MICROPROPAGATION OF FIG (*FICUS CARICA*) CV. JAPANESE BTM 6

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MICROPROPAGATION OF FIG (*FICUS CARICA*) CV. JAPANESE BTM 6

by

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LIST OF ABBREVIATIONS

%	Percentage
µM	Micromolar
2-ip	6-(γ,γ-Dimethylallylamino) purine
ABA	Abscisic acid
ANOVA	Analysis of variance
BAP	6-Benzylaminopurine
BRs	Brassinosteroid
°C	Degree Celcius
DNA	Deoxyribonucleic acid
et al.,	Et alia
g	gram
GAs	Gibberellic acid
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
JA	Jasmonic acid
kPa	Kilopascal
lm	Lumen
Mg	Miligram
mg/L	Miligram per litre
mL	Mililitre
MS	Murashige and Skoog
NAA	1-Naphthaleneacetic acid
NaOCl	Sodium hypochlorite
NaOH	Sodium Hydroxide
PCR	Polymerase Chain Reaction
PGR	Plant Growth Regulators
TZ	Thidiazuron
W	Watt
TDZ	Thaidiazuron

LIST OF APPENDICES

MIKROPERAMBATAN POKOK TIN (*FICUS CARICA*) CV. JAPANESE BTM 6

ABSTRAK

Ficus carica L., dikenali sebagai pokok ara dan termasuk dalam keluarga Moraceae, ditanam di kawasan subtropika dan Mediterranean di seluruh dunia. Pokok ini kaya dengan serat semulajadi, mineral, dan vitamin. Namun, kaedah pembiakan konvensional, seperti peluruhan, pemotongan kayu keras, dan pemacuan udara, tidak cekap untuk tujuan pertanian di Malaysia kerana kecekapan pematangan yang suboptimal. Namun, eksperimen telah menunjukkan bahawa vermicast boleh meningkatkan induksi akar, memperbaiki kadar kelangsungan hidup anak benih dalam kaedah ini. Oleh itu, kami sedang mempertimbangkan untuk menggunakan vermicast sebagai pilihan untuk membantu dengan induksi akar. Vermicast, hasil dekomposisi bahan organik oleh cacing tanah, terkenal dengan impak positif terhadap pertumbuhan tanaman. Kajian ini bertujuan untuk menetapkan kaedah mikropembiakan *Ficus carica* cv. Japanese BTM 6 dan menilai kesan larutan vermicast ke atas kultur pucuk *in vitro*. Eksplan segmen nodus steril dirawatan melibatkan dengan pelbagai jenis dan kepekatan aditif organik seperti kentang, pisang, dan air kelapa. Pucuk yang dihasilkan dirawat dengan kombinasi cytokinins (meta-topolin, BAP, 2ip, zeatin, dan TDZ) dan auxins (IAA, IBA, NAA) untuk penggandaan pucuk. Selain itu, pucuk dinilai bagi respons terhadap larutan vermicast (5% hingga 30%) dari segi regenerasi pucuk dan akar *in vitro*. Plantlet dengan akar telah diaklimatisasi dengan campuran pasu yang berbeza dan vermicast sebagai media aklimatisasi dengan dan tanpa larutan vermicast semasa penyiraman. Selanjutnya, pengekstrakan DNA dijalankan ke atas sampel daun yang dikumpulkan dari lapan kitaran subkultur yang berbeza (S1-S8), diikuti dan akan dikenakan kepada analisis polimorfisme menggunakan penanda SCoT dan DAMD.

Keputusan menunjukkan bahawa jumlah pucuk berganda tertinggi (6.39 ± 0.59 pucuk) diperhatikan dalam medium Murashige dan Skoog (MS) yang ditambah dengan 1.0 mg/L meta-topolin, manakala peningkatan tertinggi dalam ketinggian pucuk eksplan (3.43 ± 0.16 cm) direkodkan dalam MS yang ditambah dengan 0.5 mg/L zeatin. Perlu dinyatakan, rawatan melibatkan 1.5 mg/L IAA mengekalkan jumlah akar tertinggi (1.78 ± 0.48 akar) berbanding dengan semua rawatan auxin individu. Sebaliknya, medium MS yang ditambah dengan 20% larutan vermicast menghasilkan jumlah akar tertinggi (3.93 ± 0.60 akar) dan panjang pucuk purata 3.16 ± 0.59 cm, melampaui kepekatan larutan vermicast dan rawatan auxin yang lain. Seterusnya, gabungan cytokinins (meta-topolin dan zeatin) dengan larutan vermicast menunjukkan hasil yang bertentangan berbanding dengan rawatan individu. Walaupun penggunaan larutan vermicast tidak merangsang regenerasi pucuk berganda dengan efektif seperti rawatan sitokinin, ia secara signifikan meningkatkan regenerasi akar dalam kultur *in vitro* berbanding dengan rawatan auxin yang diuji. Penyelidikan ini menekankan potensi kesan merangsang akar larutan vermicast dalam kultur *in vitro*. Plantlet yang diaklimatisasi dalam campuran pasu dengan tanah vermicast yang dirawat dengan larutan vermicast memberikan pemanjangan pucuk tertinggi (4.33 ± 0.15 cm) dan memberikan kadar kelangsungan hidup yang lebih tinggi berbanding hanya campuran pasu. Analisis jalur monomorfisme melalui penanda molekul SCoT dan DAMD kini sedang dijalankan untuk mengesahkan kestabilan genetik tanaman mikropembiakan di semua lapan kitaran subkultur. Kajian ini berjaya menetapkan protokol mikropembiakan yang cekap untuk *Ficus carica* cv. Japanese BTM 6 dan mendokumentasikan kecekapan vermicast dalam merangsang regenerasi dan pertumbuhan plantlet yang sesuai untuk komersialisasi dan pembiakan di Malaysia.

MICROPROPAGATION OF FIG (*FICUS CARICA*) CV. JAPANESE BTM 6

ABSTRACT

Ficus carica L., commonly known as the fig tree and belonging to the Moraceae family, is cultivated in subtropical and Mediterranean regions worldwide. This fruit-bearing tree is rich in natural fibre, minerals, and vitamins. However, conventional propagation methods, such as grafting, hardwood cutting, and air layering, are inefficient for establishing viable plant stocks in Malaysia. The primary factor to this inefficiency is the suboptimal rooting efficiency associated with these conventional propagation techniques. However, experiments have shown that vermicast can enhance root induction, improving the survival rate of plantlets in these methods. Therefore, vermicast can be as an option to help with root induction."Vermicast, a byproduct of organic matter decomposition by earthworms, is known for its advantageous impact on plant growth in agriculture. This study aims to establish methods for the micropropagation of *Ficus carica* cv. Japanese BTM 6 and to evaluate the regeneration effects of vermicast solution on *in vitro* shoot cultures. Sterile nodal segment explants were subjected to treatments involving various types and concentrations of organic additives, including potato, banana, and coconut water. The induced shoots were further treated with different combinations and concentrations of cytokinins (meta-topolin, BAP, 2ip, zeatin, and TDZ) and auxins (IAA, IBA, NAA) to promote shoot multiplication. Additionally, the induced shoots were assessed for their response to varying concentrations of vermicast solution (ranging from 5% to 30%) in terms of *in vitro* shoot and root regeneration. Plantlets with roots were acclimatized with different potting mix and vermicast as the acclimazation media with and without vermicast solution during watering. Furthemore, DNA extraction was performed on leaf samples collected from eight different subculture cycles (S1-S8), then subjected to polymorphism analysis using SCoT and DAMD markers. Results revealed that the highest number of multiple shoots (6.39 ± 0.59 shoots) was observed in Murashige and Skoog (MS) medium supplemented with 1.0 mg/L meta-topolin, whereas the greatest increment in explant shoot height (3.43 ± 0.16 cm) was recorded in MS medium supplemented with 0.5 mg/L zeatin. Notably, the treatment involving 1.5 mg/L IAA induced the highest number of roots (1.78 ± 0.48 roots) compared to all individual auxin treatments. Conversely, MS medium supplemented with 20% vermicast solution yielded the highest number of roots (3.93 ± 0.60 roots) and an average shoot length of 3.16 ± 0.59 cm, surpassing other vermicast solution concentrations and auxin treatments. Subsequently, the combination of cytokinins (meta-topolin and zeatin) with vermicast solution exhibited a counterproductive outcome compared to individual treatments. While the utilization of vermicast solution did not stimulate the regeneration of multiple shoots as effectively as cytokinin treatments, it significantly enhanced root regeneration in *in vitro* cultures compared to the tested auxin treatments. This investigation underscores the potential root-stimulating effects of vermicast solution on *in vitro* cultures. Plantlets acclimatized in the potting mix with vermicast soil treated with vermicast solution resulted in the highest shoot elongation (4.33 ± 0.15 cm) and provided a higher survival rate compared to only potting mix. Analysis of monomorphic bands via molecular SCoT and DAMD marker were used in this experiment to confirm the genetic stability of micropropagated plants across all eight subculture cycles. This study has successfully established efficient micropropagation protocol for *Ficus carica* cv. Japanese BTM 6 and documented the efficiency of vermicast in stimulating regeneration and growth of plantlets well-suited for commercialization and propagation in Malaysia.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Ficus carica L., commonly known as Fig, holds significant botanical importance as a deciduous plant belonging to the *Ficus* genus within the Moraceae family. Fig cultivation can be recognizable by its palmate leaves and fibrous roots, this deciduous tree or shrub possesses a unique appearance. The easiest way to differentiate among fig cultivars is the according to characteristics of their fruits, including differences in skin color, size, flavor, and texture. Geographically, figs exhibit versatility, thriving in different climacteric and geographical conditions (Hiwale, 2015). Historical evidence suggests that fig cultivation dates back to 2000-3000 BC in the eastern Mediterranean area (Marpudi *et al*., 2013). Today, figs hold global significance as a crop, valued for both dried and fresh consumption, whether in dried form, canned, or through various preservation methods. Beyond its process applications, the fig is a nutritional product, rich in nutrients, amino acids, and antioxidants. It stands out for its high fiber content, as well as notable amounts of potassium, calcium, and iron, surpassing other common fruits (Lakshmi *et al*., 2018). The fig plant contains diverse bioactive constituents distributed across various parts, encompassing phenolic compounds, organic acids, phytosterols, anthocyanins, triterpenoids, coumarins, and volatile compounds such as hydrocarbons and aliphatic alcohols (Oliveira *et al*., 2009). Additionally, the leaves and latex of plant have been utilized in alternative medicinal practices for treating various health conditions, such as calluses, bee stings, gum injuries, cataract treatment, and wound healing (Hashemi *et al*., 2011). Traditional uses of fig encompass a wide range of medicinal applications, including as an soothing agent, diuretic, aphrodisiac, haemorrhoid suppressant, cough suppressor, antiulcer, and hypercholesterolemia medication (Lalitha *et al*., 2021). Fig fruits have been used as a diuretic, expectorant, and mild laxative in Indian medicine, and both traditional and modern medical procedures use fresh figs to treat skin problems (Solomon *et al*., 2006).

Currently, fresh figs are considered one of the more expensive fruits in Malaysia due to their usual importation, and there is no commercial cultivation for domestic purposes. The use of seedlings is not practical due to significant variations from the mother plants, resulting in the development of commercial propagation methods such air layering, grafting, and cuttings (Ling *et al*., 2018). However, these traditional methods do not ensure consistent plant growth and development, resulting in variable fruit output, especially on a larger commercial scale. Current fig propagation methods in Malaysia are less efficient and are not well-suited for the production of plant stocks intended for commercial planting.

To address these challenges, plant tissue culture techniques offer a continuous, sustainable, cost-effective, and viable method for producing plant stocks that are exactly like the parent plant, irrespective of environmental or geographic factors. Explants, which are tiny bits of plant tissue, can be used in a continuous process to generate a lot of plantlets quickly (Chun *et al*., 2020). Plant growth regulators, which mainly comprise different auxins and cytokinins, are added to certain plant tissues in order to manipulate them and trigger organogenesis. While previous literature has successfully optimized shooting and rooting parameters for large-scale *in vitro* propagation based on specific cultivars, it is essential to recognize that these optimizations may not be suitable for the micropropagation of the Japanese BTM 6 cultivar because different cultivars of *Ficus carica* may exhibit varying shoot and root induction responses to the same plant growth regulators, but these results can still serve as a reference for this experiment.

Vermicast is a product generated by earthworm that digested organic material in the soil into nutrient rich form (Singh *et al*., 2016). Vermicast contains specific hormones, enzymes, bacteria, as well as both inorganic and organic components. These compounds undergo a transformative process within the earthworm's intestine, and the product are vermicast (Teršič & Gosar, 2012). Various types of food sources, such as rice straw, banana peelings, mudpress, and cow manure, can be supplied to earthworms to produce vermicast (Cagasan *et al*., 2020). The quality of the vermicast is affected by the humanity, pH, temperature, organic carbon content, and the concentration of NPK in the environment, all of which can impact the health of worms involved in vermicast production (Sharma *et al*., 2009). Besides, vermicast has been prove that contain high quality of organic nutrient with high concentration of NPK as compare to commercial fertilizer found in market (Cagasan *et al*., 2020). According to the study done by (Lin *et al*., 2021), vermicast contain plant growth regulator like cytokinins, auxins and humic acids that will help in germination, growth and plant production. On the other hand, vermicast solution with plant growth regulators used in plant tissue culture have showed significant result in callus induction done by Raja *et al*. (2022) but according to current experiment result vermicast solution treatments does not showed any callus induction.

Thus, the main goal of the current study is to evaluate the effectiveness of various plant growth regulators at different doses and the influence of vermicast solution on *Ficus carica* cv. Japanese BTM 6 regeneration and micropropagation. The study involves optimizing different parameters, including the number of shoots, shoot height, number of roots, and root length, which play a specific role in determining the health of the plantlets. To ensure the true-to-type and uniform nature of the *in vitro* regenerated plantlets at various subculture intervals, their genetic fidelity will be evaluated using molecular markers to identify potential occurrences of polymorphism. In addition to these objectives, vermicast solution as a supplementation in *Ficus carica* cultivation has not been previously described in the literature, the purpose of this study is to explore the potential of vermicast solution in improving the growth of *Ficus carica* cv. Japanese BTM 6 as combination to different types and concentrations of plant growth regulators, with the ultimate goal of producing healthy plantlets suitable for commercial purpose.

1.2 Research Objective

The aim of this research is to formulate a successful protocol for the *in vitro* micropropagation of *F. carica* cv. Japanese BTM 6 using nodal explants, and secondly, to examine the genetic stability of the resulting micropropagated plantlets, facilitating their suitability for more cultivation of commercially valuable plant stocks. The objectives of this study include:

a) To induce multiple shoots from *in vitro* explants of *Ficus carica* cv. Japanese BTM 6 by using nodal segment of the stem, with a series of combination of plant growth regulators and vermicast solution.

b) To evaluate and identify polymorphism occurrences of the *in vitro* regenerative shoots by using molecular marker.

c) To induce *in vitro* rooting and to acclimatizate the *Ficus carica* cv. Japanese BTM 6 plantlets for field adaptation.

CHAPTER 2

LITERATURE REVIEW

2.1 General information on *Ficus carica*

2.1.1 Description, Origin and Distribution

Ficus carica L. from the Moraceae family is a member of the genus *Ficus* known as the common fig. Fig plants are known as keystone species in tropical rainforest ecosystems because their fruits sustain insects, birds, and other creatures throughout the year (Falistocco, 2020). Besides, the fig is one of the early cultivated fruit species by humans that possibly originated from the Middle East. Still, the fig is now widely cultivated in the subtropical and Mediterranean regions of the world, and it is recognised as an important world crop today harvested worldwide for dried and fresh consumption, either raw, canned, or in other ways of preservation (Barolo *et al*., 2014). Figs are eaten fresh and also savoured as dried fruit because of their flavour (Hssaini *et al*., 2021). Turkey, Egypt, Morocco, Spain, Greece, California, Italy, Brazil, and other regions with mild winters and scorching, dry summers are the primary producers of edible figs (Mawa *et al*., 2013). Figs can be found in tropical and sub-tropical climates because of suitable conditions and the help of animal and human selection. However, the fig tree is also a highly popular plant grown as a solitary specimen in orchards and gardens where the climatic conditions and local customs are not normally appropriate for this crop (Falistocco, 2020). This is the reason for the high population of genetic diversity of figs worldwide.

2.1.2 Economic Value

According to the Food and Agriculture Organization (FAO), fig fruit output totals more than a million tonnes per year, with the Mediterranean region accounting for 82 per cent of the industry's production (*FAOSTAT, 2015 - Google Search*, n.d.). Fig is currently a crucial crop for the entire planet. Turkey, Egypt, Morocco, Spain, Greece, California, Italy, Brazil, and other regions with generally warm winters and hot, dry summers are the main producers of edible figs (Mawa *et al*., 2013). About 27% of the fresh figs, 53% of the dried figs, and 51% of the fruit exported globally originated from Turkey. The Turkish Statistical Institute recently released data showing that the output and export volumes of dry figs are 299 and 278 thousand metric tonnes, respectively (Yilmaz *et al*., 2017). However, figs have experienced a growing trend in recent years on the global market, both for fresh and dried varieties. The fig's weight, maturity index, and shape all play a significant role in determining how desirable it is for trade, with globose-shaped fruit variants favoured for their suitability for packaging and shipping (Benettayeb *et al*., 2017). Fig cultivation has increased in various nations, including Mexico, with an estimated annual growth rate of 6% for the harvested area and 13% for production volume (Garza-Alonso *et al*., 2019). In Mexico, 1440 hectares of land were under cultivation in 2017, producing an average yield of 5.6 tonnes per hectare. However, under greenhouse conditions, yields of more than 100 tons per hectare have been noted (Garza-Alonso *et al*., 2019). As compare to Indonedia and Thailand, Malaysia in fig crop production still in the starting period, at the project site of Indonesia, Malaysia, and Thailand Growth Triangle (IMT-GT) in Chuping, Perlis, has 16,000 fig trees planted on a 10 hectare plot to increase the fig crop production in Malaysia (Kamarubahrin *et al*., 2019).

2.1.3 Morphology and Characteristics

The fig species are small deciduous trees or shrubs with slightly roughened bark and greyish-brown in colour. The fig leaves are slender and petiolated, with an acute, obtuse apex, a palmately lobed, cordate base, an ovate, almost orbiculate, or ovate leaf blade, and scabrous-pubescent surfaces (Mawa *et al*., 2013). Besides, Figs are classified as dioecious since they exist in both the capri fig and common fig tree types (Mawa *et al*., 2013). The fig fruit is a composite fruit comprising a tissue receptacle that encloses multiple pedicellate pistillate blooms that eventually turn into drupelets. In contrast to the empty drupelets in parthenocarpic fruits, tiny seeds develop from fertilised drupelets (Marcotuli *et al*., 2020). The volume and size of seeds are substantially influenced by chemotype, caprification, and ripening phases, which also affect flavour and taste in fresh and dried fig fruits (Hssaini *et al*., 2021). Depending on the fruit size, each fruit can contain from 30 to 1600 seeds (Shahrajabian *et al*., 2021).

Figs fruits is one of the easier way to recognized the cultivar of the fig that are classified into two big groups that light skin kinds include fruits with yellow, yellow-green or green hue colour fruits for example Golden Orphan and the dark skin type of fruits with red, purple, black or brown skin tone for example Japanese BTM 6 and Texas Everbearing are in this category (Bey and Louaileche, 2015). Besides, different cultivar of fig fruits have their special characteristic that can help to recognize them from other types of cultivar for example Violette de Solliès is recognized as sweetest fig fruits (Ling *et al*., 2022) and Golden Orphan with a yellow-green skin fruit has a milky taste (Sriskanda *et al*., 2021).

On the other hands, some cultivar of fig plants have some their own special morphological different between other cultivar such as ‘Kadota’ has the heaviest fruits

(61.53 ± 10.4 g) and highest stalk width (6.59 ± 1.1 nm) whereas the highest stalk is on cultivar of ‘Fassi’ (Hssaini *et al*., 2020). In general, conducting an inventory of plant material based on morphological traits is important for the effective management of genetic resources. This process is to preserve the existing genetic variability and establishing a germplasm collection (Podgornik *et al*., 2010).

2.1.4 Growing conditions

Fig species can adapt to many types of environment condition due to their adaptability to wide range of climatic and soil conditions. Fig can survive in many environmental conditions without having any significant problem related to toxicity or nutrient lost (Hiwale, 2015). Fig are commonly found in semiarid tropical, subtropical climates, coastal and inland regions whether in lowland or mountain areas (Badgujar *et al*., 2014). These fig trees can grow well in conditions of partial or complete sunlight exposure. Importantly, fig can survive in extreme condition that are not suitable for their growth for example mild winters as low as 10ºC and region with hot summers as high as 1525 meters above the sea level (Hiwale, 2015).

One of the weaknesses of figs is their sensitive fruits to rainy weather condition. Rain can affect the quality of fruits by causing premature splitting during the ripening process (Isa *et al*., 2020). Depending on the cultivation of fig fruits can be ripe into full size within three days. Therefore, to obtain quality fruits the harvesting time is very important. Figs must be harvested at the peak of ripeness because late harvesting will cause the loss of freshness, wilting and decay due to overripening (Flaishman *et al*., 2007). On the other hand, prematurely harvested fruits will be low quality, in bad condition of size, colour and taste (Flaishman *et al*., 2007).

In current agricultural practices, both greenhouse and open-field planting are widely used for the cultivation of figs because of their high productive of good quality fruits (Shamin-Shazwan *et al*., 2019). Among these cultivation methods, a greenhouse provides a more controlled and stable environment for efficient fruits production compared to the open-field planting (Ponce *et al*., 2014). The advantage of greenhouse cultivation for fig trees and their fruits is protected by most of the pests and diseases infection. Besides, these figs trees and fruits are also protected to those unpredictable weather changes (Cerri *et al*., 2009). Thus, all these factors will affect the quality and quantities of fruits production. A previous study showed that greenhouse cultivation will provide better yields, ten times greater per unit as compared to traditional field crop methods (Shamin-Shazwan *et al*., 2019).

In those countries with tropical climates such as Malaysia, the temperature ranges from 22 to 33 °C with high humidity levels ranging from 80 to 90%, except in the highland regions. These climatic conditions create a favouble environment for the cultivation of figs (Isa *et al*., 2020). Therefore, figs are one of the highly viable alternative fruits crop production in Malaysia.

2.1.5 The Different Propagation Techniques of *Ficus carica*

Traditionally, figs are grown using various techniques, most by vegetative propagation. Hardwood cutting is one of the traditional propagation methods for *Ficus carica*, in which the basal part of the stem is dipped in a rooting hormone to promote rooting and increase the plant’s chances of survival. In Aljane and Nahdi (2014) study, two-year-old shoots with a length of 40 cm and a diameter of less than 1.5 m showed the best rooting and plant growth results.

On the other hand, air layering is also one of the common propagation methods used for *Ficus carica*. By covering a portion of a tree branch without the outer skin with soil or moss treated with rooting hormone, a technique known as air layering causes roots to begin to form. Then, the entire branch with the rooting part will be transplanted into a new soil pot as a new plant following the rooting process (Tchoundjeu *et al*., 2010). In a previous study, the experiment showed up to a 96% survival rate using air layering to propagate *Ficus carica* (Wakle *et al*., 2021). However, the air layering method produces less than the time taken. Hence it is not a suitable method for mass propagating figs.

Compared to traditional propagation methods for *Ficus carica*, the plant tissue culture technique is a good option for the mass propagation of figs. The plant tissue culture technique is a process of cloning genetically identical mother plants in a sterile and controlled (*in vitro*) environment with the factors of luminosity, photoperiod, temperature, and nutrition supplementation (Smith, 2012). The plant tissue culture technique has been widely used to increase the fig plant stocks in many experiments (Azhar and Zainuddin, 2020; Boliani *et al*., 2019; Ling *et al*., 2018). According to previous experiments, plant tissue culture can be a viable option for producing a larger amount of rooted plant stock in a short time, compared to the low survival rate of traditional propagation methods.

2.1.6 Nutritional Value

Numerous scientific investigations have proved that fig is one of the most nutritious fruit cultivated by human. Besides, the United States Department of Agriculture (USDA) has state that dried figs have excellent content of nutrient content as compare to other dried fruits. This shows that fig have high nutritional levels and therapeutic attributes

(Dueñas *et al*., 2008). A previous study demostrated that dried fruits of *Ficus carica* content essential nutrients, including vitamins, minerals, carbohydrates, sugars, organic acids, phenolic compounds, as well as high concentrations of fiber and polyphenols (Mawa *et al*., 2013). Furthermore, figs contain low lipid and cholesterol content while containing high level of amino acids (Oliveira *et al*., 2009). All these contents of fig fruits can provide many essential nutrients for human body. Besides, fig fruits also contain key elements such as copper, magnesium, manganese, calcium, potassium and vitamins E and K (Khatib and Vaya, 2010).

Notably, figs also contain essential oils such as (Z)-3-hexeny benzoate, n-nonanal, n-tetracosane, (E)-2-hexenal, n-hexadecanoic acid, n-docosane, and phytol (Saif *et al*., 2020).This intricate composition further contributes to the comprehensive nutritional tapestry that figs present. According to the study done by Walia *et al*., (2022) on *Ficus* fruits, the study focused on identifying *Ficus* fruits containing substantial amounts of bioactive compounds such as luteolin, lutein and myricetin, which are known with various health benefits to the human body.

2.1.7 Traditional Uses and Medicinal Values

The composition of dried fig fruits including sugars, organic acids, vitamins, minerals and phenolic compounds provides potential health benefits (Mawa *et al*., 2013). Notably, different part of fig tree, such as leaves, fruits and roots have been proven in traditional medicine. This includes recognized attributes such as laxative, antispasmodic, anti-inflammatory, circulatory, and respiratory effects (Oliveira *et al*., 2009). Additionally, fig components have been utilized to address various health concerns, including conditions impacting the immune system (malaria), gastrointestinal tract (ulcers and vomiting), reproductive system (menstrual pain), endocrine system (diabetes), and infectious diseases (skin disorders, scabies, and gonorrhea) (Badgujar *et al*., 2014). The advantages of using herbal medicine, like fig product are their affordability, accessibility and the limited side effects. Futhermore, applications of figs in dietary contexts and as medicinal agents have shown potential in improving cardiovascular diseases and reducing blood pressure (Alamgeer *et al*., 2017).

Moreover, figs have been investigated for their potential anticancer activity against a range of malignancies, including colon, prostate, breast, cervical, and liver cancers. Most of the therapeutic attribute found in fig leaf extracts(Perez *et al*., 2000), and the fruit and stem exhibit higher antioxidant activity. Besides, fig leaves contain 121 volatile compounds, while fig fruits only contain 108 of coumpounds, but there are 18 shared volatile compounds found in both fig leaves and fruits (Li *et al*., 2012). On the other hand, previous studies also showed that figs have the therapeutic potential in the context of gastrointestinal health. Polysaccharides extracted from figs, when administered in water extracts have showed significant reduction in inflammation in animal experiment tests (Zou *et al*., 2020).

Furthermore, the ethanolic leaf extract of figs has also been found to exhibit antioxidant activity against hepatocellular carcinoma (HepG2) and human laryngeal carcinoma (Hep-2) cell lines. Moreover, the aqueous leaf extract of figs has been observed to contribute to glucose homeostasis. Specifically, it has shown the effect of lowering plasma glucose levels and enhancing glucose uptake in the skeletal muscles of diabetic rats (Perez *et al*., 2000). These findings highlighted the diverse health related properties of figs.

2.1.8 *Ficus carica* cv. Japanese Brown Turkey Modified (BTM) 6

The Ficus carica orginating in Asia and the Meditereanean region, to be believed to have been introduced to Japan during the Meji era (1868-1912), specifically the Brown Turkey cultivar, based on general historical knowledge of plant inductions and agricultural practices in Japan (Ragone *et al*., 2001). Japan have a long history of cultivating the *Ficus carica* Brown Turkey variety.

The *Ficus carica* Japanese BTM 6 is a modified version derived from the Brown Turkey cultivar. Notably, it exhibits distinctive features such as a reddish-purple skin turn into rusted red during ripening, and it contains amber-toned edible seeds. Additionally, it stands out as one of the largest fruits when compared to other cultivars.

Figure 2.1: Fruits of Fiucs carica cv. Japanese BTM 6. Scale bar represents 2 cm.

Figure 2.2: **Cross-section of *Ficus carica* cv. Japanese BTM 6.** Scale bar represents 2 cm.

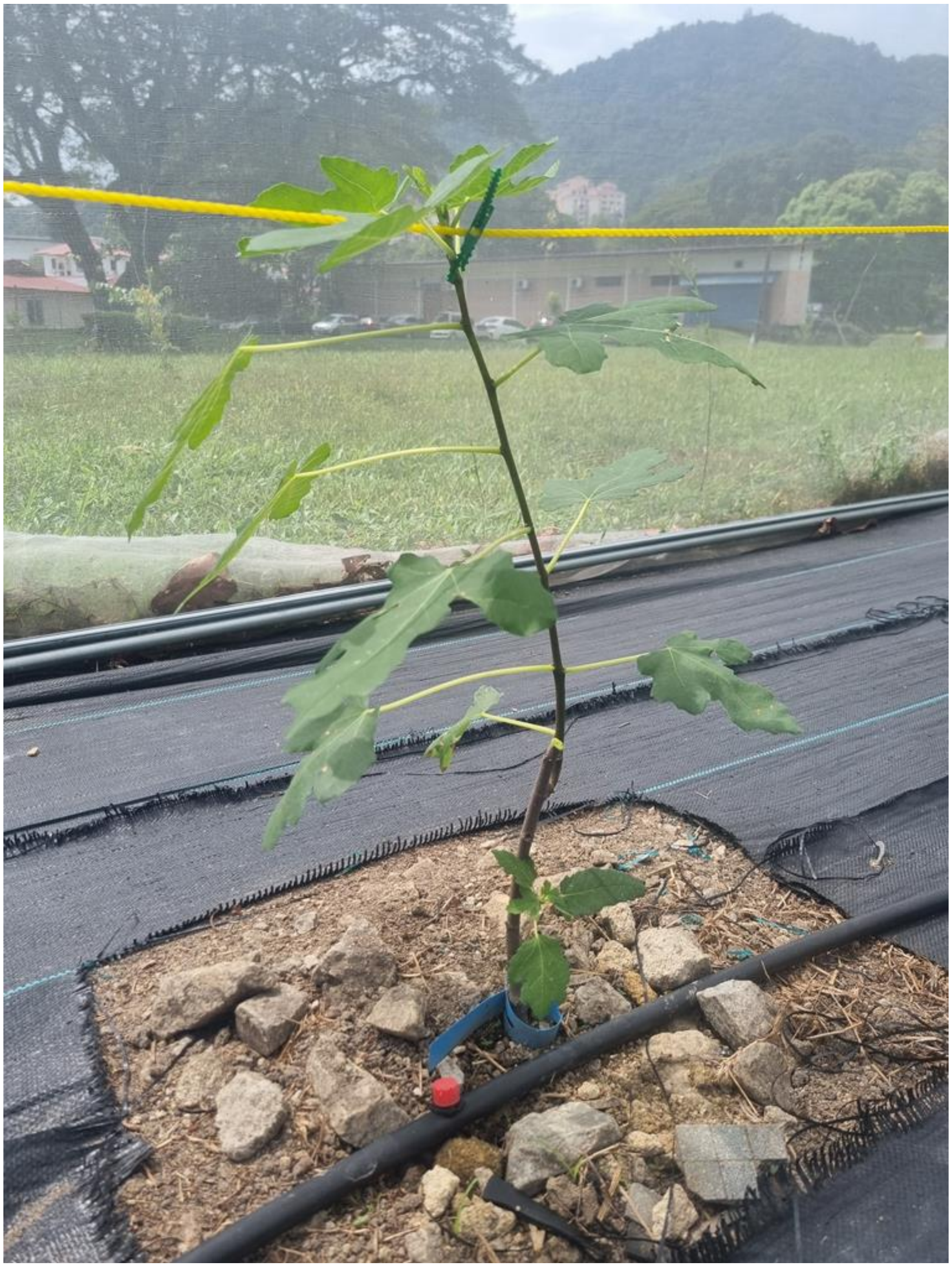

Figure 2.3: *Ficus carica* **cv. Japanese BTM 6 plant in greenhouse.**

2.2 Plant Tissue Culture

Another term for plant tissue culture is *in vitro* cultivation using an aseptic technique for plant tissues, cells, and organs. Different with traditional gardening, *in vitro* culture is based on the capacity of the cell to develop freely and requires precise, suitable physical and chemical conditions, enabling the development and differentiation of a fully-developed plant into the desired results. Micropropagation is one of the methods in plant tissue culture to produce genetically identical plants with small sections of plant tissues as explants are grown in a sterile media with different types of plant growth regulators and a controlled environment to increase the growth rate of shoots and roots (Suman, 2017).

Micropropagation is a rapid process as compared to commercial propagation. This allows some low-productivity crops to have a chance to undergo mass propagation by using micropropagation. Commercial propagation needs more human power and time to produce large amounts of new plantlets than micropropagation. Micropropagation has higher multiplication rates than commercial propagation because of the time taken and human power needed (Bhatia *et al*., 2015). Therefore, decreasing in time taken and human power will also cause the cost needed for explant production to decrease.

In plant tissue culture, after propagation methods those explants will be keep in sterile container and place in environment with constant light supply with a ratio of 16:8 light:dark photoperiod, temperature range of 25-27ºC and low humanity to avoid contamination rate increase (Smith, 2012).

2.2.1 Plant Growth Regulators (PGRs)

Plant growth regulators (PGRs) are typically used in agriculture, horticulture, and viticulture to increase the productivity of different types of crops. PGRs increase the plant growth rate as inhibitors of hormone biosynthesis or translocation and hormone receptor blocker (Rademacher, 2015). In order to direct development and growth, a mix of external and internal signals interact with the genetic makeup of the plant to govern plant form and size (Kazan, 2013).

There are different types of PGRs commonly used to help in plant growth, for example, cytokinins, auxins, gibberellins (GAs), abscisic acid (ABA), ethylene, brassinosteroids (BRs), and jasmonic acid (JA) (Jiang and Asami, 2018). The cytokinins and auxins essential in plant development. Specifically, creating and sustaining the meristems required for the entire plant body to grow, wherein the shoot meristems differentiate into the above-ground plant and the root meristems differentiate into the below-ground plant (Schaller *et al*., 2015).

Cytokinins is one of the common plant growth regulator used in plant tissue culture. That is because cytokinins can influence seed germination and also help in bud regeneration (Mok, 1994). There were many type of cytokinins used in plant tissue culture such as 6-Benzylaminopurine (BAP), zeatin, 6-γ, γ-Dimethylallylamino purine (2-ip) and also Thaidiazuron (TDZ). By using 6.0 mg/L of BAP in MS medium have increase the number of buds and fresh weight on *Musa* sp. Var. “Yangambi” explants (Ngomuo *et al*., 2013). Besides, MS medium supplemented with 5.0 mg/L of BAP was the optimal media for multiple shoot formation for Ficus carica cv. ‘Violette de Solliès’ (Ling *et al*., 2022).

On the other hands, auxins are also one of the common plant growth regulator used in plant tissue culture. Auxins such as Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA) are widely used in plant tissue culture to control the organ regeneration, callus induction and somatic embryogenesis induction (Nic-Can and Loyola-Vargas, 2016). For example MS medium supplemented with 0.5 or 0.75 mg/L NAA have been used in Henequen plant to increase the efficiency of root formation (Caraballo *et al*., 2010).

Furthermore, purpose of using gibberellins in plant tissue culture is because it can remove the growth limitation of plant because of they will degrade the growth-inhibiting

DELLA proteins in the plants (Hedden and Sponsel, 2015). The used of 10µM and 2.5µM of gibberellins in two types of sweet potato (Ukerewe and Gihingamukungu) showed increment of shooting and rooting effect (Ndagijimana *et al.*, 2014).

Besides, ethylene is rarely used in plant tissue culture as compare to cytokinins and auxins but it might mediate many auxin and cytokinin response and also improve the production of culture (Pengelly & Su, 2018). Ethylene may have promotive effect on shoot and root organogenesis (Beyl, 2011) but high concentration of ethylene may cause hyperhydricity that may lead to decrease the plant growth (Polivanova & Bedarev, 2022).

2.2.2 Tissue Culture and Micropropagation of *Ficus carica*

Various plant tissue culture techniques have been explored in prior research to understand their applicability to figs. Factors such as types of explants, hormones, carbon sources, and micropropagation methods have been investigated. Explants, including nodal segments, internodal segments, axillary buds, and leaf explants, were tested to induce organogenesis in *Ficus carica*.

Concerning carbon sources, minor differences were observed in dry and wet weights, while an increase in salinity concentration in the media was found to decrease the growth of shoots in *Ficus carica* explants (Qrunfleh *et al.*, 2013). Besides, 0.2 mol/L of fructose in MS medium resulted highest shoot length and shoot number as compare to other concentration treatment and sucrose treatment on *Ficus carica* Conadria and Black Mission cultivars (Taha *et al.*, 2013).

Numerous studies have examined the impact of cytokinins and auxins on *Ficus carica* to assess the growth rates of shoots and roots. Among different cytokinins such as 6-benzylaminopurine (BAP), Thidiazuron (TDZ), and Kinetin in the same medium, BAP demonstrated the most suitable cytokinin for multiple shootings on *Ficus carica* Black Jack cultivar (Parab *et al*., 2021). Additionally, in the 'Violettte de Solliès' cultivar of *Ficus carica*, BAP as compared to zeatin, TDZ, and Kinetin showed best result in multiple shoots (Ling *et al*., 2022).

In the rooting process, various auxins, including α-indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA), yielded positive results for *Ficus carica* cv. Sarilop plant (Hepaksoy and Aksoy, 2006). According to (Ling *et al*., 2022) studies, *Ficus carica* cv. 'Violette de Solliès' with 3.0 mg/L of IBA in MS medium exhibited the highest rooting percentage, while 2.0 mg/L of indole-3-acetic acid (IAA) in MS medium resulted in a greater number of roots with a lower rooting percentage.

Furthermore, investigations of callus formation on *Ficus carica* cv. Japanese BTM 6 leaf explants in MS media involved different combinations of benzyladenine (BA) and NAA, such as 2.0 mg/L BA, 0.5 mg/L BA combined with 0.5 mg/L NAA, and 2.0 mg/L BA combined with 0.5 mg/L NAA (Azhar and Zainuddin, 2020). Accroding to (Dhage *et al*., 2012) study, *Ficus carica* cv. Brown Turkey leaf explant showed the highest callus induction when culture in MS medium supplemented with 2.0 mg/L of TDZ and 4.0 mg/L of 2ip at 85.8% of percentage as compare to other 3 cultivar of fig.

2.3 Vermicast

2.3.1 General Information of Vermicast

Earthworms can be named as "farmer's friend" or "natural plowman" that is because they help in the soil physical, chemical, and microbiological communities (Balachandar *et al*., 2018). Earthworm will digest organic material in soil into nutrient-rich products that is vermicast. Vermicast is the product of earthworm, which digests biodegradable substances and organic waste into nutrient-rich form (Singh *et al*., 2016). Vermicast contain specific hormones, enzymes, bacteria, inorganic and organic components that transit of the soil via the earthworm's intestine and is more stable than the original soil aggregates (Teršič and Gosar, 2012). In addition, large amount of exogenous and endogenous enzymes has been found in the intestine of earthworms change the organic minerals into forms that are more easily absorbed by plants (Saha *et al*., 2012). According to the reports, the earthworm's intestine has key nutrient concecntration of carbon, nitrogen, phosphorus, and sulphur that are two to five times higher than those in original soil. Consequently, the high concentration of nutrients and bacteria in the earthworm's intestine also help in transformation of various pollutants in soil such as heavy metals, organic pollutants, microplastics, and antibiotics (Sun *et al*., 2020).

According to the studies done by Gajalakshmi and Abbasi (2004), various types of earthworms are commonly used to produce vermicast such as tiger worm (*Eisenia fetida*), European nightcrawlers (*Eisenia hortensis*), and the blue worm (*Perionyx excavates*). Additionally, different type of food sources, including rice straw, banana peelings, mudpress, and cow manure, can be provided to earthworms for vermicast production (Cagasan *et al*., 2020). However, it is important to avoid providing acidic and spicy materials as they may turn the environment toxic for earthworms that is harmful to the earthworms. The challenge in producing high-quality of vermicast depend on the changes in the environment factors such as pH, humidity, temperature, organic carbon content, and the concentration of NPK, all of which can impact the health of worms involved in vermicast production (Sharma *et al*., 2009).

In a study by Cagasan *et al*., (2020), it was found that vermicast contain high-quality organic fertilizer with high amount of NPK concentrations compared to common fertilizers available in the market. Vermicast also contains micronutrients, beneficial soil microbes such as nitrogen-fixing bacteria, and growth hormones that contribute to plant growth (Sinha, 2013).

The utilization of vermicast in agriculture offers several benefits, including 50% of reduction in the use of inorganic fertilizers (Gajalakshmi and Abbasi, 2004) and an increase in the total microbial population of N-fixing bacteria and actinomycetes (Belliturk and Aslam, 2021).

Moreover, vermicast contains enzymes that break down organic matter in the soil, releasing nutrients to enhance plant shooting and rooting (Sinha *et al*., 2002). Plant growth regulators such as cytokinins, auxins, and humic acids are also present in vermicast, promoting germination, growth, and overall plant production (Lin *et al*., 2021).

2.3.2 The Application of Vermicast in agriculture

The acclimatisation process using pure vermicast on tissue-cultured lakatan banana showed the highest number of leaves and increased plant height compared to sawdust and coco coir dust (Salvador, 2018). Moreover, in the study conducted by Abbey *et al*. (2018), vermicast is a better natural media supplement for kale plants because vermicast has an intermediate pH compared to potassium (K) humate and volcanic minerals and also has the highest micronutrient and macronutrient content. On the other hand, vermicast has proven that it will improve the content of polyunsaturated fatty acids.

Additionally, earthworms feed on powered oyster shells to make vermicast, which satisfies all requirements set by the Korean Ministry of Environment for factors like moisture content, pH, salinity, organic carbon, phosphate, and heavy metals. Introducing this product into the soil aims to increase its capacity, promote soil aeration, and enhance soil drainage (Kwon *et al*., 2009). Depending on the type of organic waste provided to the different species of earthworms, different amounts of plant nutrients, such as nitrogen, potassium and organic carbon will showed up in the vermicast product (Jansirani *et al*., 2012). Moreover, compared to inorganic fertiliser, vermicast showed better plant growth results in stem diameter, shoot mass, shoot length, number of leaves and leaf pigments in cluster beans (*Cyamopsis tetragonoloba*). Furthermore, vermicast application improved root nodule formation, decreased disease incidence and permitted fewer plants to be stunted on *Lantana camara* plant (Karthikeyan *et al*., 2014).

Vermicast contains different nutrients for plant growth, but the amount of different nutrients depends on the sources the earthworms are fed. For example, goat manures had higher total concentrations of C, P, and K than cattle manures. However, goat manures had a higher N content than cattle manures (Loh *et al*., 2005) but as compared to other commercial fertilisers available on the market, vermicast can produce high-quality organic fertilisers that are significantly better. The study showed that vermicast has almost the same nitrogen content as inorganic fertilisers, while potassium and phosphate in vermicast have a higher content (Cagasan *et al*., 2020).

Besides, using vermicast in agriculture can reduced pest attack, faster seed germination and increase yield product of vegetable and fruits (Olle, 2019). In the study done by (Katiyar *et al*., 2023), vermicast produced by *Eisenia fetida* and *Eugilius euganiae* worms increase the production of chili and brinjal plants that help in waste management and augmenting bioresources. Using organic waste to feed the earthworms for vermicast production may contribute to decrease the financial challenges faced by farmers, offering a more cost-effective alternative to replace inorganic fertilizers (Sinha *et al*., 2010).

According to the study done by Mapile *et al*. (2023) , vermicasts of *Pheretima losbanosensis* have the potential as organic fertilizer because it contain many types of bacteria that can produce many plant needed nutrients that can help in improve the plant growth and soil fertility. All these nutrients will help to improve the growth of plants to increase the yield product.

2.3.3 Application of Vermicast in Plant Tissue Culture

In a recent study done by Raja *et al*. (2022), a vermicast solution, combined with various concentrations of plant growth regulators (PGRs) such as BA, TDZ, and NAA, was administered to *Naregamia alata* plants in MS medium to investigate the response. In Raja *et al*. (2022) experiment, among the concentrations tested, the combination of 2,4-D with the vermicast solution exhibited the maximum callus induction, characterized by green nodular callus. Additionally, the combination of TDZ with the vermicast solution demonstrated the highest percentage of shoot regeneration, the greatest shoot length, and the highest number of shoots. Conversely, the combination of IBA with the vermicast solution yielded the most favorable results in root regeneration, exhibiting the highest root length and number of roots.

Sterile vermicast and cocopeat with quarter strength of MS basal salt have been used on explants treated 5 minutes in different types (IBA, IAA and NAA) and concentration (50 to 250 mg/L) of auxins in a bottle place in a greenhouse for rooting process (Sabitha *et al*., 2021). According to (Sabitha *et al*., 2021) experiment, explants treated with 200 mg/L of NAA and 200 mg/L IBA culture in sterile vermicast and cocopeat showed the highest result in number of roots, roots length and root induction percentage.

Besides, vermicast extract supplemented with different types and concentrations of cytokinins and auxins have been used on *Lindernia minima* plant (Tamilvanan *et al*., 2023). According to Tamilvanan *et al*. (2023) experiment, result showed that 1.5 mg/L of TDZ and 1.5 mg/L of IBA supplemented with 15% of vermicast extract in quarter strength MS medium given highest number of shoot, shoot length, number of roots and root length respectively.

2.4 Polymorphism Analysis via Molecular Markers

Polymorphism is the occurrence of two or more variations of the same phenotypic in the same population in any morphological, behavioural, or physiological attribute, as well as in any coding or noncoding region of DNA, such as the nucleus, mitochondria, or chloroplast (Singh and Kulathinal, 2013). Since polymorphism forces the segregation of relatively frequent changes among populations, it stands out as a distinctive aspect of genetic variation and suggests the presence of underlying evolutionary mechanisms to maintain survival. Polymorphisms do not always alter the way genes function, despite being based on insertion, deletion, point mutations, duplication, and translocation (Nadeem *et al*., 2018). In plant breeding, polymorphism plays a significant role as a selection factor. Due to molecular marker technology, plant breeders can choose certain plants based on their marker pattern (genotype) rather than outward characteristics (phenotype). PCR, DNA sequencing, and Southern blotting are a few DNA markers that have evolved into several systems based on different methods or procedures for polymorphism identification. Molecular markers are made up of nucleotide sequences that