IMPROVED *IN-VITRO* PROPAGATION PROTOCOL FOR HERMAPHRODITE PAPAYA (*Carica papaya* L. cv. Eksotika)

By

HALIMAH BINTI HASHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is lovingly dedicated to who have given me the drive and discipline to tackle and task with enthusiast and determination. Without their love and support, this mammoth undertaking would not have been made possible.
With much love, and eternal appreciation.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Uma Rani a/p Sinniah, PhD
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Carica papaya cv. Eksotika is one of the most popular papaya varieties with high economic value in Malaysia. Generally, papaya is propagated by seeds. Being a dioecious species, bearing exclusively either male or female flowers on separate plants, seed propagation yields a mixture of male, female, and hermaphrodite papayas, each type bears variations of fruiting capabilities and fruit qualities. Micropropagation has been labelled as the most reliable system of propagation producing true-to-type hermaphrodites suitable for large-scale cultivation of papaya. However, at the onset of the procedure, micropropagation is often hampered, among others, by slow explant initiation and multiplication, as well as production of abnormalities in shoot and root structures. The present study was conducted to establish a reliable set of procedures in the production hermaphrodite papaya, Eksotika through selection of various explants' maturity stages, culture media manipulations by optimizing requirements for plant growth regulators (PGR), procedures in subculture, pretreatments in root initiation, *in vitro* root media substrates and plantlet performance in *ex vitro* environment. Shoot initiation with different explants maturity were conducted on 3-, 6-, 9- and 12-weeks old explants sourced from verified hermaphrodite papaya seedlings on Murashige & Skoog (MS) and De Fossard (DF) media, each supplemented with 1-Naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) in a complete randomized design (CRD) with four replications. Six-week-old explants recorded the highest rate of survival and number of shoots and leaves when cultured on DF medium supplemented with 0.1 and 0.5 mg/L NAA and BAP respectively. Shoot length was highest on DF medium supplemented with 0.1 and 0.25 mg/L NAA and BAP respectively. Multiplication rate on subculturing of adventitious buds increased gradually until the eight subcultures, but declined after the ninth. In rooting, indole-3-butyric acid (IBA) at 2.0 mg/L significantly stimulated root growth, and increased root number and length. In acclimatization, growing medium of mixture of peat moss, sand, and vermiculite in a ratio of 2:1:1 (v/v) showed significant positive effects on survival percentages, plant height, leave number, and stem diameter. Vermiculite in half-strength DF liquid medium promoted root development, producing higher root formation, number of roots, and root length. Plantlets treated with zeolite in

the same medium or sterile water yielded lower rate of growth and abnormal roots development. Anatomical observations revealed that length of root hairs became significantly shorter and scattered in distribution with unicellular root hairs on the epidermal cell surfaces. In *ex vitro* conditions, rooted plantlets grown in vermiculite substrate showed highest survivability after transplantation in a mixture of peat moss, sand, and vermiculite in a ratio of 2:1:1 (v/v) compared to those plantlets grown in perlite, rock wool and zeolite substrates. Overall, the results proved the efficiency of micropropagation procedure to produce in vitro hermaphrodite *Carica papaya* L. cv. Eksotika. Papaya using shoot tips as a potential region for shoot initiation and proliferation of explants to ascertain an effective protocol for the multiplication of planting materials. Additionally, the high potential to improve the rooting phase with these findings suggests that the rooting substrate may induce considerable changes in root quality which in turn affects plantlets survival during acclimatization. Solving these issues, it can help improve the protocol that will produce a high success rate in the mass production of true-to-type hermaphrodite *Carica papaya* cv. Eksotika and high-quality plants

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAMBAHBAIKAN PROTOKOL PEMBIAKAN BETIK HERMAPHRODITE (*Carica papaya* L. cv. Eksotika) SECARA *IN VITRO*

Oleh

HALIMAH BINTI HASHIM

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Carica papaya cv. Eksotika adalah satu daripada variti betik yang popular dengan nilai ekonomi yang tinggi di Malaysia. Secara amnya, pembiakan betik adalah melalui biji benih. Oleh kerana betik adalah spesis *dioecious* iaitu mengeluarkan bunga jantan atau betina pada pokok yang berasingan, pembiakan dengan menggunakan biji benih menghasilkan betik jantan, betina atau hermafrodit yang masing-masing mengeluarkan hasil dan kualiti buah yang berbeza. Kaedah pembiakan mikro merupakan kaedah yang berupaya mengeluarkan tanaman hermafrodit sesuai untuk penanaman betik dalam skala besar-besaran. Walau bagaimanapun, pada permulaan prosidur, pembiakan mikro sering terhalang, antara lain, oleh masalah permulaan dan pendaraban kultur, serta pengeluaran kelainan struktur pucuk dan akar. Kajian ini dijalankan untuk membangunkan satu set prosedur dalam pengeluaran betik hermafrodit cv. Eksotika melalui pemilihan kematangan eksplan, manipulasi media kultur dengan pengoptimuman keperluan bahan penggalak pertumbuhan (PGR), prosidur sub-kultur, prarawatan bagi pengakaran, substrat pengakaran secara *in vitro* dan prestasi pertumbuhan di persekitaran *ex vitro.* Kajian bagi menggalakkan pertumbuhan pucuk telah dijalankan ke atas eksplan pada peringkat 3, 6, 9, dan 12 minggu dari sumber hermafrodit yang telah diuji di atas media Murashige & Skoog (MS) and De Fossard (DF) masing-masing dengan penambahan 1-asid naftalenasetik (NAA) and 6-benzylaminopurine (BAP) dalam rekabentuk penuh rawak (CRD) dengan empat replikasi. Eksplan di peringkat enam minggu pertumbuhan telah merekodkan kadar hidup dan bilangan pucuk yang tertinggi apabila dikultur di atas media DF yang diperkaya, masing-masing dengan 0.1 and 0.5 mg/L NAA and BAP. Kadar pembiakan tunas adventitious bertambah sehingga lapan minggu pertama subkultur tetapi merosot selepas subkultur yang ke sembilan. Bagi pengakaran, asid butirik (IBA) pada 2.0 mg/L menggalakkan pertumbuhan akar dan pertambahan bilangan dan panjang akar dengan ketara. Di peringkat penyesuaian pertumbuhan, media campuran gambut, pasir dan vermiculite dalam nisbah 2:1:1 (v/v) menunjukkan kesan positif ke atas peratusan hidup, ketinggian, bilangan daun dan diameter batang. Vermiculite dalam media cair DF menggalakkan pertumbuhan akar dengan

menghasilkan pembentukan, bilangan dan panjang akar yang tinggi. Anak pokok yang dirawat dengan media yang sama atau air steril menghasilkan kadar pengakaran yang rendah dan pembentukan akar yang tidak normal. Pemerhatian anatomi menunjukkan pemendekan ukuran akar rambut yang ketara dan bertaburan bersama akar rambut satu sel di permukaan sel epidermis. Dalam persekitaran *ex vitro* anak pokok dari substrat vermiculite menunjukkan kadar boleh hidup lebih tertinggi selepas penanaman semula di dalam media gambut, pasir dan vermiculite dalam nisbah 2:1:1(v/v) diikuti oleh anak pokok yang ditanam dalam substrat perlite, rock wool dan zeolite. Secara keseluruhan, keputusan membuktikan kecekapan prosedur pembiakan mikro untuk menghasilkan in vitro hermaphrodite Carica papaya L. cv. Eksotika. Betik menggunakan hujung pucuk sebagai kawasan yang berpotensi untuk permulaan pucuk dan pembiakan eksplan untuk memastikan protokol yang berkesan untuk pendaraban bahan tanaman. Selain itu, potensi tinggi untuk memperbaiki fasa pengakaran dengan penemuan ini menunjukkan bahawa substrat pengakaran boleh menyebabkan perubahan besar dalam kualiti akar yang seterusnya menjejaskan kemandirian tumbuhan semasa penyesuaian. Menyelesaikan isu-isu ini ia boleh membantu meningkatkan protokol yang akan menghasilkan kadar kejayaan yang tinggi dalam pengeluaran besar-besaran *Carica papaya* cv. Eksotika hermaphrodite dan tumbuhan berkualiti tinggi

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Halimah binti Hashim
Serdang.
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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µL	micro liter
BAP	Benzyl aminopurine
cm	centimetre
dH2O	Distilled water
DF	De Fossard Medium
g/L	gram per liter
HCL	Hydrochloric acid
IBA	Indole-3-butryic acid
mg/L	milligram perliter
mm	millimeter
MS	Murashige and Skoog
NaOH	Natrium hydroxide

CHAPTER 1

INTRODUCTION

1.1 Background of study

Papaya (*Carica papaya* L.) is a member of the *Caricaceace* family which comprises of twenty-two species in four genera, three of which from America (*Carica, Jacaritia* and *Jarilla*) and one genus from equatorial Africa (*Cylicomorpha*). Papaya is believed to be native to southern Mexico and neighbouring Central America. Presently, it is cultivated in most tropical and subtropical countries using seeds for propagation.

Its wide and extensive production scale reflects papaya's importance in agriculture and the world's economy. In 2020, total world production was recorded at approximately 13.9 million metric tons (FAOSTAT, 2021). India was the largest producer, accounting for nearly 43% of global production (FAOSTAT, 2021). Malaysia ranked two one in world production in the same year with approximately 0.45% production (Fruit Crops Statistics, 2021).

Locally, the most popularly grown papaya variety is Eksotika. The variety is a cross pollination of Subang 6 and Hawaiian Sunrise Solo and it was released by Malaysia Agriculture Research and Development Institute (MARDI), Serdang, in 1987 (Chan, 1987). It has vigorous growth features with production more than 60 tonnes hectares every year and can grow in multiple soil types provided with suitable drainage systems. Eksotika fruit is relatively small to medium-sized, weighing around 400 to 800 g with orange-red flesh and it has pleasing aroma with high of sucrose content (13–15°Brix) which suitable for consumption. These appealing characteristics together with high yield and aesthetic values makes the variety to be chosen variety grown either for commercial as well as export purposes, especially to East Asian countries, Singapore, Middle East, and Europe (Sew et al., 2011).

Papaya is polygamous with three sex forms: male, female and hermaphrodite (Salinas et al., 2018), differentiated by their inflorescences and fruit shapes (Ming et al., 2012). Male and female plants produce staminate and pistillate flowers, respectively. Whereas hermaphrodite plants produce bisexual flowers. Due to its high yield and pear-shaped fruits with a lower ovarian cavity, hermaphrodite papaya is the major type for commercial cultivation in tropical regions (Chávez-Pesqueira et al., 2014). Female plants are grown primarily for papain production (Reddy et al., 2012). which are commonly used in meat marinating, productions of cosmetics products, tannin hides, and many others (Ming et al., 2012), while male plants has no significant economic purposes (Urasaki et al., 2002).

In Malaysia, papaya is grown from seeds due to the non-availability of hermaphrodite planting materials. Studies have shown that the sex type of papaya plant is only recognizable only after 4 to 5 months when planted by seeds or once plant starts to

produce flowers. Since that period is relatively long, it would be beneficial for the growers to know the sex type at the seedling stage. In the export market, hermaphrodite papaya fruit has higher commercial value. A setback in the cultivation of papaya occurs when plants are propagated from seeds due to the production of non-true-to-type planting materials resulting from segregation of offsprings at the second filial generation. Identification of papaya sex type before the flowering stage would be helpful as it is cost saving especially in manpower and time allocated in removing plants with unsought sex types (male and female). Clonal propagation of selected hermaphroditic plant offers an efficient and valuable alternative in papaya field planting, ensuring 100% production of hermaphrodite plants. Propagation via tissue culture has been proposed for the rapid and large-scale production of planting materials. Tissue culture or micropropagation has been cited as the only cost-effective procedure to constantly produce desirable planting materials with suitable sex type in papaya (Salinas et al., 2018).

Although various studies on the micropropagation of papaya have been documented, information on the specific subject matter is still lacking, and protocols are still being perfected. Yie and Liaw (1977) first reported on induction of callus from stem section of papaya seedling using semi-solid culture medium. The most common micropropagation procedures used include by callus or somatic embryogenesis (Anandan et al., 2012; Kabir et al., 2016) and culture of shoot tips, axillary buds or shoot buds (Rohman et al., 2007, Hidaka et al., 2008; Bindu., 2017). Although a few protocols have been used as routine procedures, there are no suitable for extensive commercial production because of issues and challenges such as microbial contaminations by endogenic microbes, especially when matured papaya plant at the field is being use as explants sources. Low proliferation rates of matured materials have also been documented. These problems are further amplified by the lower rooting efficacy in regenerated shoots as well as lower survival rate of rooted papaya plants during acclimatization (Wu et al., 2012). Additional significant trait of responses of this plant to tissue culture is the genotype, which has been considered sensitive to plant growth regulators (Setargie et al., 2015).

Thus, a study was conducted to investigate the efficiency of papaya shoot tips as a potential region for shoot initiation and proliferation of explants to ascertain an effective protocol for the multiplication of planting materials. The study also aimed to demonstrate that shoots produced from this system could be successfully rooted and acclimatized by optimising various auxin concentrations, substrates, and potting media.

1.2 Aim and Objectives of Study

The general goal of this study was to establish an effective micropropagation procedure to produce *in vitro* hermaphrodite *Carica papaya* L. cv. Eksotika with specific objectives of the study were as follows:

a. To identify the potential of shoot tips sourced at different stages of explants' maturity for shoot induction in *Carica papaya* L.cv. Eksotika;

b. To determine the effects of different concentrations of 6-benzylaminopurine (BAP) on shoot proliferation;

c. To investigate the effects of different concentrations of indolebutyric acid (IBA) and substrates on rooting potentials of differentiated materials produced;

d. To determine the best potting medium for acclimatization of plantlets.

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