disease of watermelon (*Citrullus lanatus*) in Turkey. *Crop Protection* 90: 150-156.

Chiu, W.F. & Walker, J.C. 1949. Physiology and pathogenicity of cucurbit black-rot fungus. *Journal of Agricultural Research* 78: 589-615.

Food and Agriculture Organization of United Nation. 2019. *FAO Reports Watermelons*. http://www.fao.org/faostat/en/#data/QC.

Garampalli, R.H., Gapalkrishna, M.K., Li, H.X. & Brewer, M.T. 2016. Two *Stagonosporopsis* species identified as causal agents of gummy stem blight epidemics of gherkin cucumber (*Cucumis sativus*) in Karnataka, India. *European Journal of Plant Pathology* 145: 507-512.

Huang, C.J. & Lai, Y.R. 2019. First report of *Stagonosporopsis citrulli* causing gummy stem blight of watermelon in Taiwan. *Journal of Plant Patholology* 101: 417.

Ismail, S.I. & Abd Razak, N.F. 2020. First report of *Nigrospora sphaerica* causing leaf spot on watermelon (*Citrullus lanatus*) in Malaysia. *Plant Disease* 105: 2. doi: 10.1094/PDIS-07-20-1491-PDN.

Keinath, A.P. 2011. From native plants in central Europe to cultivated crops worldwide: The emergence of *Didymella bryoniae* as a cucurbit pathogen. *HortScience* 46: 532-535.

Keinath, A.P. 2002. Survival of *Didymella bryoniae* in infested muskmelon crowns in South Carolina. *Plant Disease* 86: 1223-1228.

Keinath, A.P., Farnham, M.W. & Zitter, T.A. 1995. Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. isolated from cucurbits. *Phytopathology* 85: 364-369.

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.

Li, P.F.F., Ren, RS.S., Yao, X.F.F., Xu, J.H.H., Babu, B., Paret, M.L. & Yang, X.P.P. 2015. Identification and characterization of the causal agent of gummy stem blight from muskmelon and watermelon in East China. *Journal of Phytopathology* 163: 314-319.

Mahapatra, S., Rao, E.S., Sandeepkumar, G.M. & Sriram, S. 2020. *Stagonosporopsis cucurbitacearum* the causal agent of gummy stem blight of watermelon in India. *Australasian Plant Disease Notes* 15: 7-9.

Newark, M.J., Li, P., Yang, X.P., Paret, M.L. & Dufault, N.S. 2020. Comparing *Stagonosporopsis* spp. Fungicide resistance profiles in Florida and East China cucurbit production systems. *Plant Disease* 104: 129-136.

Nuangmek, W., Aiduang, W., Suwannarach, N., Kumla, J. & Lumyong, S. 2018. First report of gummy stem blight caused by *Stagonosporopsis cucurbitacearum* on cantaloupe in Thailand. *Canadian Journal of Plant Pathology* 40: 306-311.

St. Amand, P.C.S. & Wehner, T.C. 1995. Greenhouse, detached-leaf, and field testing methods to determine cucumber resistance to gummy stem blight. *Journal of the American Society for Horticultural Science* 120(4): 673-680.

Stewart, J.E., Turner, A.N. & Brewer, M.T. 2015. Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. *Fungal Biology* 119: 370-382.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725-2729.

White, T.J., Bruns, T.D., Lee, S.B. & Taylor, J.W. 1990. *PCR Protocols: A Guide to Methods and Applications.* New York: Academic Press. pp. 315-322.

Yao, X., Li, P., Xu, J., Zhang, M., Ren, R., Liu, G. & Yang, X. 2016. Rapid and sensitive detection of *Didymella bryoniae* by visual loop-mediated isothermal amplification assay. *Frontiers in Microbiology* 7: 1-11.

Zhao, Q., Wu, J., Zhang, L., Xu, L., Yan, C. & Gong, Z. 2019. Identification and characteristics of *Stagonosporopsis cucurbitacearum* pathogenic factors influencing pumpkin seedling survival in north-east China. *Journal of Phytopathology* 167: 41-55.

Zhao, Q., Wu, J., Zhang, L., Xu, L., Yan, C. & Gong, Z. 2018. Identification and characterization of *Cucurbita* gummy stem blight fungi in Northeast China. *Journal of Phytopathology* 166(5): 305-313.

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