EFFECTS OF GROWTH REGULATORS ON PLANT TISSUE CULTURE OF *Ficus carica* L.

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Effects of Growth Regulators on Plant Tissue Culture of *Ficus carica* L.

ABSTRACT

Ficus carica L. is one of Moraceae family which natively to Westerns Asia and distributed worldwide and economically important especially in sericulture, horticulture, food and cosmetic industries. *Ficus carica* L. contain numerous of nutrients, minerals and vitamins. This plant also produces secondary metabolites, latex cells. The richness of nutrients and latex cells make this plant possessed many pharmacological effects which is importantly to medicinal and drugs industries. The demand for this plant is very high despite the production of fig is very inconsistent from year to year. So, this study aims to establish and determine the optimum concentration of benzylaminopurine (BAP) and napthelene acetic acid (NAA) for *Ficus carica* L. through plant tissue culture system by following completely randomized design. The shoots were able to be regenerated from stems explants at most optimum concentration of 1.0 mg/L BAP. For callus induction, all stem, shoot, petiole, and root explants able to emerge calli when cultured in MS medium with optimum combinations of BAP and NAA; 1.0 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L BAP + 1.0 mg/L NAA, 1.5 mg/L BAP + 1.0 mg/L NAA, and 1.0 mg/L BAP + 1.0 mg/L NAA respectively. The explants also were tested with other plant growth regulators such as thidiazuron (TDZ), Kinetin, indole-3-butyric acid (IBA), and 2,4-dichlorophenoxyacetic acid (2,4-D) for a comparison. The results still showed that BAP and NAA were the best plant growth regulators used compared to others combinations. The micromorphology study shows the presence of trichomes on adaxial and abaxial of *in vitro* and *in vivo* leaves. Stoma was also visible on all surfaces except on adaxial of *in vivo* leaf. The acclimatization process allowed the plantlets survived in garden soil with 86.67% after weeks 8 cultured. Lastly, synthetic seeds of *Ficus carica* L. were produced with stem explants that encapsulated in 4% of sodium alginate supplemented with 1.0 mg/L BAP. In conclusion, this study was successively established an alternative way in propagating *Ficus carica* L. through plant tissue culture system. The implication of this study would able to help in give a good information to public and improving agricultural sector with new technologies.

ABSTRAK

Ficus carica L. merupakan salah satu daripada keluarga Moraceae yang berasal dari Asia Barat dan dipasarkan secara meluas kerana kepentingan ekonominya dalam sektor pakaian, hortikultur, makanan dan juga kosmetik. *Ficus carica* L. mempunyai kepelbagaian nutrisi, galian dan vitamin. Tumbuhan ini juga mengeluarkan hasil metabolit sekunder seperti sel lateks. Kekayaan nutrisi dan sel lateks menjadikan tumbuhan ini mempunyai kelebihan dalam kesan farmakologi terutama untuk industri perubatan. Permintaan terhadap tumbuhan ini melonjak tinggi walaupun penghasilannya tidak begitu konsisten dari tahun ke tahun. Sehubungan itu, matlamat kajian ini untuk menunjukan dan menentukan kepekatan benzylaminopurin (BAP) dan naphtelen asetik asid (NAA) untuk *Ficus carica* L. melalui sistem tisu kultur tumbuhan. Pokok boleh dijana semula melalui eksplan batang pada konsentrasi optimum 1.0 mg/L BAP. Untuk kalus induksi, kesemua eksplan batang, daun, tangkai dan akar mampu menghasilkan kali bila dikultur atas MS media mengandungi kombinasi optimum BAP and NAA; 1.0 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L BAP + 1.0 mg/L NAA, 1.5 mg/L BAP + 1.0 mg/L NAA, dan 1.0 mg/L BAP + 1.0 mg/L NAA mengikut turutan. Eksplan juga turut diuji dengan pengawal tumbesaran pokok yang lain seperti thidiozuron (TDZ), Kinetin, indol-3-butirik asid (IBA), dan 2,4-diklorofenoxyasetik asid (2,4-D) untuk perbandingan. Walaubagaimanapun, keputusan masih menunjukan kombinasi BAP dan NAA adalah yang terbaik berbanding dengan yang lain. Mikromorfologi menunjukkan kehadiran trikome diatas lapisan atas dan bawah daun pokok *in vitro* dan *in vivo*. Stoma juga kelihatan pada semua lapisan daun kecuali lapisan atas daun pokok *in vivo*. Proses aklimitasi menunjukan pokok boleh bertahan bila ditanam didalam tanah kebun sebanyak 86.67% selepas 8 minggu kulturan. Akhir sekali, benih tiruan Berjaya dihasilkan daripada eksplan batang yang dikapsulkan dengan nitrium alginate yang mengandungi 1.0 mg/L BAP. Secara keseluruhannya, kajian ini berjaya membangunkan alternatif lain dalam pembiakan *Ficus carica* L. melalui sistem tisu kultur tumbuhan. Implikasi kajian ini dapat membantu dalam memberi informasi yang baik kepada awam dan memajukan sektor agrikultur dengan teknologi baharu.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BAP	Benzylaminopurine
HCL	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indolebutyric acid
2,4-D	2,4-dichlorophenoxyacetic acid
TDZ	Thidiozuron
Kin	Kinetin
mg/L	Milligram per liter
min	minute
mL	mililiter
MS	Murashige and Skoog
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
IBA	Indole-3-butyric acid
Spp.	Species
USDA	United States Department of Agriculture
FAOSTAT	Food and Agriculture Organization Corporate Statistical

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The medicinal plants have become worldwide trends which is due partly to widespread acceptance of the traditional and local medicines, and the integration of derivatives from natural sources in pharmaceutical products. Plants usually produce many secondary metabolites which were constituted an important source of pharmaceutical drugs (Al-Snafi, 2017). Moreover, the importance of medicinal plants has arisen because of the increasing of awareness in healthy lifestyle as well as belief that natural medicines are reliable, more effective and fewer side effects than synthetic drugs and chemical treatments (Alsarhan, 2014; Paknahad and Sharafi, 2015).

In the religious studies, there have five species of plants that been mentioned in Quran which are fig, olives, grapes, pomegranates, and dates. According to hadiths,

Prophet (PBUH) greatly emphasized on fig consumption because of its usefulness in treatment of various disease including hemorrhoids and rheumatism (Paknahad and Sharafi, 2015). Fig or its scientific name is *Ficus carica* L. is a member of Moraceae family that include *Morus* species. This plant is distributed worldwide and is economically important in many rural areas (Danial *et al.,* 2014), especially in sericulture, horticulture, food, and cosmetic industries (Aroonpong and Chang, 2015).

Commercial fig was the first plant ever to be cultivated by human and has been cultivated for a long time in various places over the world for its edible fruit. Fig is an important plant in many rural areas which natively to Westerns Asia but distributed by man throughout the Mediterranean region (Singh *et al.*, 2016). The fruits can be eaten raw, dried, canned or in other preserved forms. The dried figs have 29% water, 50% sugar, 4% protein, 5.5% nitrogen, 2% fatty material and a substance called psoralen with formula $C_{12}H_6O_3$ (Paknahad and Sharafi, 2015).

According to Al-Snafi (2017), in his preliminary phytochemical analysis and pharmacological study of *Ficus carica* L. the plants contained alkaloids, tannins, glycosides, falavanoids, saponins, coumarins, sterols, terpenes carbohydrates, phenols, essential oil, volatile oil, proteins and minerals. It also possessed many pharmacological effects such as antibacterial, antiviral, antiparasitic, antioxidant, anticancer, antimutagene, anti-angiogenic, anti-inflammatory, antipyretic, antidiabetic, antiplatelet, reproductive, endocrine, immunological, dermatological, hypolipidemic, nootropic, antispasmodic, antidiarrheal, anti-warts, nephron-protective and hepato-protective effects. Thus, this species has gained more interest in recent years for its economic importance and medicinal benefits (Mawa, *et al.,* 2013; Bayoudh, *et al.,* 2015).

Ficus carica L. or fig can be propagated through asexual and vegetative propagation. Plant tissue culture is one way of vegetative propagation. Plant tissue culture is a collection of experimental methods or set of technique of growing large number of isolated cells or tissues under sterile and controlled conditions (Singh *et al*., 2016). The control conditions such as light, humidity, and temperature (Inês *et al.*, 2016). Plant tissue culture is one of the most rapidly growing area of biotechnology because of its high potential to develop improved crops and ornamental plants. This technique has already helped in the green revolution by improving the crop yield and quality especially micropropagation (Singh *et al.*, 2016).

Micropropagation technique is one of the plant tissue culture methods which have been developed for the rapid and cost-effective propagation of plants under limited space in a short time (Aroonpong and Chang, 2015). This technique offer a viable alternative and innovative method of asexual propagation and widely used for rare, endangered and medicinal plants for commercial and conservation propagation (Baskaran and Staden, 2013). These technique involve altering several factors such as type of explants, surface sterilization method, microbial contamination, various acclimation conditions before plantlets are transferred to the field, and medium used for multiplication and rooting (Aroonpong and Chang, 2015). According to Taha, Mustafa and Hassan (2013), these factors also include culture medium type, strength, and concentration of carbon source.

Fig is common in Western Asia. Turkey is the major fig producer and exporter country in the world with total production of 270, 830 tonnes of figs with 26% of the world production and 36% of exports (Oguzhan and Polat, 2012). In 2014, the total production of figs in Turkey slightly increased to 300, 282 tonnes with 9.8% increment in

2 years making it remain on top as a leader in fig production and consumption (FAOSTAT, 2015). However, the production of fig in Turkey dropped to 262, 644 tonnes with only 20% of output are recorded in 2016 (Oberhue, 2017). Figure 1.1 shows the annual production of Figs for top 10 growing fig countries from 1960 – 2014 while Figure 1.2 is the recent data of annual production of figs recorded. In addition, the production of common fig in Jordon has dropped more than 20-folds during the last five decades (Shomali *et al.,* 2017). The fluctuation in Figure 1.1 shows that the production of common fig faces many challenge including biotic and abiotic stresses such as drought, salinity, alkalinity, soil borne disease and nematodes.

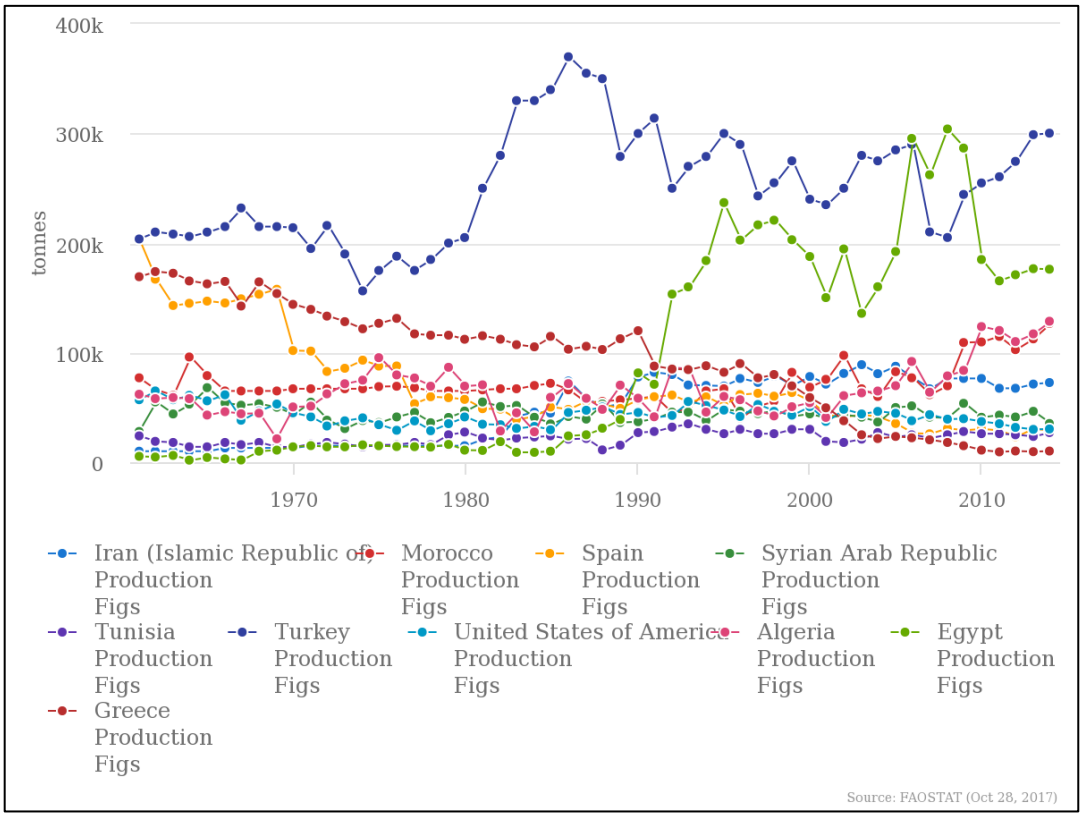

Figure1.1. Annual Production of figs by top 10 growing fig countries from1960 – 2014 (FAOSTAT, 2017)

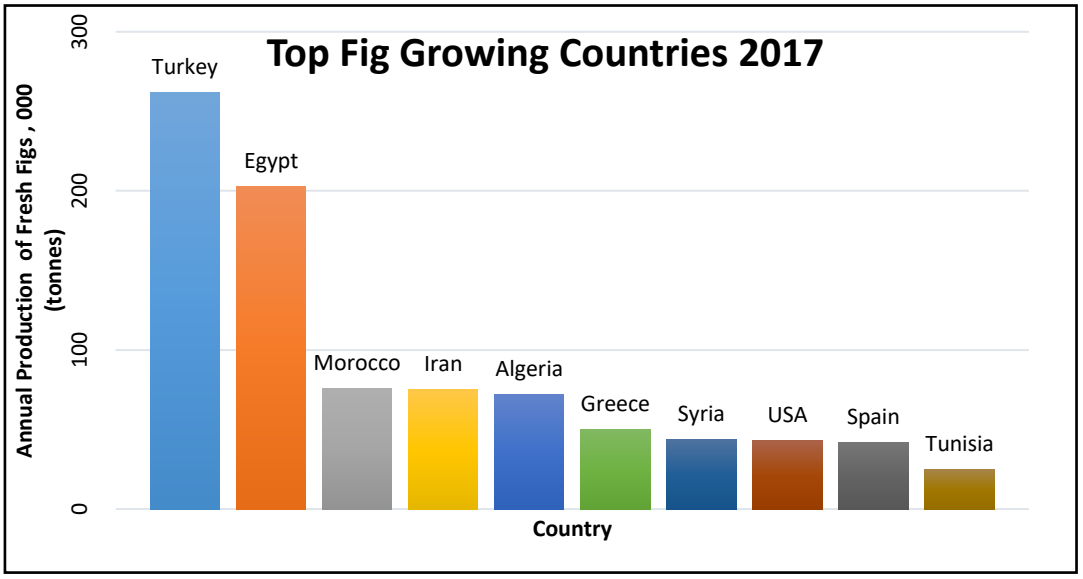

Figure 1.2. Annual Production of figs by top 10 growing fig countries 2017 (Oberhue, 2017)

From early observations, a few of farmers in Malaysia has an interest piqued in figs production. They started invest in figs production by importing varieties of figs and planting it. Figs can be considered as an introductory species or new type of plant that has been cultivated in agricultural and horticultural sectors in Malaysia. This is according to Zieman (2015), which had interviewed Ridzuan Abdullah one of fig farmers in Malaysia who had planted 700 varieties of figs around the world on his one-acre land in Kampung Merbau Sempak, Sungai Buloh. Besides that, Azmi Mohd. Sufian a teacher who also a part-time fig farmer had planted 215 types of figs around his house in Bandar Baru Beris Jaya (Ramli, 2017). Both farmers have stated that figs usually known as a season fruit, but in Malaysia figs can be cultivated and harvested all year round. However, there still no vibrant data can be found in term of market and production of fig in Malaysia.

The cultivation and production of fig in Malaysia are still new and in a small scale because fig farmers still depend on conventional methods such as cutting and air-layering in propagating the figs which consume a lot of cost and time. So, the help of biotechnology such as plant tissue culture can bring a new phase in agricultural and horticultural sectors in Malaysia specifically in breeding of figs.

1.2 Problem Statement

Largest fig growing countries use vegetative propagated plants, especially by the rooting of cuttings. However, the propagation by conventional method of cuttings, grafting and layering is slow, limited and results in poor rooting which only 20 – 30% of the cutting survive (Dessoky *et al.*, 2016). According to Dessoky (2016), conventional methods also contributed significantly to the dissemination of pathogens which affect the yield potential of the crop. *Ficus carica* L. are threatened by the attack of Fig Mosaic Disease (FMD) on leaves and fruits caused by viruses. Besides, *Ficus carica* L. are pollinated by a small wasps of the family Agonidae. So, the lack of selected pollinators according to the needs of female cultivars, and difficulties of their multiplication in fields are major constraints (Bayoudh *et al.*, 2015; Gaaliche *et al.*, 2013). *Ficus carica* L. are propagated via cutting of mature wood or grafts also resulting nonviable seeds (Singh *et al.*, 2016). So, this study will be focusing on establishing a feasible and efficient method of plantlets production through *in vitro* micropropagation using seeds, shoots, stems, roots and petioles.

1.3 Significant of Study

The significance of this study is to suggest that plant tissue culture such as *in vitro* micropropogation and regeneration is also a good technique and as an alternative to production of *Ficus carica* L. in a large quantity and considering it advantageous in producing clean plantlets. Hence, establishing a better propagation for figs and increasing a mass-scale production of good quality fig plants in Malaysia.

1.4 Objectives study

The objective of this study are:

1. To investigate a complete regeneration process of *Ficus carica* L. through tissue culture system.
2. To study callus induction *Ficus carica* L. through tissue culture system.
3. To study complete acclimatization process of *Ficus carica* L..
4. To study the production of synthetic seeds of *Ficus carica* L. from micro stem obtained *in vitro* culture.

1.5 Scope and Limitation of Study

Ficus carica L. was a new cultivation crops in Malaysia. The suitability of the climate in Malaysia has caused the cultivation of this crop among farmers have increased. However, to commercial them in locally mass production was a biggest obstacle because most of

this plant highly dependable imported from producer country. In addition, Malaysia has been burdened with hefty bills about US$3.5 billion per year on the importation of food with the current deficit in agriculture especially food about US$1.35 billion (Kamarubahrin *et al.,* 2019). Meanwhile, conventional methods that were still practiced in propagating this plant caused the acquisition process was slowed and limited despite the demand from the consumer increased due to increase of awareness about its health benefits.

Kamarubahrin *et al*., (2019) also have mentioned *Ficus carica* L. as a potential crop that could contributes to the Malaysian Gross Domestic Product (GDP). In Malaysia, fruits were an important component of agricultural production. Yet, Malaysia facing poor trade performance of fresh fruits despite of various incentive programmes implemented by government through its Third National Agricultural Policy.

Thus, production of food using a biotechnology must be in line with NAP 3 new strategies approach in achieving objectives of NAP 3 such as to enhance food security, to increase productivity and competitiveness of the sector, to deepen linkages with other sectors, to create new sources of growth for the sector, and to conserve and utilize natural resources on a sustainable basis by maximizing the income through to optimal utilization of resources in the sector (FAMA, 2020).