CHARACTERISATION OF FUNGI ASSOCIATED WITH DISEASED *Sansevieria trifasciata* IN MALAYSIA

by

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LIST OF SYMBOLS

%	Percentage
&	And
®	Registered
°C	Degree Celsius
µl	Microlitre
µm	Micrometre
µM	Micromolar
g	Gram
L	Litre
mA	Milliampere
mg	Milligram
ml	Millilitre
mm	Millimetre
ppm	Part per million
psi	Pounds per square inch
™	Trademark
V	Voltage

LIST OF ABBREVIATIONS

ACT	Actin
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
CAL	Calmodulin
C_2H_5OH	Ethanol
CHS I	Chitin
CLA	Carnation leaf-piece agar
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HIS3	Histone H3
ITS	Internal transcribed spacer
kb	Kilobase pair
M	Mean
MEGA	Molecular Evolutionary Genetics Analysis
$MgCl_2$	Magnesium chloride
min	Minute
ML	Maximum likelihood
MP	Maximum parsimony
NaOCl	Sodium hypochlorite

NCBI	National Center for Biotechnology Information
NJ	Neighbor-joining
PCA	Potato carrot agar
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
RAM	Random amplified microsatellite
RAPD	Random amplification of polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minute
SD	Standard deviation
SDHI	Succinate dehydrogenase inhibitor
SFP	Single feature polymorphism
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
STR	Short tandem repeat
TBE	Tris-Borate-EDTA
TEF1-α	Translation elongation factor 1-α
VNTR	Variable number tandem repeat
WA	Water agar

PENCIRIAN KULAT YANG BERASOSIASI DENGAN *Sansevieria trifasciata* BERPENYAKIT DI MALAYSIA

ABSTRAK

Sansevieria trifasciata merupakan tumbuhan hiasan yang popular yang boleh dijumpai secara meluas di Malaysia. Tumbuhan ini mudah dijaga kerana toleran terhadap bekalan cahaya dan air yang rendah. Walau bagaimanapun, *S. trifasciata* mudah dijangkiti oleh pelbagai jenis penyakit, terutama yang disebabkan oleh kulat. Kajian ini dijalankan untuk memencil, mengenal pasti dan mencirikan kulat yang berasosiasi dengan *S. trifasciata* berpenyakit di Malaysia. Seterusnya ujian kepatogenan dijalankan untuk mengesahkan kepatogenan pencilan kulat. Sejumlah 100 pencilan telah diperoleh daripada *S. trifasciata* berpenyakit dari Pulau Pinang, Perak, Kedah, Kelantan, Pahang, Kuala Lumpur, Selangor, Melaka, Negeri Sembilan, Johor, Sabah dan Sarawak. Berdasarkan ciri morfologi dan molekul (jujukan DNA), pencilan kulat telah dikenalpasti sebagai *Colletotrichum sansevieriae* (44 pencilan), *C. truncatum* (satu pencilan), *C. endophytica* (satu pencilan), *C. tropicale* (lima pencilan), *C. fructicola* (satu pencilan), *C. alienum* (enam pencilan), *Lasiodiplodia brasiliense* (satu pencilan), *L. hormozganensis* (lapan pencilan), *L. iraniensis* (satu pencilan), *L. pseudotheobromae* (tiga pencilan), *L. theobromae* (dua pencilan), *Neoscytalidium dimidiatum* (lapan pencilan), *Fusarium oxysporum* (11 pencilan), *F. solani* (dua pencilan), *F. brachygibbosum* (satu pencilan), *Stemphylium lycopersici* (dua pencilan), *Curvularia eragrostidis* (satu pencilan), *Cu. geniculata* (satu pencilan) dan *Diaporthe tectonae* (satu pencilan). Pengenalpastian pencilan *Colletotrichum* telah disahkan melalui analisis filogenetik gabungan jujukan penjarak transkripsi dalaman (ITS), β-tubulin dan gliseraldehid-3-fosfat dehidrogenase (GAPDH).

Spesies *Lasiodiplodia* telah disahkan berdasarkan analisis filogenetik gabungan jujukan ITS dan TEF1-α, manakala spesies *Fusarium* telah disahkan melalui analisis filogenetik jujukan TEF1-α. Analisis filogenetik jujukan ITS mengesahkan pengenalpastian spesies *Neoscytalidium*, *Stemphylium*, *Curvularia* dan *Diaporthe*. Tujuh puluh lima pencilan kulat dipilih berdasarkan perbezaan morfologi untuk ujian kepatogenan. Ujian kepatogenan menunjukkan 66 daripada 75 pencilan kulat yang dipilih adalah patogenik terhadap *S. trifasciata*. Pencilan *C. tropicale*, *C. fructicola*, *F. solani* dan *D. tectonae* tidak membentuk lesi pada daun yang diinokulasi. Hasil kajian ini menunjukkan bahawa terdapat beberapa spesies kulat yang berasosiasi dengan *S. trifasciata* yang berpenyakit di Malaysia.

CHARACTERISATION OF FUNGI ASSOCIATED WITH DISEASED *Sansevieria trifasciata* IN MALAYSIA

ABSTRACT

Sansevieria trifasciata is a popular ornamental plant that can be widely found in Malaysia. It is an easy to be taken care plant as it is tolerant of low light intensity and water supply. However, *S. trifasciata* is susceptible to various diseases, particularly those caused by fungi. The present study was conducted to isolate, identify and characterise the fungi associated with diseased *S. trifasciata* in Malaysia. Pathogenicity test was then conducted to confirm the pathogenicity of the isolates. A total of 100 fungal isolates were recovered from diseased *S. trifasciata* from Penang, Perak, Kedah, Kelantan, Pahang, Kuala Lumpur, Selangor, Melaka, Negeri Sembilan, Johor, Sabah and Sarawak. Based on morphological and molecular characteristics (DNA sequences), the isolates were identified as *Colletotrichum sansevieriae* (44 isolates), *C. truncatum* (one isolate), *C. endophytica* (one isolate), *C. tropicale* (five isolates), *C. fructicola* (one isolate), *C. alienum* (six isolates), *Lasiodiplodia brasiliense* (one isolate), *L. hormozganensis* (eight isolates), *L. iraniensis* (one isolate), *L. pseudotheobromae* (three isolates), *L. theobromae* (two isolates), *Neoscytalidium dimidiatum* (eight isolates), *Fusarium oxysporum* (11 isolates), *F. solani* (two isolates), *F. brachygibbosum* (one isolate), *Stemphylium lycopersici* (two isolates), *Curvularia eragrostidis* (one isolate), *Cu. geniculata* (one isolate) and *Diaporthe tectonae* (one isolate). Identification of the *Colletotrichum* isolates were confirmed via phylogenetic analysis of combined internal transcribed spacer (ITS), β-tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequences. *Lasiodiplodia* species were verified based on phylogenetic analysis of combined ITS

and TEF1-α sequences, whereas *Fusarium* species were confirmed via phylogenetic analysis of TEF1-α sequences. Phylogenetic analysis of ITS sequences confirmed the species delineation of the isolates of *Neoscytalidium, Stemphylium, Curvularia* and *Diaporthe*. Seventy five isolates were selected based on morphological differences for pathogenicity tests. Pathogenicity tests revealed 66 out of 75 selected isolates were pathogenic towards *S. trifasciata*. Isolates of *C. tropicale, C. fructicola, F. solani* and *D. tectonae* did not form lesion on the inoculated leaves. Results of the present study indicated that there were numerous fungal species associated with diseased *S. trifasciata* in Malaysia.

CHAPTER 1

INTRODUCTION

Sansevieria trifasciata, a member of the family Asparagaceae is commonly known as snake plant or mother-in-law's tongue. It is an introduced, succulent, evergreen ornamental plant that is widely grown in Malaysia. This remarkably easy care plant is always a popular choice of plant for architecture and interior design, as well as for air filtering (Wolverton et al., 1989; Sriprapat et al., 2014). It can be found in pots, containers or beds in landscapes.

Despite being one of the toughest ornamental plants that can survive virtually any environmental conditions, *S. trifasciata* is infected by various diseases, especially fungal diseases. The most prominent disease of *S. trifasciata* worldwide is anthracnose caused by *Colletotrichum sansevieriae*. The disease was diagnosed 12 years ago in Japan following the discovery of the pathogen (Nakamura et al., 2006). Then, this pathogen-host relationship was revealed in several other countries, such as Australia (Aldaoud et al., 2011), India (Gautam et al., 2012), Korea (Park et al., 2013) and Iran (Karimi et al., 2017). The disease even greatly impacted *Sansevieria* productions in South Florida when the disease spreaded in several nurseries until the producers stopped growing the plants (Campoverde & Palmateer, 2012).

In addition, *S. trifasciata* was reported to be associated with other fungal diseases such as leaf spot and rhizome rot. The pathogens were comprised of *Aspergillus, Lasiodiplodia*, *Fusarium, Sclerotium* and *Stemphylium* (Chase & Conover, 1981; Urtiaga, 1986; Henley et al., 1991; Ahmadpour & Poursafar, 2018).

For diagnoses of fungal diseases, some could be recognised from the symptoms observed such as rust, mildew and smut diseases. Otherwise, identification involving observations of the morphological characteristics such as colony appearance, pigmention on culture medium, conidial size and shape, and structures of conidiomata under microscope may be required (Waller, 2002; Agrios, 2005).

Even though the fungal taxonomy has until recently been based on morphological characteristics, molecular and phylogenetic inference involving analysis of sequence data for targeted DNA loci of the plant pathogenic fungi has considerably increase the resolution of species discrimination. Such approaches enable more accurate and authentic fungal taxonomy especially when dealing with overlapping or contradicting morphological characteristics (Cai et al., 2009; Hyde et al., 2009; Phillips et al., 2013).

For molecular characterisation of fungi, the internal transcribed spacer (ITS) is the most prominent fungal marker gene as it has the highest possibility to successfully identify a broad range of fungi. It also clearly defined the barcode gap between inter- and intraspecific variations (Schoch et al., 2012). Along with the emerging of molecular data for systematic classification of plant pathogenic fungi, analysis of multi gene loci including internal transcribed spacer (ITS), β-tubulin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and translation elongation factor 1-α (TEF1-α) has proved to give more definitive results, particularly when a species could not be resolved by a single gene (Cai et al., 2009; Hyde et al., 2014).

After identifying the fungi that presented in a diseased plant, their roles as either pathogenic or non-pathogenic inhabitants could be assessed by conducting pathogenicity test. Re-introduction of the isolated fungi back to a healthy host plant

followed by re-isolation of the inoculated fungi provide defining insights of the lifestyles of the fungi (Agrios, 2005).

As mentioned, *S. trifasciata* is widely distributed in Malaysia. At the same time, diseases of varying degree of severity and symptoms were observed over the plants. Despite the discoveries of various diseases on *S. trifasciata* in other countries, there is a lack of documentation of the fungal diseases or fungi associated with diseased *S. trifasciata* originated from Malaysia. Aside from inspection of disease symptoms and signs, characterisation and identification of the causal pathogen are some of the dominant approaches to diagnose a disease or even enable early detection of infection before the occurrence of epiphytotics. Rapid and correct diagnosis of disease is essential before proper control measures or management can be taken (Maloy, 2005; Martinelli et al., 2015).

1.1 Objectives

Therefore, the objectives of the present study were:

(i) to isolate and identify the fungi associated with diseased *S. trifasciata* in Malaysia based on morphological characteristics and DNA sequences;

(ii) to evaluate the phylogenetic relationships of fungal isolates based on DNA sequences;

(iii) to determine the pathogenicity of the fungal isolates towards *S. trifasciata*.

CHAPTER 2

LITERATURE REVIEW

2.1 *Sansevieria trifasciata*

Sansevieria trifasciata is a xerophytic, herbaceous plant with common names including snake plant, mother-in-law's tongue, viper's bowstring hemp, lidah buaya (in Malay) and hu wei lan (in Chinese) (Macmillan, 1991; Zhou et al., 2011). It is a succulent, acaulescent, upright perennial plant with slightly concave, lanceolate leaf (30.5-121.9 cm long and 2.5-10.2 cm wide) arises from narrowed, thickened base to tapered apex. The variegated leaf has transverse, contrasting light and dark green bands (Figure 2.1A). Inflorescences are pedunculate, 30-75 cm long and flowers are borne on pedicels, yellowish-white or greenish-white in colour, solitary or in clusters of two or three (Figure 2.1B and C). Fruits are subglobose to oblong ellipsoid, bright orange in colour when matured (Stover, 1983; Acevedo-Rodríguez & Strong, 2005) (Figure 2.1D).

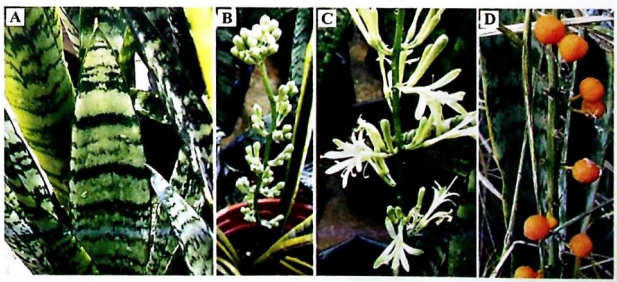

Figure 2.1 *Sansevieria trifasciata.* **(A)** Variegated leaves; **(B)** inflorescence; **(C)** flowers in clusters; **(D)** mature fruits[i].

(Source: [i]Navie, 2016)

Bantel's Sensation, Moonshine, Hahnii, Golden Hahnii, Robusta and Futura are among the varieties of *S. trifasciata* based on their leaf characteristics (Henley, 1982) (Figure 2.2). Another popular variety of this species which characterised by yellow margin along the leaf edges is *S. trifasciata* var. *laurentii*. The variegated form of this chimera is due to regional chlorophyll deficiency in the leaf (Blaydes, 1953).

Figure 2.2 Varieties of *S. trifasciata*. **(A)** *S. trifasciata* var. *trifasciata*[i]; **(B)** *S. trifasciata* var. *laurentii*[i]; **(C)** *S. trifasciata* var. 'Bantel's Sensation'[i]; **(D)** *S. trifasciata* var. 'Moonshine'[i]; **(E)** *S. trifasciata* var. 'Hahnii'[i]; **(F)** *S. trifasciata* var. 'Golden Hahnii'[i]; **(G)** *S. trifasciata* var. 'Robusta'[ii]; **(H)** *S. trifasciata* var. 'Futura'[ii].

(Sources: [i]Llifle, 2005; [ii]Longwood Gardens, 2012)

Sansevieria trifasciata could be propagated by using leaf cuttings and rhizome divisions. When leaf cutting is used as a propagule, *S. trifasciata* var. *laurentii* will lose its variegation and grows without the yellow margin on leaves, whereas rhizome division would produce leaves typical of parent plant. To propagate

using tissue culture method, all parts of leaf (apical, middle and bottom) can be used as they showed no significant effect on shoot proliferation rate and average shoot length (Sarmast et al., 2009).

Sansevieria trifasciata thrives in well-drained soil with high organic content in shady areas but tolerates varying light and water conditions. Besides attractive appearance, its adaptation to different habitats and requirement of minimum care makes it suitable for and favoured by most people as houseplant as well as for landscaping.

2.1.1 Taxonomic classification

According to Brown (1915), the genus *Sansevieria* comprised 54 species. Till 2005, new species were discovered with a total of 60 species included in the genus and increased to 73 species in recent years, with many cultivars or varieties (Takawira & Nordal, 2001; Acevedo-Rodríguez & Strong, 2005; Baldwin & Webb, 2016).

The classification of *Sansevieria* had been disputed due to divergences between authors regarding morphological characterisations, phylogenetic relationships and taxonomic interpretations. Lu & Morden (2014) considered *Sansevieria* as part of the genus *Dracaena* but several authors recognised it as an individual genus. One of the distinct features was *Sansevieria* being herbaceous plant with creeping rhizomes and succulent leaves, in contrast to *Dracaena* as a shrubby plant with woody stem and relatively thin leaves. Palynologically, *Sansevieria* was also being treated as a separate taxon from *Dracaena* (Brown, 1915; Acevedo-Rodríguez & Strong, 2005; Walters et al., 2011; Klimko et al., 2017, Klimko et al., 2018).

The various placements of *Sansevieria* which were not tenable included families Liliaceae (Brown, 1915; Benson, 1957; Stover, 1983), Agavaceae (Hutchinson, 1959; Benson, 1962), Ruscaceae (APG II, 2003), Dracaenaceae (Acevedo-Rodríguez & Strong, 2005; Walters et al., 2011), and currently Asparagaceae. The family Asparagaceae belongs to the order Asparagales under Monocots (APG III, 2009; USDA-ARS, 2014; Baksh-Comeau et al., 2016). In spite of that, Benson (1962) suggested the eligibility of removing the genus from Agavaceae was based on some structural characters of the fruits and seeds. For authors who considered the genus belonged to Dracaenaceae, *Sansevieria* was distinguished by its mucilage-filled cells with crystal raphides in leaves of certain species of the genus (Kim et al., 2010). In 2009, Ruscaceae was combined into the family Asparagaceae by researchers to reduce confusion when such families show not much distinctive features phylogenetically (APG III, 2009). Occasionally, *S. trifasciata* has been called as *S. zeylanica*, which has been bewildered with several species of the genus (Jacobsen, 1960). Based on Integrated Taxonomic Information System (ITIS, 2018), *S. trifasciata* can be classified as below:

Kingdom: Plantae – plants
Subkingdom: Viridiplantae
Infrakingdom: Streptophyta – land plants
Superdivision: Embryophyta
Division: Tracheophyta – vascular plants
Subdivision: Spermatophytina – spermatophytes, seed plants
Class: Magnoliopsida
Superorder: Lilianae – monocotyledons
Order: Asparagales
Family: Asparagaceae
Genus: *Sansevieria*
Species: *Sansevieria trifasciata*

2.1.2 Distribution and significance

Sansevieria trifasciata is a cosmopolitan plant native to Africa and widely cultivated around the world, including United States of America, Australia, Anguilla, Puerto Rico and Democratic Republic of the Congo among other countries. This plant is usually cultivated in gardens or pots, but it was also found growing in natural vegetation in KwaZulu-Natal Province of South Africa (Acevedo-Rodríguez & Strong, 2005; GISD, 2010; Walters et al., 2011). The economic value of *Sansevieria* plant is fairly high. At least 12 million cuttings were produced annually for local market as well as exports from Malaysia (Musa, 2005). *Sansevieria* was also one of the top five most imported ornamental plants originating from East Asia in the Netherlands (van Valkenburg et al., 2014). Besides being well known for its aesthetical value as ornamental plant, *S. trifasciata* is usually used for other purposes as well.

Sansevieria trifasciata was one of recommended air purifying plants by NASA that removed trichloroethylene (TCE), benzene and formaldehyde, indoor air pollutants (Wolverton et al., 1989). Besides, *S. trifasciata* was efficient in removing toluene from surrounding air (Sriprapat et al., 2014). It could also be used to absorb humidity in small type houses where the plant absorbs humidity from the environment as a source of water and undergoes photosynthesis via Crassulacean Acid Metabolism (CAM) mechanism. The process also indicated that the plant photosynthesised at night and produced oxygen at the same time, which made it suitable to be placed inside a room (Wahjutami et al., 2016).

Traditionally, the fibre extracted from the leaves of *S. trifasciata* was used by natives for bowstrings. It was also a material used for fine mats and twine weaving

(Macmillan, 1943). In more recent times, fibre of *S. trifasciata* has high potential to serve as a renewable source, environment-friendly substitute for synthetic materials and compensates the increasing demand of natural fibres in textile industry. This fibre has physical properties that are suitable for textile applications, such as good strength with low elongation and high fibre fineness. In addition, it is capable of withstanding high temperature without fibre degradation. Examples of producible products are ropes, handicrafts, sacks and mattresses (Kanimozhi, 2011; Kant & Alagh, 2013).

In medicinal aspects, *S. trifasciata* had been proven to have therapeutic effect on corns treatment. Higher concentration of the extract gave better effect with recovery time of involved patients who used 20% extract ointment was the shortest among other patient groups (Afrasiabian et al., 2016). Antidiabetic, analgesic and antipyretic potentials of *S. trifasciata* were also being evaluated on rats as animal models with assured results. Compounds such as phenolics, saponins, flavonoids, alkaloids, terpenoids, glycosides and steroids were found present in the plant extracts (Sunilson et al., 2009; Qomariyah et al., 2012; Dey et al., 2014).

The leaves of *S. trifasciata* were also used as ethnomedicine to reduce ear pain and inflammation in Malaysia (Samuel et al., 2010). The anti-inflammatory effect of *S. trifasciata* extract was also demonstrated by Anbu et al. (2015). In the evaluation, extract using methanol extraction showed significant anti-inflammatory effect, followed by aqueous extract and petroleum ether extract (Anbu et al., 2015). As traditional Chinese medicine, the plant (leaf part) is used for indications like poisonous snake bite, knocks and falls, lung heat cough and common cold. It is able to disperse swelling, clear heat and resolve toxin in the body (Zhou et al., 2011).

Moreover, *S. trifasciata* exhibited prospective control over harmful algal bloom, explicitly involving *Alexandrium tamiyavanichii*. Extracts of fresh and dried plants from aqueous extraction could inhibit the growth of *A. tamiyavanichii* significantly with suitable extract's concentration (Noor et al., 2018). Occurrence of the bloom impacts the aquaculture industries and can be harmful to animal health. In Malaysia, *A. tamiyavanichii* was reported to be responsible for three hospitalisations involving contaminated mussels in Melaka (Lim et al., 2012).

2.1.3 Diseases associated with *Sansevieria*

Like most other angiosperms, *Sansevieria* is susceptible to a number of diseases with symptoms such as rhizome rot and soft rot. In particular, diseases with various symptoms such as anthracnose, leaf spot, Pythium rot, leaf blight and soft rot of *S. trifasciata* are illustrated in Figure 2.3 and documented as described in the following sub-sections.

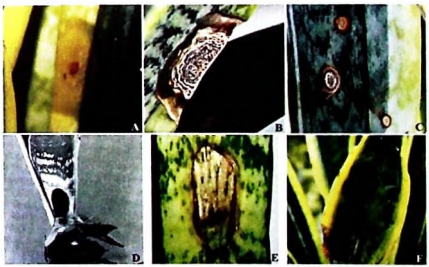

Figure 2.3 Disease symptoms on *Sansevieria trifasciata*. **(A)** Water-soaked lesion on infected leaf caused by *Colletotrichum sansevieriae*[i]; **(B)** anthracnose caused by *Colletotrichum sansevieriae*[ii]; **(C)** leaf spots caused by *Colletotrichum neosansevieriae*[iii]; **(D)** Pythium rot caused by *Pythium spinosum*[iv]; **(E)** leaf blight caused by *Stemphylium vesicarium*[v]; **(F)** bacterial soft rot caused by *Pectobacterium carotovorum*[vi].

(Sources: [i]Campoverde & Palmateer, 2012; [ii]Palmateer et al., 2012; [iii]Crous et al., 2015a; [iv]Takeuchi et al., 2002; [v]Ahmadpour & Poursafar, 2018; [vi]Maung & Bugwood, 2011)

2.1.3(a) Anthracnose

Anthracnose is the most common fungal disease associated with *S. trifasciata* worldwide. The symptom is typically observed as water-soaked lesions with orange conidial masses and erumpent black acervuli (Figure 2.3A and B). In 2006, a new fungal species known as *Colletotrichum sansevieriae* was first discovered in Japan causing anthracnose on *S. trifasciata* (Nakamura et al., 2006). This pathogen was then reported to occur in Australia (Aldaoud et al., 2011), Florida (Campoverde & Palmateer, 2012; Palmateer et al., 2012), India (Gautam et al., 2012), Korea (Park et al., 2013), Costa Rica (Pérez-León et al., 2013) and Iran (Karimi et al., 2017).

2.1.3(b) Leaf spot

Another pathogen with high host specificity towards *S. trifasciata* is *C. neosansevieriae*. This pathogen was documented as the causal agent of leaf spot disease of *S. trifasciata* in South Africa. Both *C. sansevieriae* and *C. neosansevieriae* are morphologically similar but phylogenetically clearly discrete. As *C. neosansevieriae* leaf spots coalesced with age, with visible signs on *S. trifasciata,* the symptom looked similar to those caused by *C. sansevieriae* (Figure 2.3C). In spite of that, the occurrence of *C. neosansevieriae* on *S. trifasciata* was only documented once which was from South Africa (Crous et al., 2015a).

In China, leaf spot on *S. trifasciata* was detected when sunken brown lesions with the presence of black pycnidia were observed on diseased *S. trifasciata* in an ornamental market in Luoyang City in February 2011. In this case, *Chaetomella* sp. was identified as the causal agent of the disease symptom (Li et al., 2013).

Besides, leaf spot on *Sansevieria* (Bowstring hemp) caused by *Fusarium moniliforme* was also reported. The fungus caused sunken reddish brown spots (up to 0.5 inch in diameter) with yellowish borders on either one side of the leaf or extended through it. When numerous spots merged together and encircled the leaf, the infected part above dried and died (Pirone, 1978).

2.1.3(c) Pythium rot

Diseases on *S. trifasciata* are not only detected in recent years. A study by Takeuchi et al. (2002) revealed *Pythium spinosum* as the pathogen causing Pythium rot of *S. trifasciata* var. 'Laurentii Compacta' in Japan in the year 1999. For plants infected by the pathogen, the basal part of leaf and even root turned brown to dark brown, macerated, with roots became fall out in severe cases (Takeuchi et al., 2002) (Figure 2.3D).

2.1.3(d) Root knot

In New Delhi, India, diseased *S. trifasciata* that showed stunted growth and formation of irregular root knot galls were detected. After examining the infected roots, the causal pathogen was identified as a nematode named *Meloidogyne incognita.* The nematode in various life stages, to wit, egg masses, second-stage juveniles and adults were found in the infected roots (Misra et al., 2002). This pest and host interaction had also been reported earlier in Texas and a nursery in north Florida (Mumford, 1959; Stokes & King, 1972).

2.1.3(e) Leaf blight

According to literatures, there was a fungal species which was identified as the pathogen causing leaf blight of *S. trifasciata.* Infections by *Stemphylium*

vesicarium were reported to occur in Iran. The pathogen caused grey to pale brown elongated lesions with brown margins on the infected leaves (Ahmadpour & Poursafar, 2018) (Figure 2.3E).

2.1.3(f) Rot

Apart from fungal diseases, *Sansevieria* was prone to bacterial infections. *Erwinia aroideae* and *E. carotovora* were identified and reported to cause bacterial soft rot on *Sansevieria* (Horst, 2013). The infected basal leaf tissues softened, became watery and putrid. Bacterial soft rot caused by *Pectobacterium carotovorum* was detected on *Sansevieria* in Singapore (Maung & Bugwood, 2011) (Figure 2.3F).

2.1.4 Disease control management of *Sansevieria*

Several species of the genus *Sansevieria* were grown commercially for ornamental purposes in many parts of the world. The occurrences of plant diseases lead to production losses and economic value reduction, especially in nursery situations.

In order to manage the fungal diseases as well as bacterial soft rot of *Sansevieria*, effective preventative approaches should be implemented in nurseries. One of the approaches was modifying the irrigation system to avoid creating overly humid environment which favoured the growth and spreading of pathogen. Frequent inspection for early symptom development was also important. The diseased leaf should be eradicated under dry conditions. Other than maintaining a weed free environment and using well-drained soil in the nurseries, good sanitation and disinfection ought to be practised while handling the disposal of diseased plants (Pirone, 1978; Campoverde & Palmateer, 2012). Propagation from diseased plants

should be strictly avoided. When disease occurrence was discovered, steam sterilised the soil before growing new plants (Henley et al., 1991).

The fungal disease problem could also be managed using chemical control. Chemicals such as pyraclostrobin + boscalid and thiophanate-methyl + mancozeb, had been tested positive as preventive treatments on anthracnose caused by *C. sansevieriae* whereas epoxiconazole + carbendazim, azoxystrobin and carbendazim + mancozeb were relatively more effective in inhibiting the pathogen establishment (Campoverde & Palmateer, 2012; Pérez-León et al., 2015).

Two new fungicides were introduced in 2016, namely Mural (azoxystrobin + benzovindiflupyr) and Orkestra (pyraclostrobin + fluxapyroxad). Both fungicides worked well in controlling anthracnose of *Sansevieria* caused by *Colletotrichum* (Chase & Palmateer, 2016). However, rapid growth in terms of production and usage of fungicides, especially which from the succinate dehydrogenase inhibitor (SDHI) class, had raised concern about resistance development, and thus resistance management or prevention was recommended. According to manufacturers' recommendations, fungicides should be applied at effective rates, crop stages and appropriate intervals. Mixtures or alternation of fungicides from different classes were also applicable when required (McKay et al., 2011; Sierotzki & Scalliet, 2013).

Besides the application of fungicides for fungal disease management of *Sansevieria*, nematicides namely Mocap, Nemacur and Vydate were used to control root-knot nematode infection on *S. trifasciata*. Treatments could be applied using drench application or assimilation of chemical granules into a steam sterilised soil (Stokes & King, 1972). Apart from nematicide, a suitable amount of trichothecene

(about 500 ppm) could be applied as a nematostatic agent to inhibit nematode infection of *Sansevieria* (Devidas & Rasmussen, 1991).

2.2 Fungi as plant pathogens

Fungi are small, generally minuscule or microscopic, eukaryotic, heterotrophic, usually filamentous and spore-bearing organisms that do not have chlorophyll. The majority from all fungal species that have been described is from Ascomycota (about 64000 known species) followed by Basidiomycota (about 32000 known species). More than 10000 fungal species are pathogenic and can cause plant diseases (Agrios, 2005; Moore et al., 2011). Collectively, fungi cause lots of plant diseases with about 85% of which were caused by fungi (Pernezny et al., 2008).

For most phytopathogenic fungi, they commonly disseminated through spore or conidia. Generally, wind, water, insects, apparatus, humans and other animals could be the agents that help in spreading of plant pathogenic fungi within the same plant or different plants and to other locations (Agrios, 2005).

Some pathogenic fungi produce special structures such as chlamydospores and sclerotia for survival during extreme conditions and remain viable which then germinate when the environment is favourable. For example, *Fusarium* produces chlamydospores while *Rhizoctonia* produces sclerotia. Some surviving structures astonishingly induce a higher degree of disease severity, which indicates those surviving structures have higher inoculum potential compared to conidia (De Cal et al., 1997; Ritchie et al., 2013).

2.2.1 Lifestyles of plant pathogenic fungi

Some plant pathogenic fungi depend strictly on their hosts and are known as

biotrophs. In order to complete their life cycles, they rely on living hosts for reproduction and growth. Instead of killing the host plants, they keep the hosts alive for continuous nutrients supply during infection. Examples of biotrophs are mildew, smut and rust fungi such as *Puccinia graminis, Uromyces fabae* and *Hemileia vastatrix*. Crop yield loss usually comes as the consequences of the occurrence of these biotrophs (Staples, 2000; Schulze-Lefert & Panstruga, 2003; Voegele, 2006; Lewis et al., 2018).

Contrary to biotrophs, plant pathogenic fungi which are necrotrophs kill the hosts during or prior to colonisation and feed saprotrophically on the contents (van Kan, 2006; Li Wen, 2013). *Botrytis cinerea, Sclerotinia sclerotiorum* and *Fusarium oxysporum* are examples of fungal necrotrophs that caused bunch rot of grapes, stem and crown rot of berseem, and wilt of tomato plants, respectively (Jacobs et al., 2013; Javed et al., 2017; Saira et al., 2017).

Nevertheless, most fungi live within healthy plant tissues as endophytes without any visible disease symptoms. These fungi establish varying symbiotic and ecological relationships which are usually mutualistic with the colonised plants (Rodriguez et al., 2009). Under certain circumstances, some endophytic fungi may be considered as latent or opportunistic pathogens. This could happen especially when host plants are under stress due to dramatic climate changes, disturbed or non-optimal growing environments, or when the respective beneficial endophytes are absent. Fungal species in the family Botryosphaeriaceae such as *Botryosphaeria dothidea* and *Neofusicoccum australe* were reported as endophytes or latent pathogens which became aggressive and infectious during the onset of stress conditions (Slippers & Wingfield, 2007; Dakin et al., 2010; Marsberg et al., 2017).

2.2.2 Ascomycota

Ascomycota is the largest group in the Kingdom of Fungi. It is a phylum encompassing many diseases caused by plant pathogens. Fungi of this phylum produce usually sexual spores called ascospores within an ascus, as well as asexual spores called conidia. Most of these fungi infect plants with conidia, which is during asexual stage. The sexual structures are usually produced on or in infected plant organs or tissues when the growing season comes to an end or the environment is unfavourable with scarce food supply (Agrios, 2005; Moore et al., 2011). In other cases, some fungi infected host plants during sexual stage before the rapidly producing conidia further infecting within the host (Vaillancourt & Hartman, 2000) (Figure 2.4).

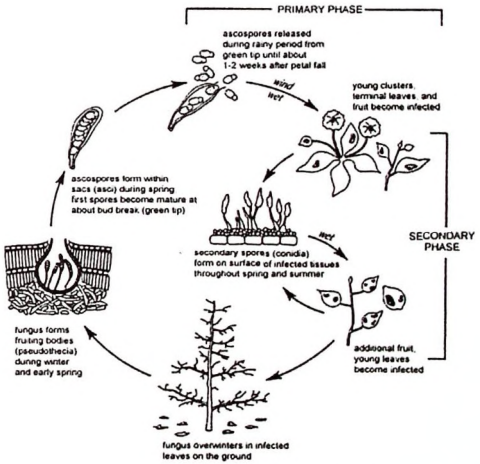

Figure 2.4 Disease cycle of *Venturia inaequalis* causing disease with symptom of apple scab.

(Source: Vaillancourt & Hartman, 2000)

Historically, many agriculturally important pathogenic fungi were classified into Deuteromycetes based on morphology. However, Deuteromycetes is no longer accepted as a taxonomic rank in current fungal taxonomy as numerous fungi from this group were in fact the anamorphs of those from Ascomycota. Even for anamorphic fungus lacking sexual form, analyses of DNA sequences and phylogeny are able to relate it to the respective teleomorphic genera. For instance, *Aspergillus niger* and *Stemphylium lycopersici* with no known sexual cycle are phylogenetically close or clustered in a monophyletic group with the teleomorphs once named *Emericella nidulans* (anamorph: *A. nidulans*) and *Pleospora allii* (anamorph: *S. vesicarium*), respectively (Câmara et al., 2002; Samson et al., 2014; Spatafora et al., 2017).

Infection or colonisation by ascomycete usually causes stunted or abnormal growth of plant organs or entire plants. The disease symptoms include leaf curl, powdery mildew, cankers, leaf spot, blight, anthracnose, wilt and scab (Agrios, 2005).

A number of diseases associated with ornamental plants caused by ascomycetes were documented around the world. *Yucca filamentosa* planted in two ornamental nurseries in Minas Gerais, Brazil was found infected by *Phyllosticta yuccae* with symptom of leaf spot (Silva et al., 2013). Anthracnose of pleomele (*Dracaena reflexa*) and Chinese evergreen (*Aglaonema crispum*) caused by *Colletotrichum gloeosporioides* was detected in a nursery and garden, respectively in India (Banerjee et al., 2017; Mounika et al., 2017). In addition, *C. liriopes* was reported to cause leaf anthracnose on *Rohdea japonica* in the United States (Trigiano et al., 2018).

2.3 Morphological characterisation of plant pathogenic fungi

Generally, morphological characterisations of plant pathogenic fungi are the primary assessment in fungal identification and classification. Macroscopically, culture characteristics of fungi on agar media including colony morphology (surface texture and colour), pigmentation and mycelial growth rates are usually being assessed. Potato dextrose agar (PDA) produces reasonably consistent colony characteristics. It has been extensively used as a medium for characterisation of various fungal genera, such as *Colletotrichum*, *Fusarium* and those in the Botryosphaeriaceae (Leslie & Summerell, 2006; Weir et al., 2012; Phillips et al., 2013).

Other than culture morphology, fungal identification has long relied on microscopic criteria such as morphology of hyphae (septate or aseptate), asexual and sexual reproductive structures. Identifying those characteristics were the essential steps when using fungal identification keys. Ellis (1971) once pointed out that morphological identification of fungi relied mainly on experience gained by examining enormous specimens and isolates, as well as information gleaned from available monographs or standard works done as reference. Characters of special taxonomic significance were particularly vital to enhance the diagnostic value (Ellis, 1971). In some cases, the varieties of certain fungal species were able to be distinguished by examining the unique and typical structures formed (Peixoto et al., 2013).

Apart from characterising the phytopathogenic fungi isolated and cultured on culture media, diseased or symptomatic plants could be examined directly, whether any visible signs present on the hosts. Many plant diseases were diagnosable based

on ascomata or conidiomata appeared on the surfaces (Sharifi et al., 2014). In other words, observation of disease signs such as perithecia and ascospores, as well as penetrating organ if exists, designate the possible identity of the phytopathogenic fungi (Peixoto et al., 2013; Guyot & Eveno, 2015).

In the past, fungal morphology had been widely illustrated in hand drawn illustrations or guide books, in addition to the descriptions in words (Ellis, 1971; Coomaraswamy & de Fonseka, 1981; Barnett & Hunter, 1998). Rapid development of tools and techniques in the later years had improved the quality of characterisation of fungal morphology progressively. Digital image analysis systems with microscope, image capture device and analytic software packages permitted a more comprehensive and detailed examination of morphological characteristics and extraction of quantitative information such as measurement (Papagianni, 2014).

Despite the advantages of morphological characterisation such as distinctive, easily discernible under light microscope, illustratable and cost effective, characterising and classifying fungi based on morphology solely is challenging when a fungus does not sporulate or worse (cannot be cultured) on common culture medium (Su et al., 2012). Moreover, characterisation of the sexual structure becomes difficult when a teleomorph could not be produced *in vitro* or under controlled condition (Trapero-Casas et al., 2012; Sharifi et al., 2014).

Phillips et al. (2013) suggested that morphological characteristics alone were inadequate to elucidate fungal species or even genera. Several fungi had conidia indistinguishable from each other within the same genus or even family. Crouch et al. (2009b) had also indicated the meagreness of morphological characters such as shape and size of conidia and appressoria in taxonomic classification of *Colletotrichum*.

The same goes for culture morphology which varied and inconsistent due to growth conditions and storage. These criteria should be evaluated with cautions over the types of culture media and growth conditions (Crouch et al., 2009b). Even with unvarying culture media and growth conditions, the culture morphology can vary extensively between isolates of a species (Alves et al., 2008; Abdollahzadeh et al., 2010; Ismail et al., 2012).

Therefore, assembling morphological characterisation and other parameters like molecular and phylogenetic analysis in modern fungal identification is necessary. The correct identification of fungi is of substantial practical importance plus it is also important to understand the evolution of morphological characters (Raja et al., 2017).

2.4 Molecular characterisation of plant pathogenic fungi

To overcome the limitation of morphological approach, DNA barcoding alone or which done along with phylogenetic analysis using one or multiple genes have become reliable and powerful tools to characterise and classify plant pathogenic fungi. Species identification using DNA barcoding is denoted by the employment of standardised, universal and short specific DNA sequences, or known as barcodes in comparative research with a well-established reference database. The rapid development of primers has also benefited the application of barcoding (Stielow et al., 2015; Meyer et al., 2019). DNA barcoding followed by sequence comparisons with existing sequences deposited in sequence databases such as NCBI (National Center for Biotechnology Information) and Fusarium-ID are usually being applied when characterising or identifying fungal species, with some findings which backed by phylogenetic analysis (Sun et al., 2016; Cardoso et al., 2017; Du et al., 2017; Lin et al., 2017; Munirah et al., 2017; Tariq et al., 2017). For fungal taxonomy involving

novel species, both DNA barcoding and phylogenetic analysis are often indispensable (Burgess et al., 2006; Alves et al., 2008; Abdollahzadeh et al., 2010; Manamgoda et al., 2012a; Huang et al., 2016).

In current classification within the kingdom Fungi, phylogenetic analysis had shown numerous morphological-based classifications were actually more diversified and were frequently paraphyletic (Spatafora et al., 2017). Given its high qualities in uniformity, reliability and advancement, sequence-based phylogenetic study has gain more popularity for not only identification, but also to understand the evolutionary relationships (Shenoy et al., 2007). In aspect of plant pathology, Hyde et al. (2014) presented a treatise on phylogenetic backbone trees of phytopathogenic fungal species. The commonly applied methods include Neighbor-Joining (NJ), Maximum Likelihood (ML) and Maximum Parsimony (MP). NJ is a prominent distance-based method, an efficient and fast way of classifying organisms into phylogenetic trees. It was also the most highly cited phylogenetic method (Van Noorden et al., 2014). Besides, NJ and its variant (BIONJ) have been widely used to produce starting points for more complicated methods (Guindon et al., 2010; Nguyen et al., 2015). On the other hand, ML and MP are character-based methods that based on probabilistic models and minimisation of number of character changes, respectively. In term of tree reconstruction accuracy, ML actually outperformed NJ and MP, especially in case like increase of multiple sequence alignment error (Ogden & Rosenberg, 2006; Yang & Rannala, 2012).

Internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) is a suitable universal DNA barcode marker for molecular identification of most fungi (Schoch et al., 2012). The ITS region is situated between the 18S small subunit and 28S large subunit genes in the nuclear DNA repeat unit, comprises ITS1

and ITS2 regions, separated by the 5.8S gene (Bellemain et al., 2010) (Figure 2.5). Analysis of ITS sequence data had been extensively used to identify various fungal species. For instance, *Neoscytalidium dimidiatum, Neoscytalidium orchidacearum*, *Stemphylium lycopersici* and *Curvularia clavata* (Huang et al., 2016; Sanahuja et al., 2016; Zhong et al., 2016; Yang et al., 2017a).

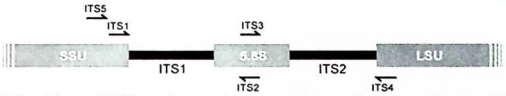

Figure 2.5 Position of ITS region and commonly used primers for amplifying this region.

(Source: Bellemain et al., 2010)

The translation elongation factor 1-α (TEF1-α) gene is useful and informative for identification of *Fusarium* at species level (Figure 2.6). It is a gene that encodes an essential part of the protein translation machinery and non-orthologous copies of the gene have not been discovered in the genus. For *Fusarium*, this gene performs effective DNA barcoding function by being matched to the sequences in database of Fusarium-ID because this database serves similarly as GenBank, but with accurately identified and validated sources (Geiser et al., 2004; O'Donnell et al., 2010). Combined sequence data of partial ITS region and TEF1-α gene were recommended to characterise and identify species within the genus *Lasiodiplodia* (Burgess et al., 2006; Alves et al., 2008; Abdollahzadeh et al., 2010; Ismail et al., 2012; Hyde et al., 2014).

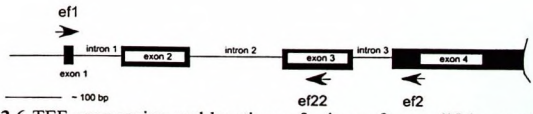

Figure 2.6 TEF gene region and locations of primers for amplifying parts of this gene.

(Source: Geiser et al., 2004)

Colletotrichum has long been reported to be associated with a wide host range of plants as symptomatic pathogen or asymptomatic endophyte (Photita et al., 2005; Rojas et al., 2010; Rabha et al., 2016; Shivas et al., 2016). Nonetheless, the genus comprises several species complexes such as *C. acutatum*, *C. boninense*, *C. gigasporum* and *C. gloeosporioides*, which require multi-locus sequence dataset for accurate species identification and classification. The universal fungal barcode marker ITS alone is insufficient to resolve species in this genus, but it separates the taxa into species complexes. For species delineation, phylogenetic analyses of multiple genes including β-tubulin 2 (*TUB2*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), actin (*ACT*) and calmodulin (*CAL*) among other markers are required to give more reliable result (Damm et al., 2012a; Damm et al., 2012b; Weir et al., 2012; Liu et al., 2014) (Figure 2.7).

Figure 2.7 β-tubulin gene and locations of primers for amplifying parts of this gene.

(Source: Glass & Donaldson, 1995)

Although analysis of DNA phylogeny could compensate for the weaknesses of morphological characterisation, the importance of the latter criterion cannot be overlooked. For example, species discrimination with the absence of extant type specimen can only rely on the morphological characteristics in original descriptions and their miscellaneous synonyms, or representatives derived from those descriptions (Abdollahzadeh et al., 2010; Cannon et al., 2012; Phillips et al., 2013).

2.5 Pathogenicity test

Pathogenicity test plays an important role based on Koch's postulates in considering a microorganism as a cause of disease. The pure culture of the suspected causal agent must produce the specific disease after being inoculated onto a healthy susceptible host plant.

The necessity of introducing a pathogen into a host in determining the cause of a disease is also illustrated in the disease triangle which comprised of pathogen, host and environment. Each of the three major components plays a significant role in determining the cause of a disease and subsequently its degree of severity (Agrios, 2005).

Another factor concerning pathogenicity test is the environmental condition. Temperature and humidity are the most important factors that influence the survival, germination, establishment and virulence of pathogenic fungi. In order to germinate, spores landed on a host plant require a condition with high relative humidity and a favourable temperature. The available moisture shall sustain until the pathogen penetrates or accommodates the host successfully. Relative humidity of approximately 85% especially at the early stage of invasion was essential for pathogenicity tests on *S. trifasciata* (Li et al., 2013; Ahmadpour & Poursafar, 2018).

Plant pathogenic fungi enter host plants through either natural openings or wounds. Of those fungi that enter the hosts through natural opening, some of them form a specialised structure known as appressorium for adhesion when in contact with the plant surface. Otherwise, fungi require wounds or openings formed due to mechanical forces such as pruning, animal feeding or wounds caused by other pathogens (Agrios, 2005). As for pathogenicity tests on *S. trifasciata* involving