EFFECTS OF PLANT GROWTH REGULATORS TOWARD CALLUS REGENERATION OF CAVENDISH BANANA

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EFFECTS OF PLANT GROWTH REGULATORS TOWARD CALLUS REGENERATION OF CAVENDISH BANANA

By

NUR SURAYA ANIS BINTI ZAINAL ABIDIN

A Project Report Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry Universiti Putra Malaysia

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DEDICATION

For my beloved family:

Zainal Abidin Bin Mohd Yusof

Latifah Binti Ahmad

My fellow siblings.

To all my friends,

Under-graduate students, laboratory assistant, and practical students, for helping me during the entire project.

Thank you for your suggestion, opinion, sacrifices and comments for my project.

Thank you for everything. May God bless all of us.

ABSTRACT

Male flowers of banana have potential to be used as explants for tissue culture of Cavendish banana which is in a sub-group of AAA. The aim of this study was to investigate the effects of concentration of different plant growth regulators toward callus induction and to identify the best part of male flowers for callus initiation. The male flowers of Cavendish banana were cultured on MS medium supplemented with 2,4-D (0.1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml), NAA (0.1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml), 2,4-D + NAA (0.5 mg/ml + 0.5 mg/ml, 0.5 mg/ml + 0.25 mg/ml, and 0.5 mg/ml + 0.125 mg/ml) and MS medium itself by using part 1 of male flowers. Part 2 of male flowers were cultured with MS medium supplemented with BAP (1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 8.0 mg/ml, 12.0 mg/ml), 2,4-D (1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 8.0 mg/ml, 12.0 mg/ml), 2,4-D + IAA (2.0 mg/ml + 0.1 mg/ml, 2.0 mg/ml + 0.5 mg/ml), 2,4-D + NAA (2.0 mg/ml + 0.1 mg/ml, 2.0 mg/ml + 0.5 mg/ml) and 2,4-D + IAA + NAA (2.0 mg/ml + 0.1 mg/ml + 0.1 mg/ml, 2.0 mg/ml + 0.5 mg/ml + 0.5 mg/ml). Explants that consist of BAP turned into green color. While other responsive explants formed globar shape of creamy and the morphology of explants were changed. BAP concentrations of 2.0 mg/ml, 4.0 mg/ml and 12.0 mg/ml showed the most responsive explants. While for 2,4-D plant growth regulator with concentration of 1.0 mg/ml and 2.0 mg/ml resulted to be the most optimum concentrations for callus induction. The present study was established for making some analysis depending on the parts of male flowers and also by using difference plant growth regulators with difference concentrations.

ABSTRAK

Bunga jantan pisang mempunyai potensi untuk digunakan sebagai bahan sampel tisu untuk kultur tisu Cavendish pisang yang berada dalam sub-kumpulan AAA. Tujuan kajian ini adalah untuk mengkaji kesan penumpuan pengawal selia pertumbuhan tumbuhan yang berbeza ke arah induksi kalus dan untuk mengenal pasti bahagian terbaik bunga jantan untuk pemula kalus. Bunga jantan pisang Cavendish telah dibiakkan pada medium MS ditambah dengan 2,4-D (0.1 mg / ml, 0.5 mg / ml, 0.25 mg / ml, dan 0.125 mg / ml), NAA (0.1 mg / ml, 0.5 mg / ml, 0.25 mg / ml dan 0.125 mg / ml), 2,4-D + NAA (0.5 mg / ml + 0.5 mg / ml, 0.5 mg / ml + 0.25 mg / mg / ml) dan medium MS itu sendiri dengan menggunakan bahagian 1 bunga jantan. Bahagian 2 bunga jantan telah dibiakkan dengan medium MS ditambah dengan BAP (1.0 mg / ml, 2.0 mg / ml, 4.0 mg / ml, 8.0 mg / ml, 12.0 mg / ml), 2,4-D (1.0 mg / , 2.0 mg / ml, 4.0 mg / ml, 8.0 mg / ml, 12.0 mg / ml), 2,4-D + IAA (2.0 mg / , 2,4-D + NAA (2.0 mg / ml + 0.1 mg / ml, 2.0 mg / ml + 0.5 mg / ml) dan 2,4-D + IAA + NAA (2.0 mg / 0.1 mg / ml, 2.0 mg / ml + 0.5 mg / ml + 0.5 mg / ml). Sampel tisu yang terdiri daripada BAP berubah menjadi warna hijau. Sedangkan sampel tisu yang lain membentuk globar berkrim dan morfologi sampel tisu berubah. Kepekatan BAP 2.0 mg / ml, 4.0 mg / ml dan 12.0 mg / ml menunjukkan penemuan yang paling responsif. Sementara untuk pengatur pertumbuhan tanaman 2,4-D dengan kepekatan 1.0 mg / ml dan 2.0 mg / ml menghasilkan konsentrasi yang paling optimum untuk induksi kalus. Kajian ini ditubuhkan untuk membuat beberapa analisis bergantung kepada bahagian-bahagian bunga jantan dan juga dengan menggunakan pengawal selia pertumbuhan tumbuhan yang berbeza dengan kepekatan yang berbeza.

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APPROVAL SHEET

I certify that this research project report entitled “Effects of Plant Growth Regulators Toward Callus Regeneration of Cavendish Banana” by Nur Suraya Anis Binti Zainal Abidin has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.

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%	percent
µM	micro molar
2,4-D	2,4-Dichlorophenoxyacetic acid
cm	centimeter
et al.	et alia.
IAA	indole acetic acid
mg/ml	milligram per millilitre
p-value	probability value
NAA	Naphthaleneacetic acid
BAP	6-benzyl aminopurine
MS	Murashige and Skoog
BA	6-Benzyladenine

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

There are more than 500 varieties of banana plants in the world. But the most common kinds are Dwarf Cavendish, Valery, and Williams Hybrid bananas. The Cavendish banana is generally-grown cultivar. This type of banana usually can be found in the Latin America, Africa, and Southeast Asia. They are belonging to the genome group, which have three sets of chromosomes donated by *Musa acuminata* known as wild species. Cavendish banana originated from Southeast Asia especially Vietnam, and they became extensively cultivated in the 1950s. Since many consumers does not know about this, the story of Cavendish is fascinating glimpse into the world of commercial banana cultivator. They are increasingly produced for export market.

According to Anny Jong (2011) , she said that Malaysia is the second most widely cultivated banana crop with a total production of more than 50,000 metric tones and can be consumed as an export commodity. Generally, bananas are grown from their vegetative suckers. Even they are mostly seed sterile, the traditional planting is not the excellent method because the method planting itself may carry fungal pathogen, viruses, weevils and nematodes. The disease that usually attacks the banana plants known as

Fusarium wilt which is a very harmful fungal. The disease caused by the *Fusarium oxysporum f. sp. Cubense*

(Foc). The existent of *Foc* in the soil, cannot be eliminated once they are present. There are four recognized races of the pathogen which are separated according to the host susceptibility.

Race 1, usually attacks 'Gros Michel' plantations, and also attacks 'Lady Finger' (AAB) and 'Silk' (AAB) varieties. While race 2 affects the cooking bananas (ABB). *Heliconia spp* is affected by the race 3. *Heliconia spp* is close relative of banana and is not considered to be a banana pathogen. For the Cavendish bananas (AAA), usually attacks by the race 4. This race 4 is divided into 'sub-tropical' and 'tropical' strains. The one that capable to attack Cavendish banana is present as 'Tropical' race 4 and causing disease growing under any conditions, whereas the 'sub-tropical' race 4 normally causes disease in plants growing sub-optimally for example consists of cool temperature, water stress and poor soil. To manage the *fusarium* wilt disease is extremely difficult as the fungus remain alive in the soil as once it is present.

Besides of traditional planting, another alternative method of propagation is taken up through tissue culture. Tissue culture is propagation technique used to clone a single cell in a culture medium under hygienic conditions. This technique has many advantages. By doing tissue culture technique, they tend to be pest and disease- free seedlings as they are easily affected by the tropical race 4. Besides that, they growth uniformly and increase the yield.

Moreover, this technique takes shorter time compared to traditional planting. Shoot or meristem tips are commonly used in tissue culture technique for large scale production of uniform vigorously growing propagules for field establishment. To make a new improvement of banana production, the *in vitro* plant regeneration from male flowers of banana has been suggested as an alternative method to regenerate plants (Sultan et al., 2011).

Thus, the present research proposed to regenerate plants by using male flowers of banana as an explant through tissue culture technique by reducing the concentration of auxins. According to Machuki (2013), he said that higher level of auxins cut off organized growth promoting growth of meristem-like cells. Auxins act as growth regulator and promote cell division in tissue culture. They support callus induction. The mostly used of auxin for tissue culture is 2,4-Dichlorophenoxyacetic acid or easily known as 2,4-D. Other growth regulators used in tissue culture are 1-Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP). The present study is to carry out an efficient protocol on callus induction of male flowers of cavendish bananas.

1.2 PROBLEM STATEMENT

The huge problem of handling Cavendish banana is the disease. They are easily affected by the Tropical race 4 of *Fusarium* wilt disease. As the Cavendish bananas are important for domestic markets, the prevention is needed to produce quality bananas and higher yield in short duration of time.

Thus, tissue culture technique is used to control the disease as the method produced disease-free seedlings and this technique also can produce a lot of plants in a short time.

1.1 OBJECTIVES

The main objectives of this study is to investigate the effects of concentration of different plant growth regulators toward callus induction. In addition, this study also attempts to evaluate the best part of male flowers in regenerating the callus.

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