MORPHOGENESIS STUDIES AND GROWTH OPTIMIZATION OF *Carica papaya* L. var Eksotika THROUGH TISSUE CULTURE SYSTEM

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ABSTRACT

The research aimed to study the morphogenesis and growth optimization of *Carica papaya* L. var Eksotika through tissue culture system. A study was conducted to test the effect of 16 different concentrations and combinations of Benzylaminopurine (BAP) and Napthalene-Acetic Acid (NAA) on three different types of *Carica papaya* L. var Eksotika explants, namely leaf, stem and root. Complete plant regeneration was successfully achieved when root, stem and leaf aseptic explants were cultured on MS medium supplemented with various combinations of BAP and NAA. MS medium supplemented with 2.0 mg/L BAP was found to be the optimum medium and stem explants was the most responsive explants producing 3.800 ± 0.323 shoots per explants. MS media supplemented with 0.5 mg/L BAP and 2.0 mg/L NAA was most optimum for root induction. Optimum callus induction was obtained when root explants were culture on MS medium fortified with 2.0 mg/L BAP + 1.0 mg/L NAA. Meanwhile, synthetic seeds of *Carica papaya* L. were produced when microshoots were encapsulated with 4.0% sodium alginate solution added with 2.0 mg/L BAP. Finally, acclimatization of *Carica papaya* L. was accomplished when plantlets were transferred to the soil. The research showed that *in vitro* propagation of *Carica papaya* L. through tissue culture system was successfully achieved. In conclusion, the regeneration process, callus induction, synthetic seed, microscopic studies and acclimatization of *Carica papaya* L. var Eksotika were achieved. Micropropagation had been successfully obtained from the explants culture on combination and concentration of BAP and NAA. This research has provided positive implications towards the development of agriculture industry especially in Malaysia and also worldwide.

KAJIAN MORFOGENESIS DAN PENGOPTIMUMAN PERTUMBUHAN BAGI *Carica papaya* L. var Eksotika MELALUI SISTEM TISU KULTUR

ABSTRAK

Kajian ini bertujuan untuk mengkaji morfogenesis dan pengoptimuman pertumbuhan *Carica papaya* L. var Eksotika melalui sistem kultur tisu. Kajian telah dijalankan untuk menguji kesan 16 kepekatan dan kombinasi BAP dan NAA yang berbeza pada 3 jenis eksplan yang berbeza *Carica papaya* L. var Eksotika, iaitu daun, batang dan akar. Penjanaan semula tumbuhan yang lengkap berjaya dicapai apabila akar, batang dan daun aseptik dikultur pada medium MS ditambah dengan pelbagai kombinasi BAP dan NAA. Medium MS ditambah dengan 2.0 mg/L BAP didapati sebagai medium optimum dan eksplan batang adalah eksplan paling responsif yang menghasilkan 3.800 ± 0.323 pucuk bagi setiap eksplan. Medium MS dengan 0.5 mg/L BAP dan 2.0 mg/L NAA adalah paling optimum untuk induksi akar. Induksi kalus yang optimum diperolehi apabila eksplan akar dikultur pada medium MS yang ditambah dengan 2.0 mg /L BAP dan 1.0 mg /L NAA. Sementara itu, biji benih tiruan *Carica papaya* L. berjaya dihasilkan apabila pucuk mikro dikapsulkan menggunakan larutan natrium alginat 4.0% ditambah dengan 2.0 mg/L BAP. Akhir sekali, aklimatisasi lengkap *Carica papaya* L. ke persekitaran luar telah berjaya dicapai apabila plantlet dipindahkan kepada campuran tanah. Kajian yang telah dilakukan ini menunjukkan bahawa propagasi pesat tanaman *Carica papaya* L. melalui sistem kultur tisu telah berjaya dicapai. Kesimpulannya, proses regenerasi, induksi kalus, benih tiruan, kajian mikroskopik dan aklimatisasi lengkap *Carica papaya* L. var Eksotika telah berjaya dicapai. Mikropropagasi ekplan dikultur dengan kombinasi BAP dan NAA telah berjaya diperolehi. Kajian ini juga mempunyai implikasi yang positif terhadap perkembangan industri pertanian di Malaysia khususnya dan juga di seluruh dunia amnya.

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2,4-D	2,4-dichlorophenoxyacetic acid
BAP	Benzylaminopurine
HCL	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indolebutyric acid
Kinetin	6-furfurylaminopurine
mg/l	Milligram per liter
min	minute
ml	mililiter
MS	Murashige and Skoog
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
PGR	Plant growth regulators
Rpm	Rotation per minute
USDA	United States Department of Agriculture

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis (Anis and Ahmad, 2016). Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

The *Carica papaya* L. var Eksotika has become an important fruit having great nutritive and commercial value. Vegetative propagation of papaya through conventional methods has not been successful. To overcome this limitation, papaya can be propagated through *in vitro* propagation. Papaya Ring Spot and Papaya Leaf Curl are two major viral diseases that threaten papaya cultivation worldwide (Pandey, 2017). An efficient *in vitro* regeneration protocol is therefore imperative for papaya improvement (virus resistance) through recombinant DNA technology. Micropropagation of papaya began three decade ago. Papaya is a polygamous fruit crop and both dioceous as well as gynodioceous varieties of papaya are being cultivated. Cloning of female papaya plants through *in vitro* shoot bud culture is an ideal approach. *In vitro* regeneration with shoot tip, excised from mature papaya plant, has been attempted (Nguyen *et al*., 2018). However, most of the protocols are genotype dependent and could not be reproduced. Callus culture has also been reported in papaya (B and Podikunju, 2017). Papaya is recalcitrant to tissue culture. Slow rate of proliferation, poor establishment of axenic cultures and high mortality of plants during acclimatization are some of the main problems of papaya

1.2 Tissue Culture *In Vitro* Regeneration

Tissue culture has been applied to diverse research techniques such as viral elimination, clonal propagation, gene conservation, *in vitro* fertilization, mutation, induction for genetic diversity, genetic transformation, protoplast isolation and somatic hybridization, secondary metabolite production and other related techniques (Mohanraj, 2016). The commercial production of ornamental plants is growing

worldwide. Its monetary value has significantly increased over the last two decades and there is a great potential for continued further growth in both domestic and international markets. About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production were reported (Teymourian *et al*., 2017). These plants are over exploited due to their high medicinal value and hence, propagation of the plants by tissue culture may be mandatory, which offers a greater potential to deliver large quantities of disease-free, true-to-type healthy stock within a short span of time. Biotechnological interventions for *in vitro* regeneration, mass micropropagation and gene transfer methods in forest tree species have been practiced with success, especially in the last decade. Against the background of the limitations of long juvenile phases and lifespan, developments of plant regeneration protocols of ornamental species are gaining importance. Ornamental industry has applied immensely *in vitro* propagation approach for large-scale plant multiplication of elite superior varieties (Shekhawat *et al*., 2016). During *in vitro* condition, plantlets are grown under fixed and controlled environment in sterile formulated medium which contained macronutrients, micronutrients, vitamins and plant growth regulators. After the plantlets reached optimum growth in the culture containers after a certain growth period, it can be transferred to *ex vitro* condition to allow continuous growth of the plantlets. As a result, hundreds of plant tissue culture laboratories have been set up worldwide, especially in the developing countries due to cheap labour costs (Kabir *et al*., 2016).

1.3 The Importance of Tissue Culture Technique

Tissue culture has great importance in studies of plant morphogenesis, physio-logy, biochemistry, pathology, embryology and cytology (Mahalakshmi *et al*., 2018). From tissue culture studies it is possible to know bow simple cells differentiate and become specialized to perform special functions. Various changes taking place in a cell can be noted from clonal culture (Balilashaki *et al*., 2016). Interrelationship between two cells can be studied in tissue culture. With the help of phase contrast cine-photomicrography a very clear understanding of mitosis and meiosis is possible in tissue culture.

1.4 Introduction of Plant Tissue Culture

Plant tissue culture can be defined as culture of plant seeds, organs, explants, tissues, cells, or protoplasts on nutrient media under sterile conditions (Anis and Ahmad, 2016). The science of plant tissue culture takes its roots from path breaking research in botany like the discovery of cell followed by propounding of cell theory. There are several advantages of tissue culture that benefits food production, medical-benefit herbs production and economy of some countries. Small pieces of tissue (named explants can be used to produce hundreds and thousands of plants continuous process). Tissue culture can begin with very small pieces of plants explants and only take a small space to grow them, this can greatly increase the plant number (Bhatia *et al*., 2015).

Tissue culture is also identified as *in-vitro* and micro propagation based on the protocol of their procedure and the final result of the plant in tissue culture. *In vitro* refers to the usage of the glass jar in the planting of seeds or other part of plant (Bae and Yoon, 2015). Cultivation in a glass jar with aseptic technique provides protection to the sources of seeds or other plant parts from the infected organism that can damage plant growth.

There are five types of *in-vitro* culture which are called as callus culture, cell suspension culture, protoplast culture, anthers culture and organ culture (Sharma and Alam, 2015). Selection in implementing this type of *in-vitro* culture depends on several criteria. Among them are, the purpose of the investigation, the involvement of the species, the origin of the plant materials used and the size of the operations required. These factors need to be investigated in order to carry out the elections in accordance with the kind of tissue culture plants that want to be propagated. Elections in the culture that is appropriate to the type of plant are also the cause in the success of tissue culture techniques.

Other than that, tissue culture may be possible to produce the clones of certain kind of economically important plants that are slow or difficult to grow by other vegetative propagation methods (Bhatia *et al.*, 2015). Along with it, micropropagation save resources since the plant materials need little attention between subculture and there is no labour or material requirement for watering, weeding, spraying etc.

1.5 Problem Statement

Papaya is an important crop especially in African Region and Asia. However there is less tissue culture research having been done on this crop. Previously, many studies focus on the effect of fertilizer, cultivation technique and microorganism on papaya. Tissue culture can be a leading gate to explore biochemical composition or genetic improvement on papaya (Silva, 2016). The more advance study on papaya can improve the quality and quantity of yield, increase disease resistance ability and increase the toleration towards different growing environment. Micropropagation can be an alternative method to propagate papaya by using plant tissue instead of seed.

Development of an efficient morphogenesis and micropropagation procedure play significant roles in meeting the requirement of planting material for breeding improvement (Silva, 2016). In this experiment, papaya tissue is propagated and multiplied in *in vitro* condition. In this study, morphogenesis and micropropagation method is in cooperated with different concentration of these two types PGR benzyladenine (BAP) and naphthalene acetic acid (NAA). Four sets of experiment were conducted to identify optimum concentration these two types of PGR for the development of plant tissue on different types of explants.

1.6 Research Objectives

Main Objectives:

1. To establish *in vitro* of *Carica papaya* L. var Eksotika through tissue culture system.
2. To study the production of synthetic seeds of *Carica papaya* L. var Eksotika from micro shoots obtained *in vitro*.

Specific Objectives:

1. To determine the optimum concentration of BAP and NAA in medium for shoots production on different explants.
2. To determine the optimum concentration of BAP and NAA in medium for roots production on different explants.
3. To determine the optimum concentration of BAP and NAA in medium for callus induction on different explants.
4. To study and compare the acclimatization and morphological characteristics by using Scanning Electron Microscope (SEM) for *in vitro* and *in vivo* plant.

1.7 Scope of Research

This study was focused on the effect of 16 different concentrations and combinations of BAP and NAA on the micropropagation of three different explants (leaf, stem and root of papaya (*Carica papaya* L. var Eksotika). The observation on callus, shoot and root development were recorded at week 4, 8 and week 12. At the final week, the weight of callus, number of shoots and number of roots were recorded.The plantlets were undergo acclimatization process to test the adaptation behavior of *Carica papaya* L. var Eksotika and determine a suitable medium soil. The production of synthetic seeds of *Carica papaya* L. var Eksotika produced were stored and will be used for the next study.

1.8 Limitation

Contamination is one of the issues in tissue culture techniques. Contamination happened on a number of factors such as environmental conditions, lack of skills in implementation and also improper sterilization techniques (Ali *et al*., 2018). Contamination problem is common to happen among novices in this field because they have poor skills. But it can be prevented with training exercises performed in the laboratory. The use of laminar flow chamber and autoclave manufacturer need to consider on the percentage contamination reduction (Rawal and Keharia, 2019). Communication with the lab assistant is also important in getting the proper use of tutoring before, during and after the related to activities both the equipment.