UNIVERSITI PUTRA MALAYSIA

TISSUE CULTURE, ITS MORPHOLOGICAL AND BIOCHEMICAL INTEGRITY AND ANTIOXIDANT PROFILING ON Cucumis sativus L. cv. MTi2

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By

TG AZIA FARAHIN KU HASAN

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

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DEDICATION

Special dedication goes to my loving parents, families, my supervisor, my co-supervisor, and fellow friends for all their continuous support, guidance, motivation and love.

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

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Chairman : Rosimah Nulit, PhD
Faculty : Science

Cucumber is one of the high-nutritional vegetable and is one of the most important crops worldwide. It is rich in vitamin B, vitamin C, proteins, minerals, amino acids and many other constituents. It is beneficial for people to consume cucumber which offer health benefits and aid in disease prevention such as cancer. Cucumber variety MTi2 is one of the famous cucumber variety in Malaysia that consumed by local people. Therefore it is important to maintain the good supply of this favorable variety and to conserve the species to ensure its continuance in the future. Also, the outbreak of viral disease prompts us to look alternative method to safe keep viable stock plant. Besides, the conventional means of propagation commonly yield non uniform plants that may reduce the crop quality. This might be a huge problem for large scale multiplication industries and for conservation purpose of the species. As an alternative, *in vitro* regeneration was carry out to optimize the protocol in obtaining uniform plantlet through culturing shoot apical meristem (SAM) of cucumber. Nevertheless, *in vitro* regeneration is also associated with somaclonal variation that may result of producing non uniform plantlet. Therefore the SAM culture was optimized in this study to generate identical plantlet. The effect of plant growth regulators (PGR) in *in vitro* regeneration was also studied through application of 10 treatments of full strength MS media supplemented with different concentration and combination of PGR, with MS basal media served as control treatment. The result showed that meristem tissue of cucumber can be regenerated into whole plant through *in vitro* micropropagation. The combination treatment of 0.01 mgL^{-1} IAA and 0.1 mgL^{-1} KIN produced highest percentage survival of plantlets which was 88%. Comparative study was done in term of morphology, anatomy, biochemical and antioxidant profiling between cucumber plant and cucumber plantlet. The finding showed that the plantlet possess similar characteristics as the cucumber plant in term of morphology, anatomy, total protein content, total phenolic content and total flavonoid content. In conclusion, identical plantlet can be produced through *in vitro* regeneration of SAM of cucumber, thus this technique can be

implemented for conservation and for large-scale multiplication purpose. This study had also identify and compared the antioxidant activity between seed, young fruit and matured fruit of cucumber. Antioxidant play vital roles in neutralize the effects of free radicals where free radicals are molecules that can damage the cellular components in human body. Phenolic and flavonoid are examples of antioxidant properties measured in this study. The finding showed that the seed of cucumber possess considerate amount of antioxidant properties which is 0.2 mg/ml flavonoid content and 0.02 mg/ml phenolic content. Therefore, the seed of cucumber can be utilized as a dietary source of natural antioxidant.

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

Kultur Tisu, Integriti Morfologi dan biokimia serta Profil Antioksida ke atas *Cucumis sativus* L. cv. MTi2

Oleh

TG AZIA FARAHIN KU HASAN

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Timun merupakan salah satu sayur-sayuran yang tinggi nutrisi dan merupakan salah satu tanaman yang paling penting di seluruh dunia. Ia kaya dengan vitamin B, vitamin C, protein, mineral, asid amino dan lain-lain unsur. Pengambilan timun adalah bermanfaat kepada manusia yang memberi manfaat kesihatan dan membantu mencegah penyakit seperti penyakit kanser. Timun MTi2 adalah salah satu variasi timun yang terkenal di Malaysia yang dimakan oleh masyarakat tempatan. Oleh itu, adalah penting untuk mengekalkan bekalan yang baik dari variasi timun ini dan untuk memulihara spesies untuk memastikan kelangsungannya pada masa akan dating. Juga, wabak virus penyakit memerlukan kami untuk mencari kaedah alternatif untuk menyimpan tumbuhan yang berdaya maju dengan selamat. Selain itu, cara penghasilan secara konvensional biasanya menghasilkan tumbuhan yang tidak seragam yang boleh mengurangkan kualiti tanaman. Ini mungkin menjadi satu masalah yang besar kepada industri penghasilan tanaman skala besar dan kepada pemuliharaan spesies ini. Sebagai alternatif, pertumbuhan semula *in vitro* dilakukan untuk mengoptimumkan protokol dalam menghasilkan anak pokok yang seragam dengan mengkulturkan meristem apikal pucuk timun. Walau bagaimanapun, pertumbuhan semula *in vitro* adalah berkait dengan perubahan somaklonal yang boleh menyebabkan penghasilkan anak pokok yang tidak seragam. Oleh itu, pengkulturan SAM telah dioptimumkan dalam kajian ini untuk menghasilkan anak pokok yang sama. Kesan daripada pengawal selia pertumbuhan tumbuhan (PGR) dalam pertumbuhan semula *in vitro* juga telah dikaji melalui penggunaan 10 rawatan MS media dengan kekuatan penuh ditambah dengan kepekatan dan kombinasi PGR yang berbeza, dengan media basal MS bertindak sebagai rawatan kawalan. Keputusan menunjukkan tisu meristem timun boleh ditumbuhkan semula menjadi keseluruhan pokok melalui mikropropagasi *in vitro*. Rawatan kombinasi 0.01 mgL^{-1} IAA dan 0.1 mgL^{-1} KIN menghasilkan peratusan kelangsungan hidup anak pokok yang tertinggi iaitu 88%. Kajian perbandingan telah dilakukan dari segi morfologi, anatomi, biokimia dan profil antioksida ke atas pokok

timun dan klon timun. Hasil kajian menunjukkan bahawa klon-klon menunjukkan ciri-ciri yang sama seperti pokok timun dari segi morfologi, anatomi, jumlah kandungan protein, jumlah kandungan fenolik dan jumlah kandungan flavonoid. Sebagai kesimpulan, klon yang serupa boleh dihasilkan melalui pertumbuhan semula *in vitro* menggunakan meristem apikal pucuk timun, oleh itu teknik ini boleh diaplikasikan untuk tujuan pemuliharaan dan penghasilan tanaman berskala besar. Kajian ini juga telah mengenalpasti dan membanding profil antioksida pada biji, buah muda dan buah matang timun. Antioksida memainkan peranan penting dalam meneutralkan kesan radikal bebas yang mana radikal bebas adalah molekul yang boleh memusnahkan komponen selular dalam badan manusia. Fenolik dan flavonoid adalah contoh-contoh antioxida yang diukur dalam kajian ini. Hasil kajian menunjukkan bahawa biji timun mengandungi jumlah antioksida yang boleh dipertimbangkan iaitu 0.2 mg/ml kandungan flavonoid dan 0.02 mg/ml kandungan fenolik. Oleh itu, bahagian biji timun boleh diambil sebagai sumber antioksida semula jadi.

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I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Tg Azia Farahin Ku Hasan on her thesis entitled

Tissue Culture, Morphological and Biochemical Integrity and Antioxidant Profiling of *Cucumis sativus* cv. MTi2 in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

SAM	shoot apical meristem
°c	degree Celsius
%	percentage
g	gram
mg	milligram
PGR	plant growth regulator
MS	Murashige and Skoog
DNA	Deoxyribonucleic acid
IAA	Indole-3-Acetic Acid
KIN	Kinetin
ml	milliliter
ppm	parts per million
N	normal
NaOH	sodium hypochlorite
pH	negative logarithm of hydrogen ion concentration
MgL^{-1}	milligram per liter
mm	millimeter
psi	pounds per square inch
v/v	volume per volume
LAF	laminar air flow
ANOVA	Analysis of Variance
gL^{-1}	gram per liter
CRD	completely randomized design
cm	centimeter

min	minute
cm^3	cubic centimeters
DPX	diputal petroleum xylene
nm	nanometer
µl	micrometer
H_2SO_4	sulfuric acid
mg/ml	milligram per milliliter
rpm	revolutions per minute
mM	millimolar
SE	standard error
DPPH	2, 2-diphenyl-1-picrylhidrazyl
µl/ml	microliter per milliliter
µm	micrometer
$mgCl_2$	magnesium chloride
DTT	dithiothreitol
EDTA	ethylenediaminetetraaceticacid
EGTA	ethyleneglycoltetraaceticacid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
KOH	potassium hydroxide

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Nowadays, with rapid development of fast food industries that offer delicious but disease-carried food, it is important for people to consume natural nutritious foods in order to keep the body healthy. Vegetables are one of the natural food sources that benefit people as they are the cheapest sources of proteins, vitamins, minerals and amino acids (Liu, 2004).

One of the popular vegetables crop is cucumber. Cucumber (*Cucumis sativus*) belongs to Cucurbitaceae family and is popularly used as salads. Cucumber is rich in potassium, oxalic acid and phosphorus (Ahmad and Anis, 2005). It contains phenolic content that offer health benefit to people (Liu, 2004) and could prevent cancer from its antioxidant action (Cai *et al*., 2004). It is good to eat raw cucumber as there are more nutrients in raw vegetables compared to cooked ones (Sivakumar *et al*., 2010). Traditionally, the seeds of cucumber are used for headache, burning and insomnia, while the leaves are used to treat dyspepsia (Gogte, 2000; Khandelwal, 2000; Shah *et al*., 2013). The leaf of cucumber has also been used for jaundice and bleeding disorder (Shah *et al*., 2013).

People love to eat raw cucumber as realizing its nutritional value besides enjoying its uniquely cool, refreshing taste and great crunchiness. In Malaysia, cucumber has great demand from local consumers. Cucumber variety MTi2 are the local variety of cucumber in Malaysia that famously consumed by Malaysian and widely grown throughout the country. Besides, cucumber seed that imported from outside of the country are expensive. It is important to maintain or increase the self sufficiency of this cucumber variety in order to maintain good plant stock.

Nevertheless, the conventional means of propagation may produce non uniform plants that may diminish the quality of the yield (Yadav *et al*., 2012). Besides, the conventional propagation of cucumber always has high exposure to disease and pests, thus reducing the yield and quality. Growing cucumber in farms is highly associated with variety of diseases such as cucumber mosaic virus, angular leaf spot, bacteria wilt disease, downy mildew disease and many more. Cucumber farming is also easily affected by viruses and bacterial that may lead to insufficient cucumber supply to the world market. For example in 2011, there was an outbreak of cucumber issue

in Europe that affects hundreds of people and caused the death of 14 people (Torry, 2011). The cucumber was infected with *E. coli* from contaminated water used to water the crops (Poullter, 2011). This issue lead to the diminished of cucumber crops of many areas around the world especially Spain, and some Europe countries were reported to ban the sale of cucumbers ("E. coli Cucumber Scare", 2011).

Therefore, it was important to conserve the cucumber especially of favorable variety to ensure the continuance of cucumber stock if the world was affected by cucumber crisis. Besides, commercially-important variety of cucumber must be conserved to produce uniform products. The finding of this study may lead to production of plantlet with similar characteristics as the mother plant. Recently, *in vitro* propagation was used as alternative method to overcome the problems faced during conventional propagation. *In vitro* propagation possesses many advantages over conventional propagation. This include production of genetically stable plants and virus free plants (Moghaleb *et al*., 1999), for conservation of plant species (Yadav *et al*., 2012), multiple plant of favorable traits, introduce new traits into selected plants and growing cultivars in shorten time (Taji *et al*., 2002).

1.2 Problem Statements, Justifications and Objectives of Study

However, tissue culture is always subjected to somaclonal variation, which results to production of plants that vary from the parent plant. In order to produce plant with the same favorable characteristics as the parent plant, tissue culture techniques must be optimized. This is important as no variation wanted in the production of plant variety that was high demanded from local people and has high commercial value. Here, it is necessary to obtain protocol to produce uniform plantlets that may be useful for industrial large scale multiplication and for conservation of the species.

In this study, shoot apical meristem (SAM) was used as explants to be culture *in vitro*. Meristem culture might produce genetically stable plantlet that may result of producing plantlet with similar characteristics as parent plant, besides provide explant that is free from viruses, bacterial and fungal pathogen for the culture (Grout, 1999). Apical meristem is highly organized structure. Meristematic cells of apical meristem can direct the pattern formation of organs in plant, thus help to reduce somaclonal variation, which is the variation seen in regenerated plants from tissue culture. This study had evaluated the best treatment for culturing cucumber variety MTi2 to obtain uniform plantlet. The protocol for SAM culture would be optimized in this study by using auxin and cytokinin as plant growth regulator. Comparison study was carried out in term of morphology, anatomy, biochemical and antioxidant profiling to detect whether the plantlet produced expressed somaclonal variation. The finding was useful for the species conservation, for

large scale multiplication and for future research. To date, there is no study found on the *in vitro* propagation of cucumber by using SAM.

This study was also carried out to compare the antioxidant activity between different parts of cucumber plant. People did realize the high nutritional content in cucumber fruit. However, they didn't realize which part of the edible parts of cucumber possesses higher nutritional value. Recent researches showed that there was higher nutritional content in seed rather than in edible fruit part of many plant species. As cucumber are always eaten together with the seed, this study was done to find out whether the seed of cucumber possess higher nutrient content compare to fruit parts, in term of antioxidant profiling. Different part of the plant may contain different antioxidant activity. There is also no report found on the comparative study of antioxidant profiling between edible parts of cucumber plant. Antioxidant nutrients are important for human. A few roles possess by antioxidant are in preventing cardiovascular disease, macular degeneration, pathogenic process related to cancer, asthma, besides improve immune function (McDermott, 2000).

Hence, the objectives of this study are:

(1) To regenerate *C. sativus* cv. MTi2 plants *in vitro* by using shoot apical meristem.

(2) To compare the morphology, anatomy, biochemical and antioxidant profiling between *C. sativus* plant and *C. sativus* plantlet.

(3) To compare the antioxidant profiling in different edible parts of *C. sativus* plant.

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