MICROPROPAGATION FOR *Ficus carica* L. CV. PANACHEE USING LIGHT-EMITTING DIODES SYSTEM

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MICROPROPAGATION FOR *Ficus carica* L. CV. PANACHEE USING LIGHT-EMITTING DIODES SYSTEM

by

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
µ mol/s	Micromole per second
µg/mL	Microgram per microlitre
µL	Microlitre
µM	Micromolar
2-iP	N6-(Δ2-isopentenyl) adenine
A	Absorbance
A_{470}	Absorbance at 470 nm
A_{595}	Absorbance at 595 nm
A_{646}	Absorbance at 646 nm
A_{663}	Absorbance at 663 nm
AC	Activated charcoal
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BAP	6-benzylaminopurine
BBD	Box-Behnken Design
bp	Basepair
BR	Blue and red
BSA	Bovine serum albumin
CIHEAM	Centre International de Hautes Etudes Agronomiques Méditerranéennes
CL	Contour line
cm	Centimetre
Cur	Curve
D	Composite desirability
DAMD	Directed amplification of minisatellite DNA

DNA	Deoxyribonucleic acid
DOE	Design of experiment
EDTA	Ethylenediaminetetraacetic acid
FAA	Formalin: Acetic acid: 95% alcohol
FH	Final height
FR	Far-red
g/L	Gram per litre
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
incubati	Incubation
intensit	Intensity
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter simple sequence repeats
L. cv.	Linnaeus cultivated variety
LED(s)	Light-emitting diode(s)
MANOVA	Multivariate analysis of variance
mg/L	Milligram per litre
mL	Millilitre
mm	Millimeter
MS	Murashige and Skoog
NAA	Naphthalene acetic acid
nm	Nanometer
NoS	Number of shoots
ºC	Degree Celsius
OFAT	One-factor-at-a-time
PCR	Polymerase chain reaction
PGR(s)	Plant growth regulator(s)

pH	Potential hydrogen
PSII	Photosystem II
R^2	R-squared, coefficient of determination in regression
RAPD	Random amplified polymorphic DNA
RGB	Red: green: blue
RP	Rooting percentage
RSM	Response surface methodology
RSP	Response surface plot
SAM	Shoot apical meristem
SE	Somatic embryogenesis
SI	Similarity index
SPAR	Single primer amplification reaction
SSR	Simple sequence repeats
T_a	Annealing temperature
TBA	Tert-butyl alcohol
TBE	Tris-Borate-EDTA
TDZ	Thidiazuron
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
WPM	Woody plant medium

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MIKROPROPAGASI *Ficus carica* L. CV. PANACHEE MENGGUNAKAN SISTEM DIOD PEMANCAR CAHAYA

ABSTRAK

Propagasi *Ficus carica* L. melalui kaedah propagasi konvensional didapati mencabar dan memakan masa kerana kadar kelangsungan hidup yang rendah. Matlamat kajian ini adalah untuk mewujudkan suatu protokol dioptimumkan menggunakan metodologi permukaan balas (RSM) untuk mikropropagasi dan kultur meristem ke atas *F. carica* L. cv. Panachee di bawah aplikasi sistem diod pemancar cahaya (LED) pada pelbagai spektrum. Dalam eksperimen rekaan Box-Behken (BBD) yang melibatkan 3 faktor signifikan, kultur meristem dioptimumkan dengan inkubasi tisu meristem (0.5 – 1.0 mm) pada jambatan kertas turas yang sebahagiannya direndam dalam media cecair Murashige dan Skoog (MS) ditambah dengan 50 µM zeatin (X_1) di bawah LED merah di bawah intensity 1.26 µ mol/s (X_3) ($Y_{meristem*merah} = 0.882 + 0.271X_1 - 0.250X_{3*merah} – 0.558X_1X_{3*merah}$). Untuk mikropropagasi, eksperimen BBD yang melibatkan 4 faktor yang signifikan digunakan untuk mengembangkan model matematik urutan kedua yang menghasilkan pertumbuhan dan perkembangan yang boleh dibandingkan pada spektrum LED yang berbeza. Protokol mikropropagasi yang dioptimumkan dengan selang empat minggu untuk setiap peringkat dapat diprogramkan dengan sistem SMART LED dengan inkubasi pada MS gel ditambah dengan 30 µM IAA (B) dan arang teraktif 1.0 g/L (C) di bawah kombinasi LED merah, hijau, biru (MHB) pada 22.20 µmol/s (D_{MHB}) yang menghasilkan 1.88 pucuk (Bilangan pucuk $_{LED\ MHB} = 0.4148 + 0.1667B + 0.3000C + 0.2500C + 0.400BC + 0.350CD_{MHB}$). Selanjutnya, diikuti dengan pengoptimuman melalui MS gel yang diperkuat dengan 40 µM BAP (A), 30 µM IAA (B), dan arang teraktif 1.0 g/L (C) dengan LED merah yang mencapai kenaikan ketinggian terbesar pada 32.97 mm

setelah inkubasi selama empat minggu (Ketinggian akhir $_{\text{LED merah}}$ = 9.078 + 1.767A + 1.733B + 1.822C + 2.73A^2 + 4.68B^2 + 2.55AB + 2.95AC + 5.65BC). Akhirnya, peratusan pembentukan akar tertinggi dapat dicapai dengan subkultur ke MS gel yang dilengkapi dengan 40 µM BAP (A) dan arang teraktif 1.0 g/L (C) dalam kombinasi biru dan merah pada 1: 1 dengan pengamatan cahaya 44.80 µmol/s (D_{BM}) untuk memberikan 90.00% peratusan pembentukan akar (Peratusan pembentukan akar $_{\text{LED BM}}$ = 12.00 + 5.00A + 6.67C + 16.33A^2 + 35.00AC +15.00AD_{BM}). Analisis histologi didapati menentukan saiz tisu meristem, kajian anatomi untuk pembentukan akar, kesan arang teraktif, dan LED pada jarak gelombang yang berbeza. Dalam aklimatisasi peringkat awal didapati bahawa jarak gelombang LED yang berbeza menghasilkan perkembangan fisiologi anak pokok yang berbeza. Analisis biokimia seperti jumlah protin terlarut dan jumlah kandungan klorofil telah ditentukan dalam pelbagai rawatan LED. Analisis molecular dengan menggunakan DAMD dan ISSR didapati berkesan untuk mengkaji kestabilan genetik anak pokok *F. carica* L. cv Panachee yang diaklimatisasikan terlebih dahulu. Kultur tisu pokok tin yang dipatenkan dengan sistem LED SMART dapat dihasilkan secara komersial untuk penanaman ara untuk menghasilkan tanaman pokok tin bebas penyakit secara besar-besaran dan pertumbuhannya dapat ditingkatkan di bawah LED pada spektrum dan intensiti yang berbeza.

MICROPROPAGATION FOR *Ficus carica* L. CV. PANACHEE USING LIGHT-EMITTING DIODES SYSTEM

ABSTRACT

Ficus carica L. is challenging and time-consuming through conventional plant propagation methods due to the low survival rate. This study aims to establish an optimised protocol using response surface methodology (RSM) for micropropagation and meristem culture on *F. carica* L. cv. Panachee under light-emitting diodes (LEDs) application using different spectra. In 3-significant factors by Box-Behken design (BBD) experiment, meristem culture was optimised by incubating meristematic tissue (0.5 – 1.0 mm) onto filter paper bridge partially soaked in liquid Murashige and Skoog (MS) medium supplemented with 50 µM zeatin (X_1) under red LEDs at the intensity of 1.26 µ mol/s (X_3) ($Y_{meristem*red} = 0.882 + 0.271X_1 - 0.250X_{3*red} – 0.558X_1X_{3*red}$). For micropropagation, 4-significant factors by BBD experiment were used to developed second-order mathematical models, which resulted in incomparable growth and development at different LED spectra. Optimised micropropagation protocols with every four-weeks interval for each stage can be integrated with a SMART LED system with the incubation on gelled MS added with 30 µM IAA (B) and 1.0 g/L activated charcoal (C) under the combination of red, green, and blue (RGB) LEDs at 22.20 µmol/s (D_{RGB}) that resulted 1.88 shoots (Number of shoots $_{RGB\ LED} = 0.4148 + 0.1667B + 0.3000C + 0.2500C + 0.400BC + 0.350CD_{RGB}$). Subsequently, followed by optimisation through gelled MS fortified with 40 µM BAP (A), 30 µM IAA (B), and 1.0 g/L activated charcoal (C) under red LEDs to achieve the greatest height increment at 32.97 mm after four weeks (Final height $_{red\ LED} = 9.078 + 1.767A + 1.733B + 1.822C + 2.73A^2 + 4.68B^2 + 2.55AB + 2.95AC + 5.65BC$). Finally, the highest rooting percentage was achieved by subculturing the *in vitro* shoots onto the gelled MS

supplemented with 40 µM BAP (A) and 1.0 g/L activated charcoal (C) in the combination of blue and red at 1: 1 with the light intensity 44.80 µ mol/s (D_{BR}) to produce 90.00 % rooting percentage (Rooting percentage BR LED = 12.00 + 5.00A + 6.67C + 16.33A^2 + 35.00AC + 15.00AD_{BR}). Histological analysis was found to determine the size of meristematic tissue, anatomical study for root formation, effects of activated charcoal, and LEDs at different wavelengths. Pre-acclimatisation was found that different wavelengths of LEDs resulted in different physiological developments. Biochemical analyses such as total soluble protein and total chlorophyll content were determined in various LED treatments. Molecular analysis using DAMD and ISSR was found effective in studying the genetic fidelity of pre-acclimatised *F. carica* L. cv. Panachee. Patented fig tissue culture with SMART LEDs system can be commercially produced for fig plantation to produce disease-free fig plantlets at a large-scale, and the growth can be enhanced under LEDs at different spectra and intensities.

CHAPTER 1

INTRODUCTION

Ficus carica L. is a flowering plant from the family of Moraceae (Rønsted et al., 2005; Bussmann et al., 2019). It is one of the 850 species of the genus *Ficus* (Somashekhar & Mahesh, 2013; Burckhardt & Batta, 2018). *F. carica* is a deciduous subtropical shrub or small tree, and it is renowned as fig. It is a drought-tolerant plant that requires small amounts of water (Botti et al., 2003; Flaishman et al., 2008; Chithiraichelvan et al., 2017). The growth is limited by winter, an environment with cold temperature or high humidity such as hailing and raining seasons (Botti et al., 2003; Flaishman et al., 2008; Chithiraichelvan et al., 2017). It is a latex-producing plant in which the milky latex is secreted in wounded areas (Flaishman et al., 2008; Elsayed et al., 2018). It is believed that the latex is used as one of the plant defence mechanisms of the fig tree (Pallardy, 2010; Elsayed et al., 2018).

The fig is composed of individual drupelets developed from ovaries in an enclosed receptacle or syconium of inflorescence (Weiblen, 2002; Machado et al., 2005; Borges et al., 2011). It has an ostiole, which acts as a small entrance that undergoes coevolution to allow genera of fig wasps from the family Agonidae to enter and pollinate (Machado et al., 2005; Borges et al., 2011; Kjellberg & Lesne, 2020). The pollination of *F. carica* is known as caprification which is host-specific whereby volatile attractants are released from receptive figs of female plants to attract *Blastophaga psenes* for pollination (Weiblen, 2002; Machado et al., 2005; Kjellberg & Lesne, 2020). Therefore, fig cannot be pollinated through manual pollination. This eventually made fig becomes less available in the market due to the viability of the seeds and lack of host-specific-pollinator in nature.

The fig is cultivated worldwide because it has nutritional value to humans as it is fat as well as cholesterol-free with excellent sources of vitamins, minerals, and carbohydrates (Begum et al., 2020). It is also used for medicinal purposes. Mawa et al. (2013) and Begum et al. (2020) had pointed out that the consumption of fig has therapeutic effects on respiratory, gastrointestinal, inflammatory as well as cardiovascular disorders. It can also be used to treat constipation (Ali et al., 2012; Barolo et al., 2014; Idrus et al., 2018). Sirisha et al. (2010) had reviewed that the leaves of *F. carica* have pharmacological actions such as anti-ulcer, anti-diabetic, and anti-fungal activities. It has been one of the food industry materials for biscuits and other side products such as pudding and jam (Barolo et al., 2014; Khapre et al., 2015).

Traditional fig cultivation involved site selection, planting, pruning, fertilisation, and cultivation practices. The fig tree is commonly propagated through cuttings, air layering, or grafting in warm and temperate climates (Gholami et al., 2012; Gaaliche et al., 2016). However, the survival rate of grafted fig is too low due to the infection of the pathogens, poor rooting system, and the inability to adapt to the environment in Malaysia, although there is no report published on the survival rate (Faccoli et al., 2016). Fig is propagated through stems by air-layering and cutting method because the fig tree seeds are not viable (Flaishman et al., 2008; Gholami et al., 2012; Bussmann et al., 2019). To date, there is no effective horticultural practice on fig propagation to support the research gap on survival rate and soil media to be used in cultivation. Traditional cultivation requires controlled growing conditions, extensive labour, and knowledge on fig as well as the quality of the figs that are easily affected by pathogens (Flaishman et al., 2008; Faccoli et al., 2016). Fig is easily susceptible to diseases and pests such as rust, which can observe on the leaves and beetles, which act as a vector for carrying

diseases to the plants (Rahmani & Aldebasi, 2017). Some insects will cause the yield to be decreased drastically.

Micropropagation provides an alternative breeding method used in plant tissue culture to propagate disease-free clones at a high survival rate (George & Debergh, 2008; Kanwar et al., 2019). Plantlets will exhibit totipotency genetically identical to their parent plant (Caponetti et al., 2018). Therefore, the nutritional and medicinal extracts of *F. carica* produced from plant tissue culture are the same as the parent plants (Caponetti et al., 2018). The explants used in micropropagation are meristem tissue and shoots (apical buds) that must undergo surface sterilisation steps. Relevant research such as enhancing the production of secondary metabolites using suspension culture and elicitors from the friable callus and roots conducted to extract medicinal compounds (Devi et al., 2018).

Light plays a pivotal role in plant tissue culture. Generally, *in vitro* plants incubated in the plant tissue culture room are given white fluorescent light. However, different plants required different photoperiods. Therefore, it had proved that *in vitro,* plants could be affected by different light treatments. For example, long day plant such as spinach requires more extended exposure period to light (Utasi et al., 2019) while short-day plant such as soybean requires shorter exposure period to light for the optimal growth (Devi et al., 2018). Besides, different light spectra displayed different reactions to plants in various stages. Researchers had used light-emitting diodes (LEDs) technology with a lower cost of production to regulate and control the physiological growth of the plants (Gupta & Jatothu, 2013). Studies on the effect of LEDs on different plants in plant tissue culture were widely carried out which involving different ratios and ranges of red, green, blue, and other LEDs (Coward et al., 2016; Kim et al., 2020).

LEDs application on *F. carica* L. is essential to study the improvement of growth and development, but there is no study on the implementation of LEDs.

F. carica L. cv. Panachee was found as a chimera plant, commonly known as tiger fig (Figs4Fun, 2008). It is susceptible to diseases such as rust and fruit rot. *F. carica* cultivated in Malaysia was found to be challenging to be propagated through grafting and air-layering due to low survival rate and lack of specific pollinator in nature (Kjellberg & Lesne, 2020). Among the cultivated Fig varieties, Panachee is the most expensive in Malaysia due to distinctive traits on the fruit, which is a unique variegated colouration, green with yellow stripes. This eventually made it the most suitable cultivated varieties to be an ornamental plant for its foliage with a tasty and bountiful harvest (Kamarubahrin et al., 2019). Furthermore, there is no exact market size and niche in Malaysia due to the difficulties in propagating it (Kamarubahrin et al., 2019). Research on fig is challenging and poorly understood primarily on *F. carica* L. cv. Panachee. This project was conducted to assess the hypothesis that optimised protocol on micropropagation and meristem culture under LEDs application produced a high quality of fig plantlets with well roots system at large-scale in a short period.

1.1 Rationale and significance of the study

Through plant tissue culture technology, *F. carica* L. cv. Panachee can be propagated at a larger scale within a shorter period. However, this plant biotechnology is still not known by many farmers. Optimisation of micropropagation and meristem culture with the application of LEDs at different wavelengths and intensities by using response surface methodology (RSM) to enhance the growth and development of the plantlets. Hence, the markets on fig can be established for local supply. Post-analyses

on the tissue culture plant such as biochemical, molecular, and histology are vital to study the effects of the treatment on the cellular arrangement, the anatomical examination of the regenerated plantlets. Physiological and biochemical analyses can be carried out to study the stomatal density, total protein, and chlorophyll content. The application of LEDs on *F. carica* L. cv. Panachee was the first reported on this cultivated fig which was optimised by using RSM. The findings of the research will be implicated in a system that used the regression equations, which are then sold as a commercial prototype for the fig industries that want to start a fig laboratory to produce disease-free fig plantlets at a commercial scale.

1.2 Research objectives

The general objectives of the study are as follows:

I. To establish the optimised protocol of meristem culture and micropropagation of *F. carica* L. cv. Panachee under LEDs application at different spectra,

II. To establish a protocol on pre-acclimatisation under LEDs application at different wavelengths and evaluate the effects of LEDs through qualitative analyses using microscopy, biochemical, and molecular analyses.,

III. To study the effects of the treatments at the cellular level through histological analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 *Ficus carica* L.

2.1.1 Botanical classification, taxonomy, and distribution of *Ficus carica* L.

Ficus carica L. is a deciduous subtropical flowering woody plant from the family Moraceae with more than 1000 species classified into about 40 genera (Ronsted et al., 2005; Lanskyet al., 2008; Lansky & Paavilainen, 2010; Somashekhar & Mahesh, 2013; Bussmann et al., 2019). It is a gynodioecious fruit tree (2n = 26) and is also one of the oldest cultivated crop plants (Weiblen, 2000; Bussmann et al., 2019). It is a member of the family Moraceae (Mulberry family), which are trees with alternately arranged leaves and a unisexual inflorescence that produces milky latex (Singh, 2016; Elsayed et al., 2018).

The genus *Ficus is* classified into six subgenera, categorised by a particular reproductive system (Berg, 2004; Bussmann et al., 2019). *F. carica* is native to the Middle East and Western Asia (Somashekhar & Mahesh, 2013). The fig tree is one of the unique *Ficus* species in which it is grown everywhere in tropical and subtropical countries that possessing Mediterranean climates such as California, Australia, South America, Turkey, Egypt, and Morocco (Gozlekci, 2010; Patil & Patil, 2011; Abdelsalam et al., 2019). Among the major producing countries, Turkey is the top fig producer globally in 2018, which produced 306,499 tonnes of figs [Food and Agriculture Organisation (FAO) of the United Nations, 2018]. Bursa is a major fresh fig producer in Turkey (Çalişkan & Polat, 2008; FAO, 2018).

Natural mutations might occur within a cultivar when the fig tree is repeatedly propagated by cuttings and air layering, which eventually gives rise to phenotypic

variability (Flaishman et al., 2008). *F. carica* is now cultivated widely in the Mediterranean region from Turkey to Spain and Portugal (Patil & Patil, 2011). Since the fig tree is planted commercially as an essential crop, it has been cultivated worldwide throughout the temperate world for both the fig and as an ornamental plant.

Some cultivated varieties of the *F. carica* are cultivated in Thailand and Indonesia and Malaysia (Kamarubahrin et al., 2019). *F. carica* is commonly known as the common fig, edible fig, and just fig. In Malaysia, the Malay refer fig tree as "Pokok tin". The "Pokok" means tree in English and the "tin" refers to the fig. On the other hand, the Chinese refer to it as "fruit without flower" while known as "anjeer" for Indians. The figs must be imported first, then slowly adapt to the weather in Malaysia. Cultivated varieties found to be suitable are Panachee (Figure 2.1), Black Jack, Brunswick, BTM 6, Risa, and Violette de Solliés.

***Ficus carica* L. (Common fig)**	
	
Kingdom	Plantae
Order	Rosales
Family	Moraceae
Genus	*Ficus*
Species	*carica*
Binomial name	*Ficus carica* L.

Figure 2.1: *Ficus carica* L. cv. Panachee.
(Scale bar = 1 cm)

Four types of figs can be described based on the cropping and pollination characteristics, common fig, San Pedro type, Smyrna type, and caprifig (Labidi et al., 2018; Bussmann et al., 2019; Cui et al., 2019). Common figs such as *F. carica* L. cultivated variety (cv.) Panachee, Black Jack, and Brunswick are not actual fruit. Common figs need no pollination for fruit production along with their growth and maturity (Flaishman et al., 2008; Taleb et al., 2019; Kjellberg & Lesne, 2020). The other two types of edible figs, San Pedro, and Smyrna required caprification (pollination of fig) through a special pollination agent, fig wasp (Kjellberg & Lesne, 2020). An example of San Pedro type figs is *F. carica* L. cv. Dauphine, King and San Pedro, and the instance of Smyrna-type figs is *F. carica* L. cv. Sarilop, Marabout, and Zidi (Flaishman et al., 2008; Bussmann et al., 2019). The fourth type is caprifig which acts as a pollen source for the commercialisation of fig plantation (Flaishman et al., 2008). Caprifig is known as a male fig that responsible for pollen production since fig is a deciduous subtropical woody plant. Traditionally, cultivated variety classification is the key in fig collections in which the individual cultivars had been widely distributed. There is a possibility where the same common name is being used for different fig cultivars (Flaishman et al., 2008; Hssaini et al., 2020).

Differences in the morphology of the fruits (pomological characteristics), leaves, and stems are always aided in morphological identification to differentiate the cultivated varieties (Hssaini et al., 2020). Cultivar classification can also be done based on the pomological characteristics of the infructescence of *F. carica*. A study was done by Polat and Caliskan (2008) that the infructescence of *F. carica* L. cv. Bursa Siyahi, Yediveren, Sari Zeybek, Göklop, Morgüz, and Yeşilgüz were characterised based on their pomological characteristics such as fruit stalk, size, shape and ostiole width as well as the acidity of the fruit. Other pomological characteristics such as fruit weight, volume,

peel thickness, and skin colour were used as parameters for studying the growth pattern and fruit characteristics of *F. carica* L. cv. Ajlouni, Byadi, Khartamani, Khdari, Mwazi, and Zraki (Ateyyeh & Sadder, 2006).

2.1.2 Morphological description and development of *Ficus carica* L. cv. Panachee

2.1.2(a) Vegetative morphology and development

2.1.2(a)(i) Stem, branch, and leaf

Ficus carica L. is a deciduous tree or shrub that can grow to a height of six to ten meters. A morphological study was done by the International Plant Genetic Resources Institute (IPGRI) and Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM). They found that the growth habit of fig is one of the characteristics of the cultivar, and it is cultivar dependent. Fig trees vary in their growth habits ranged from further classified into erect, semi-erect, open, spreading, and weeping (IPGRI & CIHEAM, 2003; Bussmann et al., 2019). Shoot length and width are depending on the cultivated variety and the horticultural cultivation of the fig trees. Fig trees have different shoot colours, mainly green, brown, and other colours like greyed-green. The fig tree tends to form suckers emerging from the ground.

Ficus carica L. cv. Panachee is known as a chimera as its fruits are striped green and yellow (Figs4Fun, 2008). Chimera is a single organism composed of cells from different zygotes, and it is common in fruit tree species (Gaut et al., 2019). Plant chimera might result from the somaclonal variation or partial fusion of the plant tissues from two different genomes, species or cultivated varieties (Gaut et al., 2019). However, the origin of the parents cultivated varieties used to give rise to cv. Panachee

is not known. Panachee is an ancient variety mentioned as long ago as 1668 (Provender Nurseries, 2020).

The apical meristem found at apical buds has differentiated and elongated to produce lateral outgrowths such as scales, leaves, infructescence, and lateral vegetative buds (Figure 2.2). Each terminal bud contains four to five primordial leaves flanked by a scale (Gaaliche et al., 2016). As the bud grows and elongates, the cover scale abscised, and the apical meristem differentiated into a shoot that produces leaves and new infructescence. The formation of buds is significant for plant growth and development as it allows plants to undergo dormancy to survive in harsh conditions such as winter. The development of shoots from buds also reduces competition, such as the source of light among plant parts (Kalaitzoglou et al., 2019).

The position of buds is critical as it determines the arrangement of branches and leaves. There are two types of buds, which are the apical buds and lateral buds. The formation of apical and lateral buds is regulated by plant growth regulators, which are auxins and cytokinins. Lateral buds can be found in two different parts of a plant (Figure 2.2). Lateral bud located at the axil of leaves (axillary buds), and the shoot or stem portion of the plant develops into lateral shoots of the plant. The formation of lateral buds is stimulated by cytokinin since it inhibits apical dominance and promotes lateral dominance (Müller & Leyser, 2011; Brunoud et al., 2020).

The leaves are fragrant leaves that are deeply lobed with three or five lobes depending on the cultivars, which aid in cultivar classification (Burckhardt & Batta, 2018). Some cultivated varieties can be easily identified through the morphology of leaves that differ distinctly in terms of leaf colour, petiole colour, number, and shape of lobes, the shape of leaf base, leaf margin, and leaf margin dentation (Figure 2.3). Purple

colour pigmentation was observed at the petiole of *F. carica* L. cv. Violette de Solliés [Figure 2.4 (E)]. *F. carica* L. cv. Panachee is a chimera plant in which the leaves give dual colouration (Figure 2.3). However, the formation of leaves with dual colouration was found random throughout this study.

Figure 2.2: Stem morphology of *Ficus carica* L. cv. Panachee. (Scale bar = 1 cm)

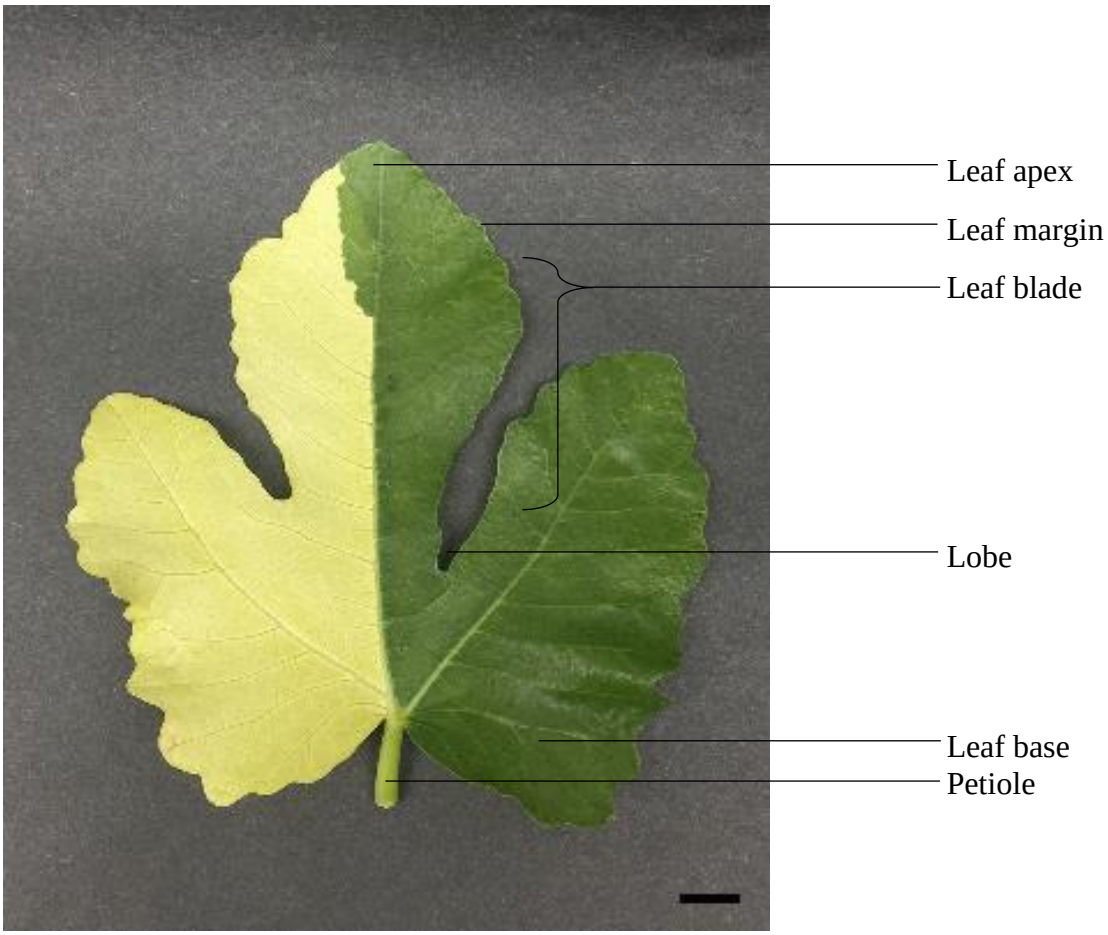

Figure 2.3: Leaf morphology of *Ficus carica* L. cv. Panachee. (Scale bar = 1 cm)

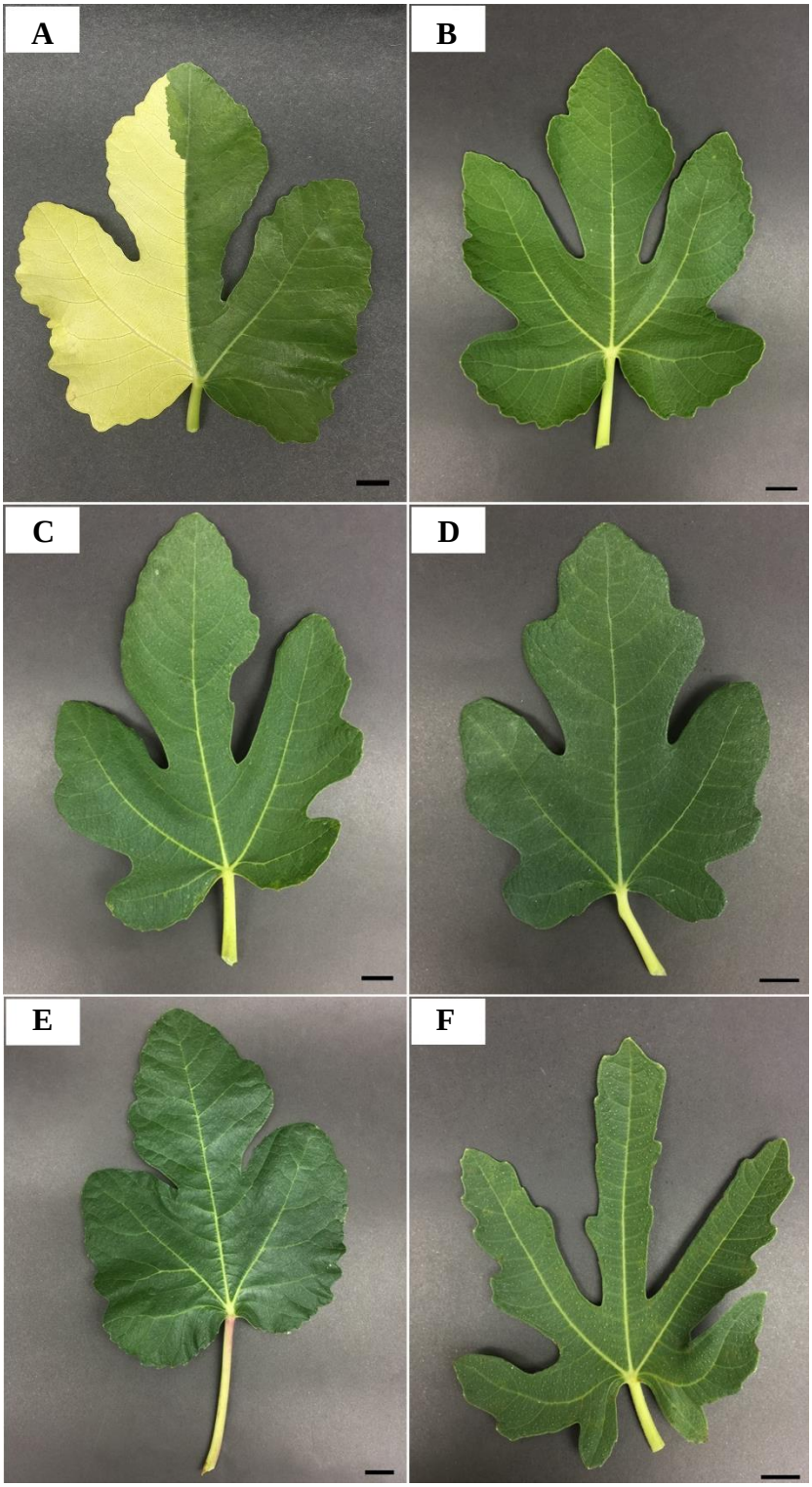

Figure 2.4: Leaf morphology of *Ficus carica* L. of the different cultivated variety.
(A) Leaf of *F. carica* L. cv. Panachee, (B) Leaf of *F. carica* L. cv. Black Jack, (C) Leaf of *F. carica* L. cv. Risa, (D) Leaf of *F. carica* L. cv. Brown Turkey, (E) Leaf of *F. carica* L. cv. Violette de Solliés, (F) Leaf of *F. carica* L. cv. Brunswick. (Scale bar = 1cm)

2.1.2(a)(ii) Latex

Plants from families such as Alismataceae, Alliaceae, Apocynaceae, Butomaceae, Cichorioideae, Convolvulaceae, Euphorbiaceae, Moraceae, Papaveraceae, and Urticaceae are latex-producing plants (Singh, 2016; Ay & Duran, 2018). Some plants from the family of Sapotaceae are latex-producing. The plants from the families Gnetaceae and Marsileaceae are the plants with enlarged secretory idioblast, latex tubes (Raskovic et al., 2016; Singh, 2016). *F. carica* is a plant from the family of Moraceae which has latex-containing saps that contain latex which is milky in colour. Latex, a sticky emulsion which is the cytoplasmic fluid containing the plant organelles in vasculatures, laticifer cells as those found in the plant cells (Chang et al., 2011; Baeyens-Volant et al., 2015; Singh, 2016). According to Agrawal and Konno (2009), latex production was proof in the microevolutionary perspective in which the plants have spurred adaptive radiation.

Latex has essential secondary metabolites used in the taxonomical study and its involvement in plant defence mechanisms. Secondary metabolites found in latex are rubber (terpenoid), alkaloids, cardenolides, terpenoids, phenolics, and proteins such as proteases, protease inhibitors, chitinases, oxidases, lectins and hevein-like chitin-binding proteins (Agrawal & Konno, 2009; Raskovic & Polovic, 2016). Cysteine proteases are involved in latex coagulation upon biotic and abiotic injuries, and this wound healing property is the most useful plant defence mechanism that protects the injured or wounded part from pathogens entry and further spread (Moussaoui et al., 2001; Azarkan et al., 2006; Ay & Duran, 2018).

Oliveira et al. (2010) had also pointed out that plant secondary metabolites can provide resistance to herbivores through toxic or antinutritive effects. The secretion of latex gives defence against wounds, predators such as herbivores, or

pathogens such as insects and microorganisms (Pallardy, 2010; Chang et al., 2011; Raskovic et al., 2014). Baeyens-Volant et al. (2015) had reported that silkworm larvae died when they were fed on latex-containing leaves of *F. carica* but alive when fed on latex-free leaves. The latex also provides the stickiness which can mire insect herbivores from physical attacks. Latex exuded from the laticifers in the plant when the laticifers are wounded or injured (Figure 2.5. However, some herbivorous insects have adaptations to cope with the secondary metabolites in the latex of plants. For example, larvae of monarch butterflies that feeding on milkweeds and silkworms that feeding on mulberries (Agrawal & Konno, 2009).

Besides, Kim et al. (2003) reported stress-related genes of rubber particles and latex in *F. carica,* which helps to tolerate abiotic stress such as cold and drought treatments and plant growth regulators treatments using jasmonic acid, abscisic acid, and salicylic acid. The presence of latex in *F. carica was* used in the phylogenetic study (Lazreg, 2011). Through scanning electron microscopy (SEM) analysis, Singh et al. (2003) had found that the rubber particles of the *F. carica*, *F. benghalensis,* and *Hevea brasiliensis* did share some degree of similarity in architecture in terms of size and form.

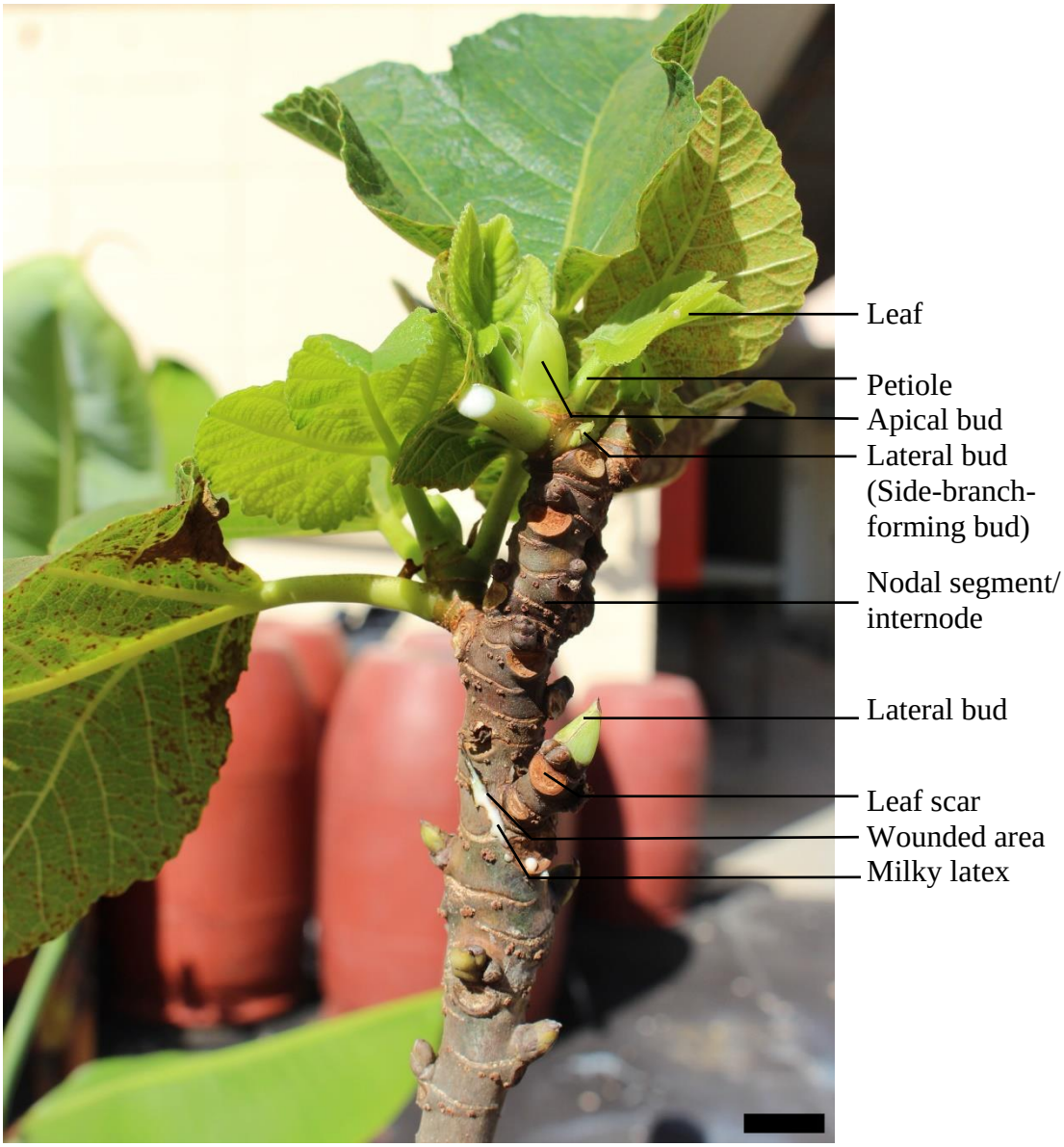

Figure 2.5: Milky latex produced at the wounded area on the shoot of *Ficus carica* L. cv. Panachee.
(Scale bar = 1 cm)

2.1.2(b) Fruit growth and development

Ficus carica is a gynodioecious plant in which it shows dioecy but having either hermaphroditic or perfect flower (female fig) on separate plants (Kjellberg & Lesne, 2020). The fig wasp, *Blastophaga psenes* from the family of Agonidae, acts as a pollinator and pollinate through the opening of the infructescence, ostiole from male plant to female plant (Machado et al., 2005; Mahmoudi et al., 2018). The process of pollinating the figs known as caprification (Kjellberg & Lesne, 2020). Rahemi and Jafari (2005) had pointed out that caprification involving pollen will influence the time of fruit ripening and skin colour of *F. carica* L. cv. Shah-Anjiri and Sabz, respectively. The formation of the infructescence is a modified inflorescence known as a false fruit or multiple fruits in which both the flowers and seeds are borne. Fig itself can produce perfect flowers or fruit without pollination. However, seeds of the fruit are not viable when there is no pollination (Kjellberg & Lesne, 2020).

Generally, the fig fruit is three to five cm long with green skin and turns purple, brown, or yellow with green stripes depending on the cultivars (Taleb et al., 2019). *F. carica* L. cv. Panachee has fruits with unique variegated colouration striped green and yellow with strawberry-like-coloured flesh. Panachee is commonly known as tiger fig, striped tiger, variegated, jaspee limone, marbled limone or plume (Figs4fun, 2008). The fruit of the fig tree is the infructescence or scion of the tree which initiated from the axillary buds located between the stem and petiole.

Dramatic pigment changes occur along with the fruit maturation as well as the fruit size and the texture, which changes from hard to soft. Among the cultivars that can be cultivated in Malaysia, *F. carica* L. cv. Panachee gives yellow with green stripes on maturation (Figure 2.6), cv. Black Jack gives a purplish colour (Figure 2.7), cv. Brunswick offers brown colour matured fruits (Figure 2.8). The maturation of the fruits

is greatly influenced by the weather and horticulture practices. The fruits take about five to six weeks to grow and mature (Figs4fun, 2020).

The fig contains numerous seeds, each representing a tiny fruit. The type of inflorescence of the genus *Ficus* is hypanthodium in which the typical inflorescence has the vessel-like receptacle with a small opening, ostiole (Singh, 2016; Bussmann et al., 2019). Ostiole is known as fig eye, a small opening visible in the middle of the infructescence (Figures 2.9 and 2.10). The flowers found along the inner wall blooming inside the infructescence, which does not have visible outwardly. The whole complex infructescence is known as syconium that consists of a hollow fleshy structure lined with numerous unisexual flowers (Somashekhar & Mahesh, 2013).

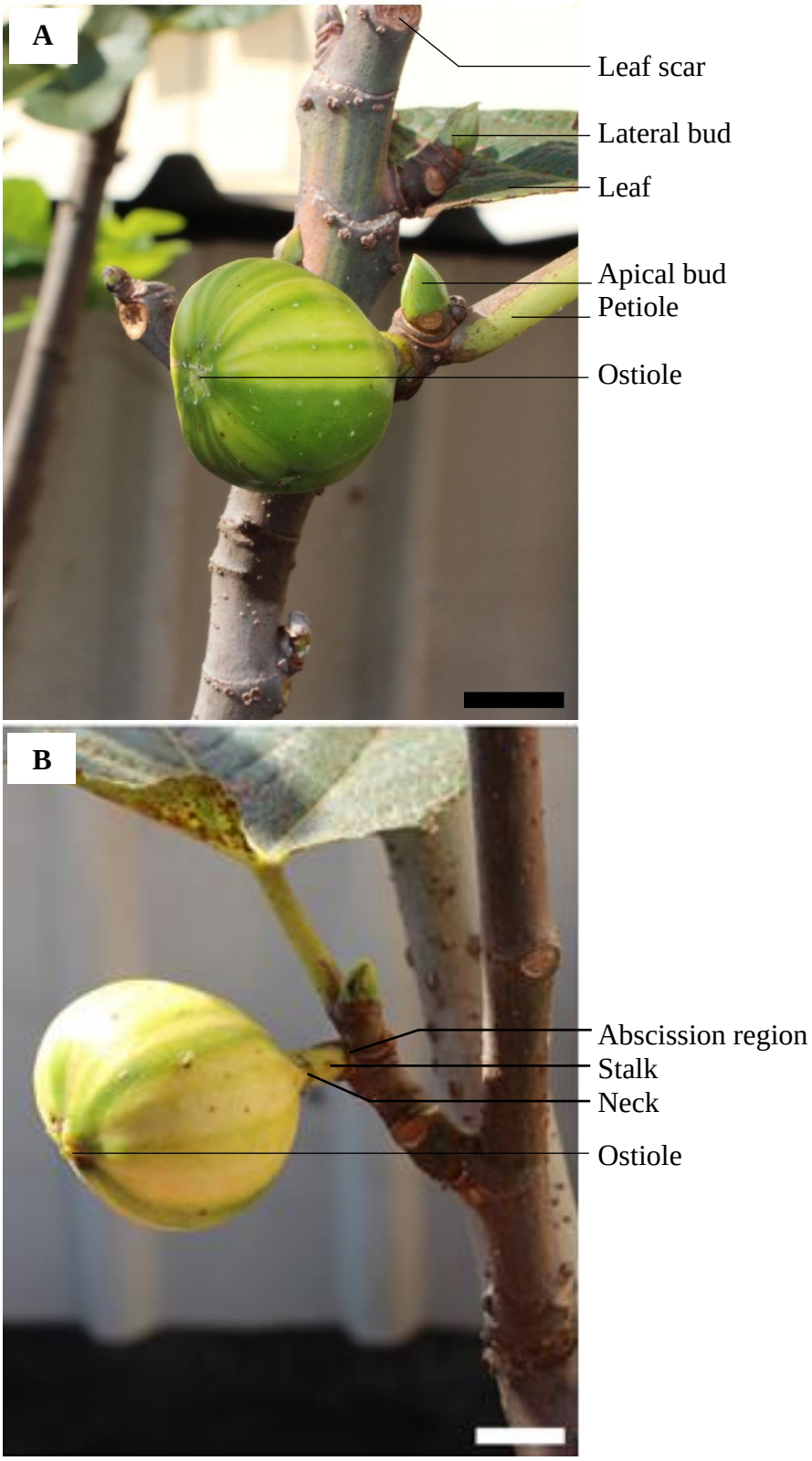

Figure 2.6: Branches of *Ficus carica* L. cv. Panachee with fruits.
(A) Branch with immature fruit, and (B) Branch with matured fruit. (Scale bar = 1 cm)

Figure 2.7: Branch of *Ficus carica* L. cv. Black Jack with fruits. (Scale bar = 1cm)

Figure 2.8: Branch of *Ficus carica* L. cv. Brunswick with fruit. (Scale bar = 1 cm)

Figure 2.9: Cross-section of a matured infructescence of *Ficus carica* L. cv. Panachee.
(Scale bar = 1 cm)

Figure 2.10: Cross-section of a matured infructescence of *Ficus carica* L. cv. Black Jack.
(Scale bar = 1 cm)

2.1.3 Horticultural practice on *Ficus carica* L.

Ficus carica is a subtropical species native to semi-desert or arid regions with summer-like temperatures and low humidity (Saddoud et al., 2008; Pakyürek, 2019). The optimal fig growth and production are depending on optimum climatic conditions. Generally, *F. carica* grows best and produces high-quality fig in high temperate climates with intense solar radiance and low relative humidity (Botti et al., 2003; Flaishman et al., 2008; Polat & Caliskan, 2008; Boudchicha et al., 2018). However, other environmental conditions such as rain, hail, haze, and wind with sudden changes in internal temperatures of internal fruit pressure that cause fruit splitting can indirectly reduce fruit quality and production, which results in fruit cracking (Flaishman et al., 2008; Bussmann et al., 2019).

Ficus carica cultivated worldwide that adapt and grow on a broad range of soils, including heavy clays, loam, and light sand provided the soil is well-drained. Fig shows good tolerance to soils with pH ranging from 6.0 to 8.0 (Flaishman et al., 2008). Since fig is a drought-tolerant plant, it requires lesser amounts of water as compared to other plants. Fig shows moderate tolerance to high salinity moderately. Metwali et al. (2014) found that *F. carica* L. cv. Black mission is the most salt stress-tolerant cultivated variety compared to Brunswick and Brown Turkey (which also known as Texas everbearing).

For commercial production, regular pruning needed to get rid of any diseased, broken or overlapping branches to ensure enough new wood for proper maintenance (Mendoza-Castillo et al., 2017). Gerber et al. (2012) highlighted that pruning one-third of the total length of the shoots cultivar “Bourjasotte Noire” produced the least negative effect on the crop yield but stimulating the induction and development of more growth

and longer shoots. Water-soluble fertiliser with a nitrogen-potassium-phosphorus ratio about 20:5:20 is commonly used and applied throughout the growing season as fruit quality highly correlated with the nutritional status of the tree and the nutrient supplemented (Flaishman et al., 2008; Pereira et al., 2017).

Ficus carica can be propagated through cuttings, air-layering, or grafting. Conventional propagation through branches usually carried out using mature wood with age two to three-year-old. However, the selection of healthy branches for propagation purposes and root induction with soil mixture media required experienced horticultural practices. Viable seeds can only be produced through caprification. Seeds found in the mature fruit without caprification are non-viable for germination and propagation purposes. Therefore, rapid mass multiplication can be done through plant tissue culture, studied on *F. carica* L. for the propagation in larger quantity and with higher survival rate.

2.1.3(a) Major diseases and pests

Ficus carica subjected to diseases that often place significant biological constraints on production (Figure 2.11). Of these, the common problems are grey mold on fruit, fig canker [Figure 2.11 (G)], coral spot and fig rust [Figure 2.11 (C)] as well as fig mosaic virus (Huseyin & Selcuk, 2004; Mikolajski, 2004; Bayoudh et al., 2017) that also observed from FigDirect Sdn. BhD., Perak, when the figs are planted outside the greenhouse, especially during the rainy season. Table 2.1 displays the diseases and disorders of fig, adapted from Diseases of Tropical Fruit Crops (Michailides, 2003). Different kinds of pathogens will infect different parts of the plant. Banihashemi and Javadi (2010) had pointed out that *Phomopsis cinerascens* are the causal agent to cause

fig canker at the branch of the tree. Banihashemi and Javadi (2010) found that delay pruning would be the best solution to manage the disease. Bayoudh et al. (2017) had found the incidence of fig mosaic virus on San Pedro, Smyrna and caprifig types of figs in the regions of Center-east of Tunisia. The symptoms of the infected fig trees are rolling up the leaves and forming scales on the fruits (Bayoudh et al., 2017). Throughout this study, it was found that figs were quickly susceptible to dieback and sooty canker which found as endogenous fungus in the stem. The infections of these diseases were characterised by branch dieback and tree death [Figure 2.11(F) and (G)] that was an endogenous fungal infection that targeted plant's xylem (Rehab et al., 2014; Alwan & Hussein, 2019).

López-Martínez et al. (2015) had concluded that buprestid and cerambycid beetles are the primary pests that would influence the yield and quality of fruits because the larvae can bore in the vascular system (Figure 2.12). The invasion of borer commonly observed on grafted plants. The grafted plant acts as a host and releases chemical compounds at the wound when it is under stressed conditions such as stress caused by phytosanitary agents, nutritional status, as well as drought which attract adult borer to lay eggs onto the wounded parts (López-Martínez et al., 2015; Faccoli et al., 2016). This invasion of borer will lead to infections by pathogens such as twig or branch dieback and canker. Some insects act as vectors to distribute the fig disease. Kajii et al. (2013) had found that *Euwallacea interjectus* (Blandford) which carries wilt fungus *Ceratocystis ficicola* can make pinholes at the lower trunk of *F. carica*. This eventually caused the xylem dysfunction of fig trees. Arthropod pests such as *Thrips* sp. and *Blastophaga psenes* found as vectors to *Fusarium*-related decay and nematodes such as *Meloidogyme* spp. were found to be root-knot nematodes which infected on the roots and led to the death of young tree (Wohlfarter et al., 2011).