MICROPROPAGATION OF FIG (*Ficus carica*) CULTIVARS ‘TEXAS EVERBEARING’ AND ‘LISA’ & THE ANTI-HYPERGLYCEMIC POTENTIAL OF FIG LEAVES

TAN LI VERN

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by

TAN LI VERN

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We did it dad! Not exactly a doctor by profession as you'd wanted but I hope it's close enough. You might not be here but I carry your strength, endurance and drive with me every day on this draining yet fulfilling journey. Looking back, it's worth it!

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LIST OF SYMBOLS & ABBREVIATIONS

°C	Degree Celsius
2,4-D	2,4-Dichlorophenoxyacetic acid
AC	Activated charcoal
ANOVA	Analysis of variance
BAP	6-Benzylaminopurine
cm	Centimetre
CRD	Complete randomized design
DMRT	Duncan multiple range test
g/100 g b.w.	Gram per 100 gram body weight
g/L	Gram per litre
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
i.p.	Intraperitoneal
KIN	Kinetin
LED	Light Emitting Diode
lm	Lumen
M	Molarity
mg/L	Milligram per litre
min	Minute
mL	Mililitre
mmol/L	Millimol per litre
MS	Murashige and Skoog

NAA	Naphthaleneacetic acid
NaOH	Sodium hydroxide
PGR	Plant growth regulator
rpm	Rotation per minute
SD	Sprague Dawley
SEM	Standard error of mean
STZ	Streptozotocin
TDZ	Thidiazuron
TE	Texas Everbearing
USM	Universiti Sains Malaysia
v/v	Volume per volume
WPM	Woody plant medium
ZEA	Zeatin

LIST OF APPENDICES

MIKROPERAMBATAN POKOK TIN (*Ficus carica*)

KULTIVAR 'TEXAS EVERBEARING' DAN 'LISA' &

POTENSI ANTI-HIPERGLIKEMIA DAUN POKOK TIN

ABSTRAK

Pokok tin (*Ficus carica*) merupakan pokok buah yang sangat dihargai untuk kandungan nutriennya yang tinggi serta kepentingannya kepada sektor ekonomi dan perubatan. Walaupun berasal dari wilayah Timur Tengah dan Mediterania, kemampuan pokok tin untuk tumbuh dan bermandiri membolehkannya ditanam juga di negara tropika. Walau bagaimanapun, kaedah penanaman konvensional semasa yang digunakan untuk pembiakan stok tanaman adalah terhad disebabkan oleh kesukaran memperoleh stok tanaman yang menghasilkan akar kuat serta bebas penyakit. Kultur tisu tanaman terutamanya mikropropagasi telah berjaya diterapkan untuk propagasi stok tanaman untuk memperbanyakkan tanaman komersial. Kajian semasa bertujuan untuk menubuhkan protokol yang cekap bertujuan untuk menghasilkan pucuk berganda dari *Ficus carica* kultivar 'Texas Everbearing' (TE) dan 'Lisa' serta untuk menilai potensi daun pokok tin dalam pengawalan penyakit diabetis. Dari keputusan yang diperolehi, pensterilan eksplan berjaya dijalankan dengan kadar kemandirian maksimum pada 50%. Keadaan optimum untuk kultivar TE adalah dengam pengkulturan segmen nodal secara mendatar dalam medium MS yang ditambah dengan 1.0 g/L AC, 10 g/L sukrosa, 3.0 mg/L TDZ dan 0.75 mg/L NAA, yang mencatat kadar penginduksian pucuk berganda maksima (100 %) serta jumlah pucuk (2.15 ± 0.10 pucuk) dan panjang pucuk (1.05 ± 0.12 cm) yang tertinggi. Untuk kultivar Lisa, kadar penginduksian pucuk maksima (100 %) serta jumlah tunas (1.92 ± 0.14 tunas) dan panjang tunas (2.11 ± 0.18 cm) yang tinggi diperoleh juga dengan mengkultur segmen nodal secara mendatar dalam medium MS ditambah dengan 1.0 g/L AC tetapi dengan penambahan fruktosa 20 g/L, 2.0 mg/L ZEA dan 1.0 mg/L NAA. Kemudiannya, penginduksian akar untuk eksplan kedua-dua kultivar berjaya dijalankan dengan menggunakan WPM dan diaklimatasikan dalam campuran tanah yang terdiri daripada tanah hitam, tanah merah dan gambut coco pada nisbah 3: 1: 1. Dalam penilaian potensi pengawal diabetis daun pokok tin dari *F. carica* kultivar ‘Texas Everbearing’ dan ‘Lisa’ pada tikus diabetes, ekstrak daun diperoleh melalui maserasi dalam 50% etanol selama 72 jam. Ekstrak pokok tin dari kultivar TE dan Lisa pada 250 mg/kg menunjukkan pengaruh yang signifikan ($p < 0.05$) terhadap penurunan kadar glukosa darah puasa pada hari ke-14 (16.3 ± 2.1 and 17.6 ± 3.1 mmol/L masing-masing) serta jumlah pengambilan air pada minggu kedua (27.2 ± 2.4 and 27.5 ± 2.0 ml/100 g b.w. masing-masing) jika dibandingkan dengan tikus kumpulan diabetis, semuanya menunjukkan penurunan hiperglikemia. Namun, tiada perbezaan yang signifikan dicatat pada berat badan dan organ jika dibandingkan dengan tikus kumpulan diabetis. Kajian ini berjaya mendokumentasikan protokol mikropropagasi yang cekap untuk pokok tin (*Ficus carica*) dari kultivar 'Texas Everbearing' dan 'Lisa' bagi pengeluaran stok tanaman yang sesuai untuk ladang komersial dan juga membuktikan potensi ekstrak daun pokok tin dari kedua-dua kultivar dalam pengawalan penyakit diabetis.

MICROPROPAGATION OF FIG (*Ficus carica*) CULTIVARS 'TEXAS EVERBEARING' AND 'LISA' & THE ANTI-HYPERGLYCEMIC POTENTIAL OF FIG LEAVES

ABSTRACT

Common figs (*Ficus carica*) are highly valued fruit crops owing to its elevated nutrient content and vast economic and medicinal importance. Although native to the Middle East and Mediterranean region, the adaptability of figs has attributed to its cultivation also in tropical countries. However, current conventional cultivation methods applied for the propagation of plant stocks are restricted due to the difficulty in obtaining a large number of well-rooted, disease-free plantlets. Plant tissue culture particularly micropropagation has been successfully applied to propagate plant stocks for many commercial crops. The current study aims to establish an efficient protocol for the micropropagation of *Ficus carica* cv. Texas Everbearing (TE) and Lisa and to evaluate the anti-hyperglycemic potential of fig leaves. From the results obtained, explant surface sterilization was successfully performed with a maximum survival rate of 50%. The optimal shoot induction condition for the cultivar TE was nodal segments cultured horizontally in MS medium supplemented with 1.0 g/L AC, 10 g/L sucrose, 3.0 mg/L TDZ and 0.75 mg/L NAA, recording maximum shoot induction rate (100%), shoot number of 2.15 ± 0.10 shoots and shoot length of 1.05 ± 0.12 cm. For the cultivar Lisa, shoot induction rate of 100%, shoot number of 1.92 ± 0.14 shoots and shoot length of 2.11 ± 0.18 cm was obtained similarly by culturing nodal segments horizontally in MS medium supplemented with 1.0 g/L AC but with 20 g/L fructose, 2.0 mg/L ZEA and

1.0 mg/L NAA. Explants of both cultivars were then successfully rooted in WPM and acclimatized in a potting mix comprising of black soil, red soil and coco peat at a 3:1:1 ratio. In the evaluation of anti-hyperglycemic potential of fig leaves from *F. carica* cv. TE and Lisa on diabetic induced rats, leaf extracts were obtained via maceration in 50% ethanol for 72 hours. Leaf extract from fig cultivar TE and Lisa at 250 mg/kg showed a significant effect ($p < 0.05$) in the reduction fasting blood glucose level at day 14 (16.3 ± 2.1 and 17.6 ± 3.1 mmol/L respectively) and average water intake in week 2 (27.2 ± 2.4 and 27.5 ± 2.0 ml/100 g b.w. respectively) when compared to vehicle treated diabetic control, all reflecting a reduction in hyperglycemia. No significant difference was recorded on the body and organ weights when compared to diabetic control rats. This study has successfully documented an efficient micropropagation protocol for fig (*Ficus carica*) cultivars ‘Texas Everbearing’ and ‘Lisa’ for the production of plant stocks suitable for commercial field establishments and also highlights the potential of fig leaf extract from both fig cultivars in controlling diabetes.

CHAPTER 1

INTRODUCTION

The Common fig is a fast growing deciduous tree with the scientific name of *Ficus carica*. In Malaysia, figs are commonly known by its Arabic or Malay term 'pokok tin'. Figs have a strong significance to many communities of the Muslim and Christian faith as they were mentioned in the Qur'an and Bible respectively. Originating from the Moraceae family and the genus *Ficus*, *F. carica* is one of the oldest and most accomplished species benefitting not just to civilizations but also various animals in the rainforest ecosystem (Berg, 1989; Rønsted et al., 2007; Mawa et al., 2013). Figs are native to the Middle East and the Mediterranean region where they were heavily cultivated for horticultural purposes since 4000 B.C. (Zohary et al., 2012). As of 2018, its cultivation and production were still centered in the Mediterranean regions with countries like Turkey, Egypt, Morocco and Algeria making up over 65% of figs produced worldwide (Patil and Patil, 2011b). The adaptability of figs to different climatic and soil conditions however have paved way for the cultivation of figs in tropical countries like Malaysia.

Figs are highly priced and well-sorted after for its abundant nutritional and medicinal benefits. They are a good source of fibre, carbohydrates, vitamins and minerals like calcium, iron, citric and malic acid besides also being free of fat, sodium and cholesterol (Jeong and Lachance, 2001; Slavin, 2006; Veberic *et al.*, 2008; Slatnar *et al.*, 2011). High amounts of phytochemicals like flavonoids and phenolic acids were also reported in figs that attributes to its superior antioxidant capacity, surpassing even red wine and tea which has high anti-oxidative abilities (Vinson *et al.*, 1998; Slavin, 2006; Veberic *et al.*, 2008). Figs were exploited as treatment for numerous metabolic, gastrointestinal, respiratory and cardiovascular illnesses as well as many other ailments (Guarrera, 2005; Patil and Patil, 2011b; Mawa *et al.*, 2013). While figs are mostly enjoyed fresh, its distribution and consumption is limited to its production areas due to its susceptibility to post-harvest disease, making it difficult for transport and storage (Stover *et al.*, 2007a).

Additionally, the cultivation of figs is also restricted by obstacles faced during propagation. Common figs are typically parthenocarpic, failing to produce sterile seeds which leads to the huge reliance on conventional methods like stem cutting, air layering and grafting for cultivation since ancient times (Zohary *et al.*, 2012; Chevalier *et al.*, 2014). However, only a small number of plants can be generated at a time which hinders large farm or nursery establishment. Plant cuttings are also more susceptible to pest infestation and microbial infection due to the stressful incidents such as wounding and drastic change in temperature and water content (Pasqual and Ferreira, 2007; Dolgun and Tekintas, 2008). Besides, Kumar *et al.* (1998) reported that *Ficus carica* cv. Gular cuttings had only a 20 to 30% chance of survival, marred by the failure to maintain high rooting capacity and form a strong network of roots.

The cultivation figs at a commercial scale require a large number of healthy plantlets and propagation via traditional methods is just not adequate and competent enough to meet the requirement. This leads to the prospect of utilizing tissue culture as an alternative for the propagation of fig plantlets to cater for the local establishment of fig farms and to meet the high market demand for fruits. Besides, production via tissue culture would also be financially more viable as it can cost as low as RM10 per plantlet compared to stem cuttings which can cost in the range of RM30 to RM120 per plantlet. This would also make figs more accessible particularly to poorer families as the current interest in exotic superfruits like figs have led to its inflated pricing. The production of fig plantlets through plant tissue culture were reported in a number of studies on various fig cultivars (Kumar *et al.*, 1998; Hepaksoy and Aksoy, 2006; Kim *et al.*, 2007; Danial *et al.*, 2014). However, up to now, there are still no studies reporting on *in vitro* propagation of 'Texas Everbearing' and 'Lisa'. The 'Texas Everbearing' cultivar is native to Texas, USA and possesses a robust production of amber coloured figs which are sweet and large in size, making them desirable for mass fruit production. Meanwhile, the 'Lisa' cultivar is from Japan that bears reddish yellow figs with comparatively smaller fruits but is also as sweet. This cultivar has a more pronounced leaf production rate and would be suitable to meet market demands for leaves for the production of herbal remedies.

Apart from the many benefits of its fruits, the leaves of fig were also found to posses potent hypoglycemic and hepatoprotective effects which explains its heavy usage during ancient times as traditional medicine to treat diabetes (Pérez *et al.*, 1996). Diabetes mellitus has long plagued humanity but showed no signs of stopping as it were projected to affect over 300 million people by 2025 (WHO, 2016). At current, diabetes is controlled by oral anti-diabetic drugs like metformin and glibenclamide but patients has a high tendency of developing undesirable side effects which necessitate the search for a novel anti-diabetic remedy which allows diabetes to be effectively controlled while giving little to no side effects.

Therefore, this study aims at establishing an efficient and effective micropropagation method for both *F. carica* cultivars 'Texas Everbearing' and 'Lisa' that can be utilized to produce large quantities of healthy robust fig plantlets for commercial farm establishments while also highlighting the anti-hyperglycemic potential of fig leaves obtained from *ex vitro* plantlets from both cultivars studied as possible anti-diabetic agents.

1.1 Objectives

The objectives in this study are:

i. To determine the optimal surface sterilization protocol for the establishment of *Ficus carica* cultivars ‘Texas Everbearing’ and ‘Lisa’ *in vitro* axillary shoot tip cultures,

ii. To evaluate the different parameters which effects the multiple shoot and root induction of *F. carica* cultivars ‘Texas Everbearing’ and ‘Lisa’,

iii. To assess the best methods for the acclimatization of *F. carica* cultivars ‘Texas Everbearing’ and ‘Lisa’ plantlets,

iv. To conduct extraction and evaluation on the anti-hyperglycemic potential of *F. carica* cultivars ‘Texas Everbearing’ and ‘Lisa’ leaf extract.

CHAPTER 2

LITERATURE REVIEW

2.1 *Ficus carica*

2.1.1 Description and origin

Ficus carica are a group of trees with the universal name of common figs, edible figs or simply just as figs (Flaishman *et al.*, 2008). The name is very much alike in, German (*feige*), Portuguese (*figo*) and in Spanish (*higo* or *brevo*) while in France, they are called *figue,* to distinguish them from the small bananas in which they call figs (Flaishman et al., 2008). In Malaysia, they are commonly referred to its Arabic term, Pokok Tin. Figs have strong religious significance to many Muslims in Malaysia. In Islam, figs together with olives are regarded as heavenly gifts from God which posses magical and divine properties as mentioned in the Qur'an (Flaishman *et al.*, 2008). Other than that, figs too have strong significance to the Christian faith as the Bible states that the fig is the Forbidden Fruit from the Tree of Knowledge of Good and Evil in the tale about Adam and Eve (Flaishman *et al.*, 2008).

F. carica are small sized, fast growing deciduous trees or shrubs which can grow up to 15 to 30 feet high with a tendency to be greater in width than in height due to its spreading habit (Stover *et al.*, 2007a; Patil and Patil, 2011b; Mawa *et al.*, 2013). The leaves are petiolated with broad ovate or orbicular leaf blade and are palmately lobed with three to five lobes while the roots are fibrous and well spread out (Soliman *et al.*, 2010; Patil and Patil, 2011b; Mawa *et al.*, 2013). The stems of figs are soft with a pithy interior, making it low in density and can break easily while its bark is greenish-brown when juvenile and grey when mature (Crisosto *et al.*, 2011; Mawa *et al.*, 2013).

The Common fig is a plant native to southwest Asia, most possibly the Middle East which later spread across throughout the Mediterranean by human cultivation, making them one of the very first cultivated fruit plant species (Zohary *et al.*, 2012; Mawa *et al.*, 2013). Figs were known to have initiated horticulture in the Mediterranean basin dating back to 4000 B.C. and this was justified with the discovery of fig remnants at Neolithic excavation sites (Morton, 1987; Zohary *et al.*, 2012). Besides, there were also other fossil records on figs distributed across the Southern Europe region which justifies how *F. carica* cultivars like 'Marseillaise' and 'Amatrice Casale' were native to South of France and the Abruzzi mountains in Italy respectively (Stover *et al.*, 2007b).

At current, figs are still heavily cultivated in countries across the Mediterranean region sharing almost similar growth conditions required by figs to achieve optimal growth such as a rainfall of 500-550 mm, humidity around 40 to 45% and average temperatures of 18 to 20°C yearly (Polat and Caliskan, 2008). However, figs are also striving in many tropical and sub-tropical countries as long as they are on soil that can range from heavy clay to rich loamy and light sandy soils equipped with good drainage (Patil and Patil, 2011b). According to Stover *et al.* (2007b) and Patil and Patil (2011b), figs thrive best in dry climates which is crucial for their fruit development and maturation but can also tolerate drought and high temperatures. The fig plant's high adaptability towards different soil and climate types made way for its cultivation across the Mediterranean basin and throughout the world and even now here in Malaysia (Mars, 2003a).

2.1.2 Taxonomy

Figs are categorized in the Moraceae (mulberry) family which has about 53 genera with over 1,100 plant species including herbs, trees and shrubs characterised mostly by the milky latex formed in lacticifer ducts (Clement and Weiblen, 2009; Barolo *et al.*, 2014). One of the subsets of Moraceae is the genera *Ficus* which holds one of the eldest and most accomplished higher plant species on earth (Lansky and Paavilainen, 2010). *Ficus* comprises of over 1,900 species of trees, shrubs, creepers and climbers in both the tropics and subtropics worldwide, making it one of the largest genera of flowering plants but only a few that can bare edible fruits (Frodin, 2004). Therefore, most *Ficus* plants that can produce fruits become an important food source for most animals in the tropical jungle, playing an important role in the biodiversity of the rainforest ecosystem, making them a significant genetic resource (Rønsted *et al.*, 2007; Mawa *et al.*, 2013). In this genus, *F. carica* specifically Common figs is the species which carries the greatest commercial importance and societal benefits (Barolo *et al.*, 2014).

The most unique and distinctive part of *F. carica* is its reproductive system. Dissimilar to most fruits, the edible part of figs are actually mature stem tissues instead of mature ovarian tissues (Lyons and McEachern, 1987). The fig fruit is actually an inverted flower with a hollow receptacle having both male and female parts of the flower enclosed in stem tissue, botanically known as a synconium (McEachern, 1996). The structure of a synconium is shown in Figure 2.1. Housing both male and female flowers, figs are morphologically gynodioecious but are dioecious in function specific wasps being their pollinators (Mars, 2003b). Female wasps utilize figs to house their eggs but in doing so they pollinate the flowers with pollen which were obtained from another fig tree. However, this mutualistic relationship does not occur in all fig varieties.

There are four different types of figs which are the Caprifig, San Pedro, Smyrna as well as the Common fig. The Caprifig is the most primitive fig variety with short-styled flowers and are non-edible instead of figs in the three other varieties which has long-styled flowers and edible fruits (Condit, 1941). However out of the edible fruit producing varieties, only the Common fig is parthenocarpic and does not necessitate fertilization for the fruits to set and mature into edible figs. This cements fig’s commercial value as being parthenocarpic increases the chances of obtaining seedless figs while decreasing the likelihood of having figs infested with wasps.

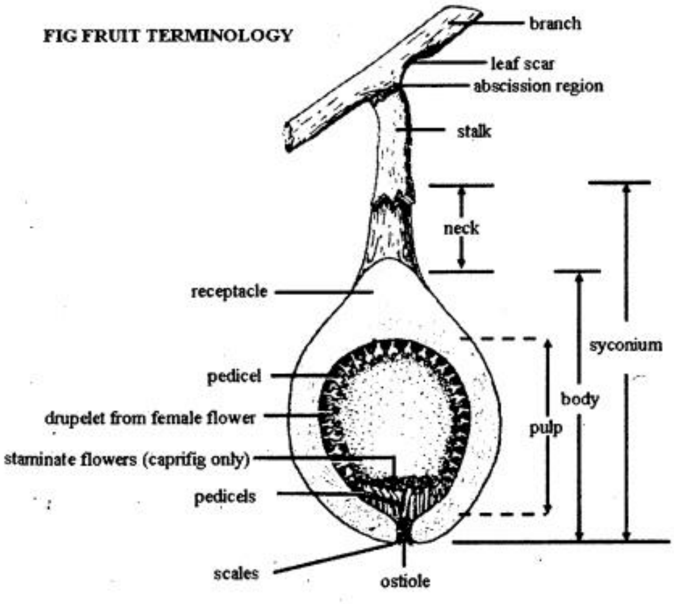

Figure 2.1: The fig fruit terminology. Image adapted from Flaishman *et al.* (2008)

2.1.3 Common fig cultivars

The Common fig comprises of many different cultivars with significant genetic diversity and can be found in many different countries around the world. The cultivars ‘Blanche’, ‘Castagnola’ and ‘Violette de Solliès’ are from France while ‘Sultani’ and ‘Abboudi’ are from Egypt, ‘Roxo de Valinhos’ and ‘Verdone’ are from Italy, ‘Brown Turkey’ and ‘Brunswick’ are from the USA and ‘Horaigaki’ is from Japan to name a few (Condit, 1955; Salhi-Hannachi *et al.*, 2006). The cultivars are mostly named based on the region they are cultivated as well as the characteristic of the fruit that it produces (Krezdorn and Adriance, 1961). Fig fruits or figs are usually pear shaped and comes in different sizes and a variety of colours ranging from the dark purple as seen in Black Mission figs to the yellowish-green in Calimyrna figs. Besides, the pulp of figs are also differently coloured from purplish-pink to orangey-yellow in colour.

One of the most sought-after cultivars is the ‘Texas Everbearing’ as shown in Figure 2.2. Native to Texas, USA, this tree is not only vigorous but also relatively large, growing up to 10 feet in height and 12 feet width (Lyons and McEachern, 1987) besides also having palmate leaves with three to five lobes. This fig cultivar is also commonly planted for the high production of fruits which are large and sweet while being reddish-purple in colour with pinkish flesh, most suitable for fresh consumption (Lyons and McEachern, 1987). Another highly desired fig cultivar is the cultivar ‘Lisa’ which originates from Japan as shown in Figure 2.3. Its leaves are dark green in colour with five to seven narrow lobes. The fruits are reddish-yellow in colour with a yellowish pulp and are also sweet. However, they are more prominently known for their robust leaf production. Most Common figs are cultivated for fresh consumption or made into different forms such as jams or tarts.

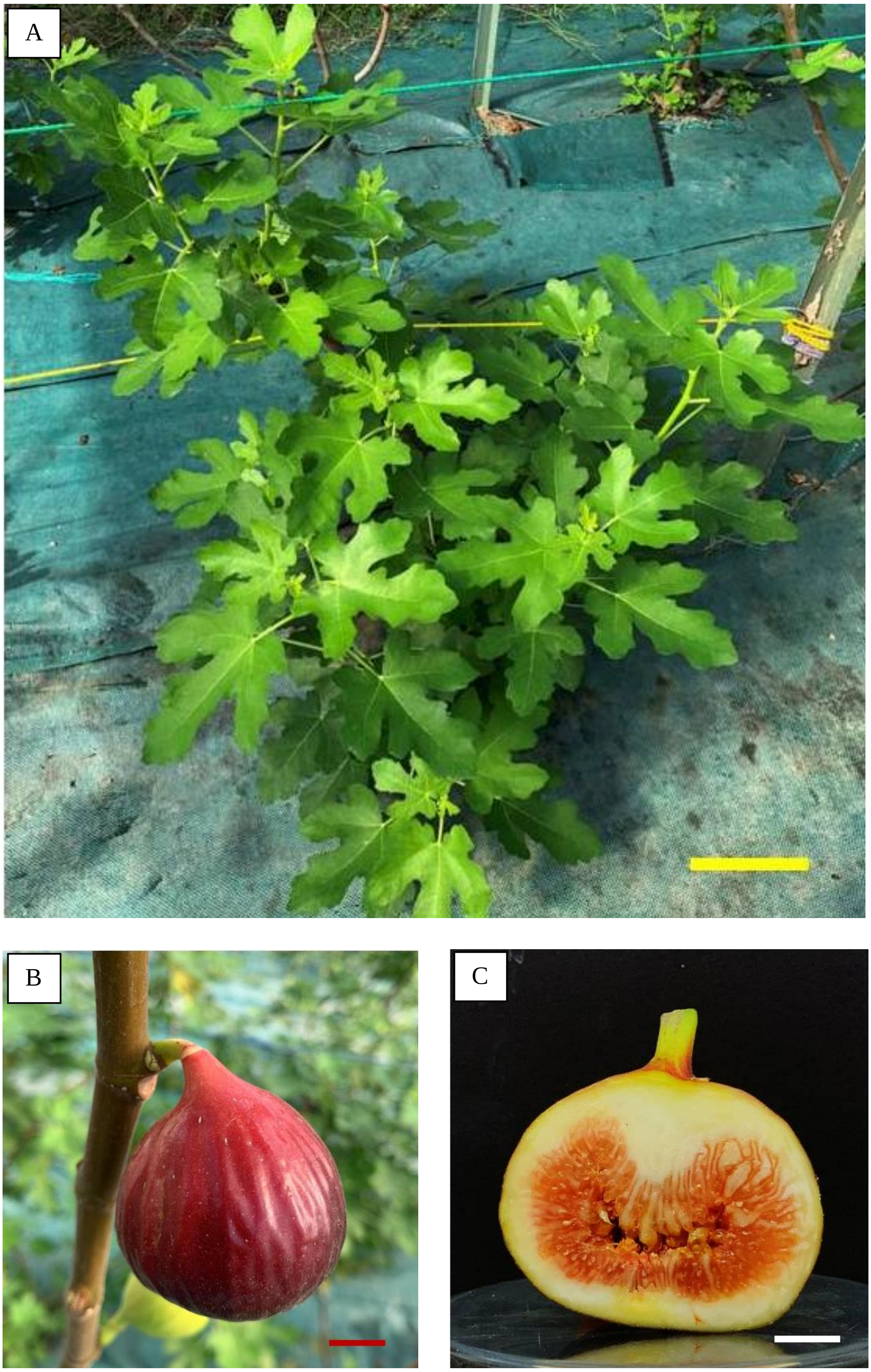

Figure 2.2: ***Ficus carica*** **cv. Texas Everbearing grown in Universiti Sains Malaysia (USM); (A)** plant (1 year old), **(B)** ripe fruit, **(C)** fruit cross-section. Scale bars : yellow = 10 cm, red = 1 cm, white = 1 cm.

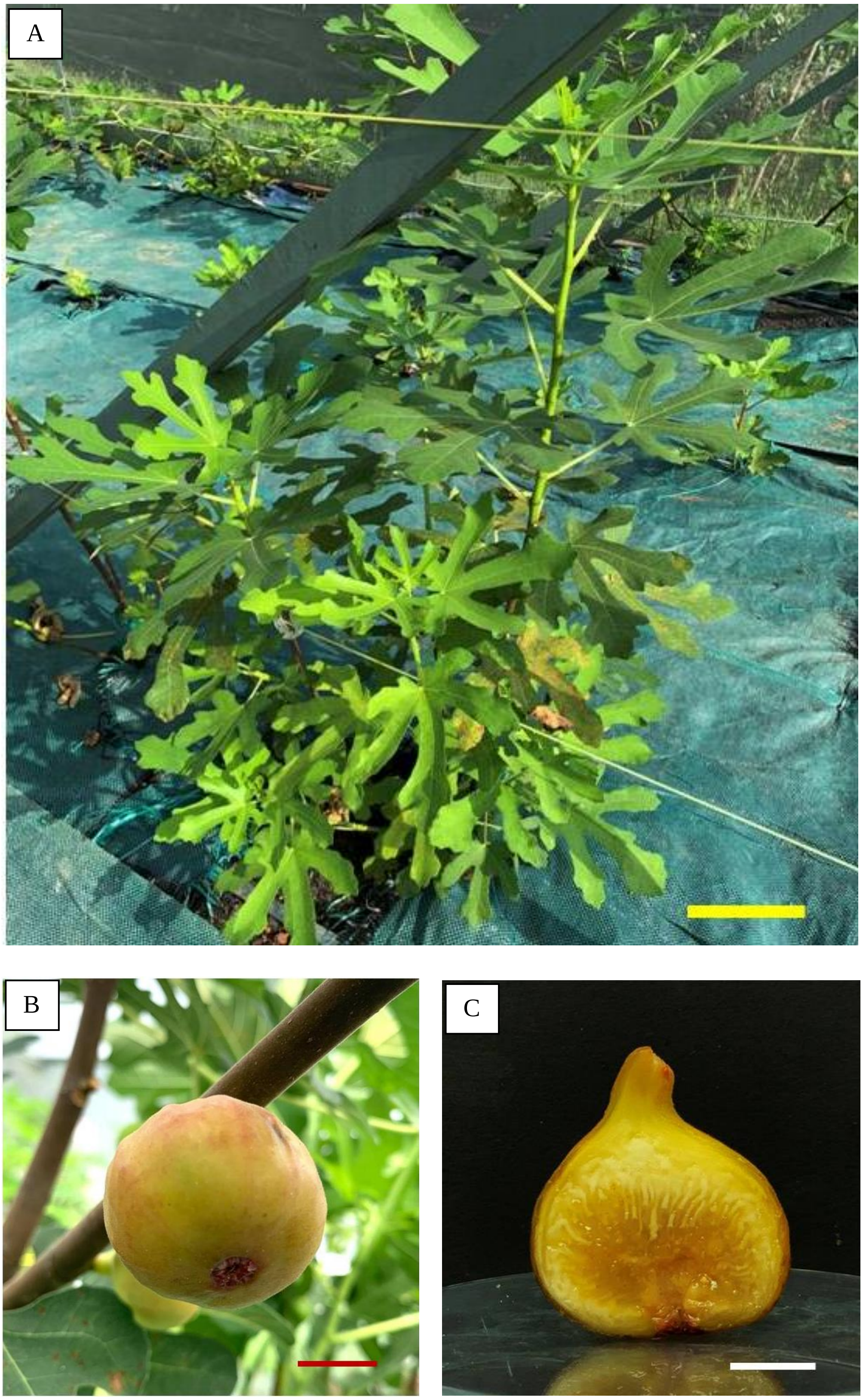

Figure 2.3: ***Ficus carica*** **cv. Lisa grown in Universiti Sains Malaysia (USM); (A)** plant (1 year old), **(B)** ripe fruit, **(C)** fruit cross-section. Scale bars : yellow = 15 cm, red = 1 cm, white = 1 cm.

2.1.4 Nutrient composition

Figs have been a health-promoting crop since thousands of years ago, nearly as old as humanity. Among other similar crops, figs were the eldest cultivated predating even cereal grains, pomegranates, olives and wine, playing an important role in many civilizations especially of the Mediterranean region since 11,000 years ago (Gibbons, 2006; Kislev *et al.*, 2006). Besides, figs have also benefitted a lot of faunas in the tropics as they are an essential food source to many herbivorous birds as well as mammals (O'Brien *et al.*, 1998). Nutritionally, figs either fresh or in their dried form are a good source of fibre, carbohydrates, vitamins and minerals (Jeong and Lachance, 2001; Veberic *et al.*, 2008; Slatnar *et al.*, 2011). Miura *et al.* (1998) had also found figs to be fat, sodium and cholesterol free while having high calcium, iron and amino acid content. Besides, figs were also found to contain high quality citric and malic acid as well as benzaldehyde which is proactively studied for its cancer prevention properties (Slavin, 2006). In addition, elevated amounts of organic acids and a wide range of phytochemicals contribute highly to the proven ability of figs in providing beneficial health effects (Slavin, 2006; Veberic *et al.*, 2008). Elevated phytochemical content predominantly flavonoids and phenolic acids has contributed to the superior antioxidant capacity of figs, even better than red wine and tea which was highly publicized for its inflated phenolic content and anti-oxidative abilities (Vinson *et al.*, 1998). The anti-oxidative ability of figs was proven in a study by Vinson *et al.* (2005) where oxidative stress observed in human test subjects induced by the consumption of high amounts of fructose corn syrup abundantly found in carbonated soft drinks was overcame after consuming figs which was found to have enriched plasma liproproteins that were responsible for preventing subsequent oxidation, providing a rise in plasma antioxidant capacity for up to four hours post fig consumption. Other than that, Solomon *et al.* (2006) have also reported on a correlation between the phytochemical content of figs to its colour as extracts of figs of darker varieties (purple and maroon) was found to have higher amounts of polyphenols and anthocyanins as compared to lighter-coloured figs (yellow and green). They added that phytochemicals were found to be highly concentrated on the skin of figs instead of the pulp.

2.1.5 Medicinal properties and importance

Plants have long been exploited by indigenous and ancient people as shelter, food, clothing, medicine and also in spiritual ceremonies (Abbasi *et al.,* 2013). At present, plants remain relevant and reliable as their high nutritional count and health benefits make them a good source of food and medicine (Pardo-de-Santayana *et al.*, 2007; Hadjichambis *et al.*, 2008). Among others, *F. carica* along with a number of plant species from the genus *Ficus* are one of most ancient and best source of cultivated medicine for animals and humans, playing an important role as a medicinal plant in the Ayurvedic and Traditional Chinese Medicine (TCM) (Flaishman *et al.*, 2008; Lansky *et al.*, 2008). The medicinal effects of *F. carica* was already described as early as 2,000 BC (Slavin, 2006). Almost the entire fig plant from its fruit to the leaves, roots and sap were utilized in traditional medicine as treatment for various metabolic (diabetes), gastrointestinal (indigestion, poor appetite and diarrhea) respiratory (cough and bronchitis) and cardiovascular ailments as well as remedies for inflammation and muscle spasms (Guarrera, 2005; Patil Vikas *et al.*, 2010; Mawa *et al.*, 2013).

Fig fruits have been a huge part of the Mediterranean diet largely in the traditional Greek diet that advocates the consumption of plant foods rich in antioxidants which provides protection against a number of degenerative diseases, cancer and coronary heart diseases (Trichopoulou *et al.*, 2006). The anti-oxidant effect of figs was attributed by its high phytochemical content together with sufficient macronutrient and inorganic compounds, contributing to the longevity observed in residents of the Mediterranean basin (Trichopoulou *et al.*, 2006). Besides, figs are also said to be effective in controlling chronic illness like diabetes and aiding liver and spleen related diseases (Mawa *et al.*, 2013). In traditional Indian medication, figs are used as an expectorant, diuretic as well as a mild laxative while in traditional Unani medicine they were taken as aphrodisiacs (Solomon *et al.*, 2006; Patil and Patil, 2011b). Plus, figs when made into a paste can help in alleviating swelling and inflammation while infusion of figs can be used as a safe laxative for children and adults (Konyalıoğlu *et al.*, 2005; Mawa *et al.*, 2013). When eaten with honey, figs can aid in stopping blood haemorrhages (Guarrera, 2005).

Fig fruits were also traditionally used together with fig leaves in the treatment of throat diseases, as coughing suppressant and an emmenagogue which is responsible in stimulating and increasing menstrual flow (Bellakhdar *et al.*, 1991), *F. carica* leaves too posses numerous medicinal benefits. One of the most reported benefit of fig leaves is its potent hypoglycaemic effect making them crucial for the treatment of diabetes, dating back to ancient times where decoctions made from fig leaves were used as folklore medication for treating diabetes (Pérez *et al.*, 1996; Khadabadi *et al.*, 2007; Cavero *et al.*, 2013). In Chinese medicine too fig leaves were processed and taken as tea to control blood sugar and high blood pressure levels (Flaishman *et al.*, 2008). Besides, fig leaf decoctions were used for the treatment of hemorrhoids and calcifications formed in the liver and kidneys as well as in the treatment of jaundice (Konyalıoğlu *et al.*, 2005; Flaishman *et al.*, 2008; Patil and

Patil, 2011b). In a study by Naghdi *et al.* (2016), it was also found that fig leaves can be an alternative treatment for infertility as the sperm count and non progressive motility of spermatozoas in mice were significantly improved and had a positive effect on the testis and epididymal sperm parameters on infertile mice.

Other than that, even the latex of *Ficus carica* possesses medicinal benefits as its medicinal usage were reported in the lands of Sumeria back in 2,900 B.C (Flaishman *et al.*, 2008). They function as ancient drugs for the treatment of cutaneous anthrax, warts and also tumours in many different cultures (Ben-Noun, 2003). Fig latex were taken as expectorant, diuretic and an anthelmintic which works by expelling parasitic worms and internal parasites from the body safely (Jeong and Lachance, 2001; Rao *et al.*, 2003; Patil Vikas *et al.*, 2010). In a study by Hashemi *et al.* (2011), fig latex, due to the presence of proteolytic enzymes, had successfully inhibited cancer cell line proliferation without exerting a cytotoxic effect on normal human cells. In addition, fig latex was also found to have controlled the proliferation of HPV related cervical cancer cells (Ghanbari *et al.*, 2019). However, fig latex should always be used with caution as it can cause allergic reactions when in direct contact with the eye and skin (Patil Vikas *et al.*, 2010).

2.1.6 Economic significance

With the vast nutritional and medicinal benefits of figs it is unsurprising that there is a high demand for it. Figs can be consumed fresh or dried as well as made into jam and paste to complement bars and pastries while a small portion are canned (Flaishman *et al.*, 2008). Fresh figs are categorized as exotic fruits, especially to the Western and Northern Europe region (Polat and Caliskan, 2008). The increasing interest in exotic, less familiar fruits in the world market have led to the surge in the demand and consumption of figs (Aksoy *et al.*, 1992; Stover *et al.*, 2007b).

The majority of fig production worldwide is centered in the Mediterranean region with Turkey being the leading country as 65% of fig trees were found in the Western Aegean region especially in Small and Big Meander valleys (Patil and Patil, 2011b). Turkey accounts for 27% of the world's fig production, producing over 306,499 tonnes of figs in the year 2018 (FAOSTAT, 2020). Countries like Egypt, Morocco and Algeria were also considered major fig producers as they have produced 189,339, 128,380 and 109,214 tonnes of figs respectively in 2018 (FAOSTAT, 2020). Together, the Mediterranean region make up 65% of the figs produced in the world. This is credited to the suitability of growth conditions in the Mediterranean shores, allowing robust fig growth and production (Kaşka *et al.*, 1990). Besides, figs could also be found in other countries with similar climate of hot dry summers and mild winters like California, Brazil, Italy, Greece and Spain (Mawa *et al.*, 2013). However, the luxury to consume fresh figs is restrained only to areas surrounding the production countries due to its fragility as well as high moisture and sugar content upon reaching maturity, making them highly perishable at room temperature (Stover *et al.*, 2007a). Besides, their susceptibility to post-harvest diseases lead to early senescence and fermentation which limits their storage period and marketing life (Cantín *et al.*, 2020). This led figs to be commonly exported as dried or processed forms instead of as fresh fruits. However, this does not devalue figs as dried figs are on par with other dried fruits primarily plums with regard to high nutrient score (Vinson *et al.*, 2005). Guvenc *et al.* (2009) mentioned that dried figs have a higher fiber content and nutrient score as compared to fresh figs although fresh figs are more suitable for people with high blood pressure and cholesterol levels due to its high calorie, fat and sugar content. However, there is also a trade concern which underlies the export of dried figs as producers struggle to compete with other dried fruits with equally high nutrition score but a lower production cost (Flaishman *et al.*, 2008). Therefore, the issues faced on fresh and dried fig production and consumption highlights the need for figs to be locally produced paired with the improvement in cultivation methods.

2.1.7 Problems affecting fig cultivation

Figs were cultivated since ancient times in all parts of the world vastly through conventional propagation methods such as stem cuttings and air layering (Zohary *et al.*, 2012). However, only a small number of plants can be produced a time due to limited number of shoots, making it difficult for nursery establishment. Besides, Dolgun and Tekintas (2008) had found that cuttings are also easily affected by ecological changes such as the sudden change in temperature or the drop in moisture content, leaving an adverse effect on the rooting and growth of cuttings. Figs characteristically possess high rooting capacity but roots produced via cuttings tend to be fragile and brittle. Buds on cuttings tend to sprout easily but the lack of proper rooting will cause a drastic loss of water leading to wilting and eventual plant death. Plantlets obtained via conventional propagation methods are also very fragile and require optimal soil and climatic conditions as their production rate can deteriorate when conditions are stressful, negatively affecting production cost, quantity and also quality (Dolgun and Tekintas, 2008). Thus, this calls for an improved, effective and sustainable propagation method for the cultivation of figs.

Other than that, figs although being well adapted to the Mediterranean climate of cool winters and hot, dry summers can also be cultivated in the tropical and subtropical regions. Figs tend to fruit well in the tropics and sub-tropics due to its high tolerance to drought and a preference for long exposure to heat to produce fruits of good quality, however frequent rainfall and high humidity levels causes figs to be commonly affected by fruit splitting and a few routine pests and diseases (Stover *et al.*, 2007b). Fruit rot and fungal attack commonly by *Aspergillus*, *Alternaria*, *Botrytis* and *Penicillium* fungi is common in areas with high humidity levels and can cause huge crop loss in many fig orchards (Tous and Ferguson, 1996). Fig rust caused by the fungus *Physopella fici* is also another major concern affecting fig cultivation. Starting off as small yellowish-orange spots on leaves, the fungus will slowly spread with spots becoming more enlarged and numerous, causing complete defoliation and loss in plant vigour (Lyons and McEachern, 1987). Besides, the fig mosaic virus which gives trademark yellow spots on the leaves and fruits of *F. carica* is also another major fig disease which stunts growth and reduce productivity of the plant leading to significant economic losses (Condit, 1941).

Other than that, plant-parasitic nematode infection is also a serious issue to fruit tree crops from the subtropical and tropical region, significantly affecting their growth and production. Besides bananas, plantains and citrus, *F. carica* plants were also commonly affected with nematode infestation. Many countries have reported of encounters with nematodes namely *Paratylenchus hamatus* which causes plant damage and growth decline as well as the cyst nematode *Hererodera fici* which affects seedling establishment as its eggs are dependent on fig root leachate (water exudates) to hatch (Di Vito, 1986; Cohn and Duncan, 1990; McSorley, 1992). However, Lyons and McEachern (1987) reported the most prominent nematode which infests figs especially those grown in Texas such as the ‘Texas Everbearing’ fig is the root knot nematode from the *Meloidogyne* genera namely *M. javanica*, *M.*

arenaria and *M. incognita*. Various methods to control the infestation of fig related diseases and infections were deployed such as the use of fungicide, pesticide and thermotherapy treatments. At current, the development of disease-free plantlets via plant tissue culture techniques has become a prominent figure on the fight against plant diseases and infestations in the agriculture industry.

2.2 Plant Tissue Culture

2.2.1 History and its applications

The concept of plant tissue and cell culture was first developed back in the 20th century by a famous German botanist named Gottlieb Haberlandt who studied and worked on developing methods to culture isolated vegetable cells systematically and effectively in nutrient mediums (Bhojwani and Razdan, 1986). Gottlieb Haberlandt's success in culturing leaf mesophyll cells was dubbed as a remarkable accomplishment considering there was very little knowledge on plant physiology during that time (Caponetti *et al.*, 2005). However, his ideas for plant development and totipotency was not successful as the cells cultured did not divide due to the absence of plant growth regulators which has not yet been discovered (Caponetti *et al.*, 2005). Although Gottlieb Haberlandt had pursued other physiological investigations, it was not long before his ideas initiated more studies on plant tissue culture related work in Robbins (1922) and White (1934) who had worked on culturing stem and root tips on medium containing inorganic salts. Rapid progress and development of plant tissue culture techniques came after 1950 and in the early 1960s with the development of the first culture medium by Murashige and Skoog (1962) which has still been the most commonly used medium for culture maintenance. New tissue culture techniques such as the establishment of protoplast and its hybrid cultures as well as the regeneration of plantlets from anthers and microspores were introduced in 1970 (Vasil and Thorpe, 1994). At the same time, knowledge and technology on genetic manipulation was emerging. This allowed the exploitation of both plant cell culture as well as molecular biology in improving plant and crop development by reducing chemical usage, improving food and fruit quality as well as producing transgenic plants equipped with highly-valued agronomic characteristics (Vasil and Thorpe, 1994; Caponetti *et al.*, 2005). Till now, there is still constant work and studies being carried out leading to the development of new tissue culture techniques and methods to improve plant micropropagation, development and modification, pathogen resistant plant production, cryopreservation and germplasm storage, cell behavior studies and secondary metabolite induction and extraction (Vasil and Thorpe, 1994).

In general, plant tissue culture is defined as the act of establishing plant cells, tissue and organs under *in vitro* or sterile conditions. The objective is to induce growth and multiplication via the manipulation of plant growth regulators and plant medium components together with external stimulus like temperature and light intensity. In *in vitro* conditions, explants are given time to develop and achieve optimal growth in formulated plant growth medium comprising of macro- and micronutrients, vitamins, carbon source and plant growth regulators and being free of other detrimental and growth-limiting organisms like bacteria and fungus. Once the plantlets were successfully developed, they can be slowly acclimatized and exposed to *ex vitro* conditions, allowing them to continue growing and striving like normal plant and crops do (Ahmed *et al.*, 2012). At current, more plant tissue culture laboratories are being established worldwide especially in developing countries as plant tissue culture make way for the multiplication of selected plant species and varieties of robust qualities equipped with good and acquired traits besides also having cheap labor and production costs (Ahmed *et al.*, 2012).

2.2.2 Plant growth media

The growth of plants in *in vitro* conditions is largely controlled by the contents of the culture medium consisting mainly of mineral salts, carbon source (sugar), water as well as other components like organic additives, plant growth regulators and gelling agent (Gamborg and Phillip, 1995). The first and most basic growth medium was the Murashige and Skoog (MS) and the Linsmaier and Skoog (LS) medium, developed in 1962 and 1965 respectively and is still widely used since its establishment (Prakash *et al.*, 2004). At current, there are more types of basal media being developed with differing chemical compositions to cater for different plant species and cultivars.

In general, the choice of medium and its composition is dictated by the objective, the cell or tissue type and the different growth stages of the culture. Even the state of media gives different effects as some plant tissues respond well to semi-solid medium while some preferred liquid medium. The type of plant growth regulators supplemented as well as the concentration and ratio between them is crucial as it controls the morphogenic response of the explant towards callus, shoots or root formation (Prakash *et al.*, 2004). Besides, the utilization of organic additives like coconut water, tomato juice and banana extract is also common as they can provide an array of organic nutrients and growth inducers. Activated charcoal is also commonly added to growth media for the purpose of withdrawing substances that may disrupt or inhibit growth while induce the release of growth stimulating compounds (Pan and Van Staden, 1998). When fully optimized, activated charcoal may reduce explant browning and chlorosis, enhance shoot multiplication and proliferation and even prevent shoots from growing in clumps (Hazra *et al.,* 2002; Fráguas *et al.*, 2004). However at incorrect concentrations, activated charcoal may give an adverse effect such as the reduction of cell proliferation or even induce shoot vitrification (Ebert *et al.*, 1993; Kadota *et al.*, 2001). Other than that, explants require a constant supply of carbon source for the maintenance of growth and overall plant health. Sucrose is the most commonly utilized carbon source with it being relatively inexpensive and easily acquired (Prakash *et al.*, 2004). However, there are also explants which preferred other carbon sources like fructose and glucose (Yu and Reed, 1993; Hsia and Korban, 1996; Cuenca and Vieitez, 2000). Different types of explant and plant species gives differing response to the culture medium and its contents which therefore cements the importance for optimization to ensure that optimal growth is achieved besides having all objectives met.

2.2.3 Plant Growth Regulators

The discovery of plant growth regulators was the first crucial breakthrough in plant tissue culture as prior to its discovery, cultured *in vitro* plant cells were unable to divide and multiply. Auxin Indoleacetic acid or IAA was the first plant growth regulator discovered, documented in the work by Fritz Went and Kenneth Thimann in the 1920s where auxins were found to be crucial for the growth of isolated meristematic plant tissues (Caponetti *et al.*, 2005). Later on in 1950s was when cytokinins were discovered in the study by Skoog and Tsui (1951) where cytokinins were utilized to induce callus and buds from stem segments of tobacco. Kinetin was later discovered followed by other cytokinins in the 1960s which have become an important component for *in vitro* shoot induction and rapid plant propagation of plant cultivars which has economic, agronomic and horticultural importance (Caponetti *et al.*, 2005).

Over the years, more studies were carried out on plant growth regulators and so far there are five different types of plant growth regulators discovered which are auxins, cytokinins, ethylene, gibberellins as well as abscisic acid. Also known as phytohormones, each plant growth regulator carries different function and effect on plant cells and tissues but with the same purpose of maintaining tissue and organ growth and development (Bhojwani and Razdan, 1986). In the induction of multiple shoots, cytokinins have been the most commonly utilized plant growth regulator. Cytokinins such as 6-Benzylaminopurine (BAP), Kinetin, Zeatin and Thidiazuron (TDZ) play a huge role in the regulation of meristem functions, induction of cellular division and differentiation into shoots from callus and plant organs as well as the preclusion of apical dominance to encourage adventitious shoot formation (Bhojwani and Razdan, 1986). Although commonly utilized alone, plant growth regulators may also interact differently when used together and at various concentrations. Most commonly, auxins when used together with cytokinins were known to have either synergistic or antagonistic interactions when utilized together and their positive interactions may induce cell and meristem development, growth and maintenance which are important for the plant to establish its full body (Su *et al.*, 2011). Thus, it is crucial to ensure the best effect and outcome is obtained from plant growth regulators by having considered its type, concentration and combinations on targeted plant species as well as explant type.