UNIVERSITI PUTRA MALAYSIA

EFFECTS OF STERILIZATION, PLANT GROWTH REGULATOR AND MEDIA FOR UPSCALING *IN VITRO* PROPAGATION OF *Musa balbisiana* Colla cv. ABU NIPAH USING TEMPORARY IMMERSION BIOREACTOR SYSTEMS

ABDUL MUHAIMIN ABDUL KADIR

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By

ABDUL MUHAIMIN ABDUL KADIR

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

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

EFFECTS OF STERILIZATION, PLANT GROWTH REGULATOR AND MEDIA FOR UPSCALING *IN VITRO* PROPAGATION OF *Musa balbisiana* Colla cv. ABU NIPAH USING TEMPORARY IMMERSION BIOREACTOR SYSTEMS

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July 2020

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The expansion of banana production, especially cv. Abu Nipah is limited due to shortage of quality planting materials available to the farmers. Hence, the study aims to establish a protocol for micropropagation of *M. balbisiana* cv. Abu Nipah and subsequent upscaling via temporary immersion bioreactor system (TIBs). The objectives of this study are; 1) to study the effect of different ethanol and Clorox ratio on surface sterilization, 2) to study the effect of cytokinins on multiplication and elongation, 3) to study the effect of single and half-cut explant on multiplication, 4) to upscale the plantlet production via shake flask and TIB system and 5) to determine suitable potting media for acclimatization of cv. Abu Nipah.

The sword suckers were surface sterilized with different ratio of ethanol to Clorox. Multiplication and elongation of cv. Abu Nipah plantlets were carried out using plant growth regulators; 6-benzylaminopurine (BAP), kinetin, thidiazuron (TDZ) and 2-isopentenyl adenine (2iP) at 5 mg/L and different explant types; single and half-cut explant. The culture was up-scaled with different systems; shake flask, TIB and solid media, and different media formulations; Murashige and Skoog (MS) and Gamborg B5 (B5) media. In vitro rooting was conducted using different auxins; 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) at 1 mg/L. Lastly, plantlets were acclimatized to *ex vitro* environment using different potting mediums.

Surface sterilization using 100%:100% ethanol to Clorox produced the highest explants survival (90%). Shoot multiplication and elongation were optimum in MS media supplemented with 5 mg/L of BAP using single explant. Upscaling

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using RITA® system produced the highest number of shoot (5.93) and shoot length (3.80 cm) while the highest number of root (11.06) and root length (10.70 cm) obtained in 1 mg/L NAA. Lastly, potting medium of peat moss : perlite (1:1 ratio) produced 92% plant survival. It can be concluded that this protocol can be used to mass propagate *Musa balbisiana* cv. Abu Nipah.

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

KESAN PENSTERILAN, PENGAWALATURAN TUMBESARAN TUMBUHAN DAN MEDIA UNTUK PENINGKATAN SKALA MENGGUNAKAN SISTEM BIOREAKTOR RENDAMAN SEMENTARA DALAM PROPAGASI *IN VITRO* *Musa balbisiana* Colla cv. ABU NIPAH

Oleh

ABDUL MUHAIMIN ABDUL KADIR

Julai 2020

Pengerusi: Mohd Hakiman Mansor, PhD
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Peluasan pengeluaran pisang, terutamanya kultivar Abu Nipah adalah terhad kerana kekurangan bahan penanaman yang berkualiti untuk para petani. Oleh itu, kajian ini bertujuan untuk menghasilkan protokol untuk pembiakan mikro Pisang Abu Nipah dan kemudianya peningkatan skala melalui Sistem Bioreaktor Rendaman Sementara. Objektif kajian ini adalah 1) untuk mengkaji kesan perbezaan nisbah etanol dan Clorox pada pensterilan permukaan, 2) untuk mengkaji kesan sitokinin pada penggandaan dan pemanjangan, 3) untuk mengkaji kesan pemotongan tunggal dan pemotongan separuh eksplan terhadap penggandaan, 4) untuk meningkatkan pengeluaran melalui sistem kelalang separa kocak dan Sistem Bioreaktor Rendaman Sementara dan 5) untuk mengenal pasti media pemasuan untuk aklimatisasi kultivar Abu Nipah.

Sulur telah disteril permukaan dengan nisbah etanol dan Clorox yang berbeza. Penggandaan dan pemanjangan anak pokok kultivar Abu Nipah dilakukan dengan pengawalatur tumbesaran tumbuhan; 6-benzilaminopurina (BAP), kinetin, thidiazuron (TDZ) dan 2-isopentenil adenina (2iP) pada kadar 5 mg/L dan dua jenis eksplan yang berbeza iaitu eksplan tunggal dan pemotongan separuh. Kultur kemudianya digandakan dengan menggunakan sistem yang berbeza iaitu sistem kelalang separa kocak, sistem bioreaktor rendaman sementara dan media pepejal, dan formulasi media berbeza iaitu media Murashige dan Skoog (MS) dan Gamborg B5 (B5). Pengaruhan induksi akar secara *in vitro* menggunakan auksin yang berbeza iaitu asid naftalena asetik (NAA), asid indolbutirik (IBA) dan asid indolasetik (IAA) pada kadar 1 mg/L. Akhir sekali, anak pokok akan disesuaikan diri kepada keadaan persekitaran *ex vitro* menggunakan medium pemasuan yang berbeza.

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Pensterilan permukaan 100%:100% menggunakan etanol kepada Clorox menghasilkan kadar kemandirian ekplan yang tertinggi iaitu 90%. Penggandaan dan pemanjangan pucuk optimum dikultur dalam media MS ditambah dengan BAP pada kadar 5 mg/L menggunakan eksplan tunggal. Penggandaan menggunakan sistem RITA® menghasilkan jumlah bilangan pucuk tertinggi iaitu (5.93) dan panjang pucuk (3.80 cm) sementara bilangan akar tertinggi iaitu (11.06) dan panjang akar ialah (10.70 cm) yang diperoleh dalam 1 mg/L NAA. Akhir sekali, medium pemasuan terdiri dari tanah gambut berlumut : perlite pada nisbah 1:1 menghasilkan kemandirian pokok tertinggi (92%). Kesimpulannya, protokol ini dapat digunakan untuk propagasi berskala besar *Musa balbisiana* cv. Abu Nipah.

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This thesis was submitted to the Senate of 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

ANOVA	Analysis of variance
B5	Gamborg B5 media
BAP	6-benzylaminopurine
cm	Centimeter
CRD	Completely randomized design
cv.	Cultivar
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kin	Kinetin
LSD	Least significant difference
NAA	Naphthalene acetic acid
PGR	Plant growth regulator
TIBs	Temporary immersion bioreactor system
TDZ	Thidiazuron
2iP	2-isopentenyadenine
%	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Scientific Scope of the Project

The project strategy is to mass propagation of banana germplasm with improved disease-free banana plantlets from *in vitro* technique. The project scope will include: 1) Development of an efficient field screening technique for early detection with improved disease-free sucker before using for *in vitro* culture, 2) Develop an efficient in vitro screening technique for early detection with improved disease-free planting material and 3) Develop low-cost propagation techniques for the distribution of disease-free tissue culture plantlets to the local farmers.

1.2 Background

Banana is an important and well-known fruit in South East Asia mainly Malaysia, due to the nutritional status and is superior to other common tropical fruits (Suntharalingam et al., 2011). They are planted in more than 120 nations in tropical and subtropical zones on five continents in the world. Banana is usually consumed fresh, and in the form of dessert, chips or cooked dishes and provide a primary food source for millions of people in developing countries. Traditionally, bananas are propagated vegetatively rather than sexually because almost all cultivated varieties are seedless, and fruits grow parthenocarpically (without seed development). The principal method of banana propagation is by using the division of sucker or pups which are the rising part at the base of the main stems or from the buried underground corms. By using this method, new plants grow but in small numbers and insufficient for large scale planting (Kaemmer et al., 1997; Crouch et al., 1998).

Banana is a large perennial herb with a pseudostem composed of leaf sheaths. It belongs taxonomically to the Musaceae family, genus *Musa*, and two sections which are Eumusa and Monocotiledonaceae. According to the classification and original source of diversity, the genus *Musa* is divided into four sections, the members of which including both seeded and non-seeded (parthenocarpic) type (Creste et al., 2003). Two of the sections (Callimusa and Australimusa) include species with a chromosome number of 2n=20 while species in the other two sections (Eumusa and Rhadochalmys) have a basic chromosome number of 11 (2n=22) (Crouch et al., 1998). The center of diversity of the banana cultivar is thought to be either Malaysia or Indonesia. The majority of cultivars are derived from two species, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome).

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Due to high demand and the continuous need for a large number of banana plants, *in vitro* method of propagation has been a way of producing plantlets on a large scale. However, the increasing demand for plantlets has caused shortages in the supply of quality planting materials. Hence, *in vitro* propagation technique was introduced to overcome this problem. It has the potential to allow cryopreservation and to provide a valuable tool for genetic improvement and enhancement programs (Mathe et al., 2012). Another advantage of this technique is a low percentage of somaclonal variation (Chinmanyee et al., 2012). Furthermore, this method can be upscaled using a bioreactor (Shibli et al., 2012). Over the years, somatic embryogenesis has been reported for more than 500 plant species, including oil palm, citrus, corn and coffee (Bhojwani and Dantu, 2013). However, only a few banana cultivars such as Bluggoe (*Musa* ABB) (Dhed'a et al., 1991), Grande Naine (*Musa* AAA) (Becker et al., 2000), Rastali (*Musa* AAB) (Ganapathi et al., 2001) and Mas (*Musa* AAB) (Jalil et al., 2003) were cultivated through embryogenesis from different types of explant.

Musa balbisiana cv. Abu Nipah (ABB genome) is also known as Saba banana in the Philippines. It is the most common banana in the Philippines, Thailand, Indonesia and Malaysia (Purseglove, 1976). It is one of the most vigorous and hardy cultivars in the ABB genome of the Eumusa section of banana cultivars. The fruits may or may not contain seeds (Stover and Simmonds, 1987). Banana cv. Abu Nipah generally is consumed as fresh or dried fruit. The banana blossom was used in many dishes in Thailand usually considered as vegetable, also has potential to be regarded as a functional food due to its high nutrient content (Thaweesang, 2019). There are five types of planting material used in the plantation; peepers, sword suckers, maiden suckers, bits of large corms and water suckers. The most commonly used planting materials in the plantation are suckers or pieces of the rhizome (Crane and Balerdi, 1998). The materials preferred for propagation differ in different places of the world (Purseglove, 1976). However, the spread of harmful nematodes, insects, Black Sigatoka and Panama disease by field-grown suckers cause a significant problem to conventional propagation in plantation (Roels et al., 2005). Smallholders typically propagate banana cv. Abu Nipah, on a small scale using suckers for chip production for local consumption in Malaysia (Jamaluddin, 1994). The unripe fruit of banana cv. Abu Nipah can be used in the production of high fiber flour (Chong and Noor Aziah, 2008), which intrigued the smallholders to do commercial planting of this cultivar, thus ultimately resulted in a shortage of plantlets for large scale planting.

Such limitations can be resolved by using bioreactors. By using a bioreactor, specific parameters can be controlled. Through controlling various chemical and physical factors, including mixing, gaseous composition, efficient oxygen transfer, pH and hydrodynamic forces in bioreactors, optimum plant growth conditions can be easily achieved in a bioreactor (Dong et al., 2013). For example, Kosky et al. (2002) used 2 L CMF-100 (CHEMAP AG) bioreactor for propagation of tetraploid banana hybrid (FHIA-18) and the plantlets produced from explants were recorded as phenotypically the same as the mother plant.

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Temporary immersion bioreactor system (TIBs) could be used to eliminate hyperhydricity and asphyxia in plants since explants came into contact with the liquid medium periodically (Berthouly and Etienne, 2005). TIBs ensured maximum growth of plantlets by providing an efficient gaseous exchange, less water retention and better nutrient medium absorption (Preil et al., 2005). TIBs were found to produce a positive result on microtuberization, somatic embryogenesis and shoot proliferation in certain plant species (Berthouly and Etienne, 2005). TIBs had been proven to be able to produce healthy sugarcane, pineapple seedlings, potato microtubers and apple rootstock (Lorenzo et al., 1998; Escalona et al., 1999; Jiménez et al., 1999; Zhu et al., 2005). Optimizing immersion time (frequency and duration) with an optimized volume of medium is very important for TIBs effectiveness in improving shoot proliferation. TIBs also enhance plant quality by producing plantlets that could acclimatize better compared to plantlets produced through conventional micropropagation methods. Besides, the upscaling of shoot proliferation using TIBs was found to reduce production costs (Berthouly and Etienne, 2005). Several studies reported shoot proliferation of banana via TIBs (Alvard et al., 1993; Matsumoto and Brandão, 2002; Colmenares and Gimenez, 2003; Roels et al., 2005). However, the *in vitro* propagation of banana cv. Abu Nipah using TIBs has not been reported.

1.3 Problem Statements

Conventional breeding has not satisfactorily produced new commercially acceptable banana varieties that are resistant to pest and disease and mass propagation is slow in conventional breeding. With the availability of more efficient biotechnology technique, new technology could be used as complementary tools to address the problem.

1.4 Objectives

The study attempts to develop an *in vitro* regeneration of banana for future breeding to produce commercially acceptable banana varieties, from diseases-free banana plantlets.

1. To study the effect of different ethanol and Clorox ratio on surface sterilization of *Musa balbisiana* cv. Abu Nipah.
2. To study the effect of cytokinins on multiplication and elongation of *Musa balbisiana* cv. Abu Nipah.
3. To study the effect of single and half-cut explants on multiplication of *Musa balbisiana* cv. Abu Nipah.
4. To upscale the production of *Musa balbisiana* cv. Abu Nipah via shake flask and TIB system.
5. To determine suitable potting media for acclimatization stage of Musa balbisiana cv. Abu Nipah.

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