UNIVERSITI PUTRA MALAYSIA

SUITABILITY OF SHOOT APICAL MERISTEM AS EXPLANT TO MINIMIZE VARIATION OF TOBACCO (NICOTIANA TABACUM L. CV. VIRGINIA) PLANTLETS

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By

AZIMAH BINTI CHE AZIZ

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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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February 2015

Chair : Rosimah binti Nulit, PhD
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A protocol for *in vitro* regeneration using shoot apical meristem (SAM) of tobacco (*Nicotiana tabacum* L. cv. Virginia) has been established and entire tobacco plantlets were produced. The explants were isolated from 5-days-old germinating tobacco seedlings. Shoot development and root formation were shown in all treatments but the most effective treatment is control with 73% of explant survival, followed by 0.5 mg/l IAA with 60% of explant survival. However, before acclimatization, plantlets from medium supplemented with 0.5 mg/l KIN showed the highest number of leaves and plantlet height with an average of 6.0 ± 0.37 leaves and 0.7 ± 0.04 cm height, followed by 1.0 mg/l KIN with an average of 5.4 ± 0.37 leaves and 0.6 ± 0.04 cm height. Number of roots was found highest in medium supplemented with 0.5 mg/l IAA with an average of 4.9 ± 0.18 roots followed by control medium with an average of 3.5 ± 0.23 roots. After 12 weeks of acclimatization, only plantlets from treatment with 0.5 mg/l IAA survived in the environmental condition. To detect the variation, the morphology, anatomy and primary metabolites between tobacco plants and its plantlets were compared. Both morphology (plant height, number of leaves and leaf morphology) and anatomy (midrib of leaf, stem and root) of tobacco plantlets were similar to tobacco plants. Besides, there is also no significant difference in the total chlorophyll, carbohydrate and protein content of both tobacco plants and plantlets. The secondary metabolites and anti-oxidative profiling between tobacco plants and its plantlets were also studied. Total flavonoid and carotenoid content were found higher than phenolic compounds in both plants. However, the antioxidant activity varied between both plants. Plantlets of tobacco have slightly higher antioxidant activity than tobacco plants. This was indicated by the significant difference ($p<0.05$) in the IC_{50} values between both plants in which plantlets have a lower IC_{50} value (16.82 ± 0.21) than tobacco plants (18.97 ± 0.28). In conclusion, no variation was expressed in tobacco plantlets regenerated from SAM *in vitro* except for a slightly different in the IC_{50} values. Tobacco plants and its plantlets can also be considered as good sources of natural antioxidant.

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

KESESUAIAN MERISTEM APIKAL PUCUK SEBAGAI EKSPLAN UNTUK MENGURANGKAN VARIASI PADA POKOK KLON TEMBAKAU (*NICOTIANA TABACUM* L. CV. VIRGINIA)

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Protokol untuk regenerasi pokok klon tembakau secara *in vitro* dengan menggunakan meristem apikal pucuk (SAM) tembakau (*Nicotiana tabacum* L. cv. Virginia) sebagai eksplan telah dihasilkan. Eksplan diisolat daripada biji benih tembakau yang sedang bercambah pada hari ke-5. Pertumbuhan pucuk dan pembentukan akar pada klon-klon ditunjukkan dalam semua medium selepas 2 minggu, walaubagaimanapun medium kawalan menunjukkan peratusan yang paling tinggi dengan 73% eksplan yang hidup, diikuti oleh medium yang mengandungi 0.5 mg/l IAA dengan 60% eksplan yang hidup. Walaubagaimanapun, sebelum proses aklimatisasi, pokok klon daripada medium yang mengandungi 0.5 mg/l KIN menunjukkan bilangan daun dan ketinggian pokok yang paling tinggi dengan purata 6.0 ± 0.37 helai daun dan 0.7 ± 0.04 sm ketinggian, diikuti oleh 1.0 mg/l KIN dengan purata 5.4 ± 0.37 helai daun dan 0.6 ± 0.04 sm ketinggian. Bilangan akar yang paling tinggi telah ditunjukkan dalam medium yang mengandungi 0.5 mg/l IAA dengan purata 4.9 ± 0.18 akar diikuti oleh medium kawalan dengan purata 3.5 ± 0.23 akar. Selepas 12 minggu proses aklimatisasi, hanya pokok klon daripada MS medium yang mengandungi 0.5 mg/l IAA berupaya untuk hidup dan menyesuaikan diri dalam persekitaran. Morfologi, anatomi dan metabolit primer antara pokok tembakau dan pokok klonnya telah dibandingkan untuk mengkaji kewujudan variasi. Kedua-dua morfologi (ketinggian pokok, bilangan daun dan morfologi daun) dan anatomi (urat tengah daun, batang dan akar) pokok klon tembakau adalah sama dengan pokok tembakau. Selain itu, tiada juga perbezaan yang signifikan pada jumlah kandungan klorofil, karbohidat dan protin dalam kedua-dua pokok tembakau dan pokok klon. Kajian perbandingan metabolit sekunder dan profil anti-oksidatif antara pokok tembakau dan pokok klonnya juga telah dijalankan. Kajian menunjukkan jumlah kandungan flavonoid dan karotenoid didapati sedikit lebih tinggi daripada sebatian fenolik dalam kedua-dua pokok. Walau bagaimanapun, aktiviti antioksidan adalah berbeza antara kedua-kedua pokok. Pokok klon tembakau mempunyai aktiviti antioksidan yang sedikit lebih tinggi daripada pokok tembakau. Ini telah ditunjukkan oleh perbezaan yang signifikan ($p<0.05$) pada nilai-nilai IC_{50} antara kedua-dua pokok di mana pokok klon mempunyai nilai IC_{50} (16.82 ± 0.21) yang lebih rendah daripada pokok tembakau (18.97 ± 0.28). Kesimpulannya, tiada variasi ditunjukkan pada pokok klon tembakau yang diregenerasi daripada SAM secara *in*

vitro kecuali sedikit perbezaan pada nilai-nilai IC_{50}. Pokok tembakau dan pokok klonnya juga boleh dianggap sebagai sumber antioksidan semula jadi yang baik.

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I certify that a Thesis Examination Committee has met on 13 February 2015 to conduct the final examination of Azimah binti Che Aziz on her thesis entitled "Suitability of Shoot Apical Meristem as Explant to Minimize Variation of Tobacco (*Nicotiana tabacum* L. cv. Virginia) Plantlets" 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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	percentage
°C	degree Celcius
µl	microliter
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
BSA	Bovine serum albumin
cm	centimeter
DPPH	2, 2-diphenyl-1-picrylhidrazyl
DPX	diputal petroleum xylene
g	gram
gL^{-1}	gram per liter
IAA	Indole-3-Acetic Acid
KIN	Kinetin
L	liter
mg	milligram
mg/µl	milligram per microliter
mg/l	milligram per liter
mg/ml	milligram per milliliter
min	minute
ml	milliliter
mm	millimeter
MS	Murashige and Skoog
Na_2CO_3	Sodium carbonate
nm	Nanometer
PGR	Plant Growth Regulator
pH	Negative logarithm of hydrogen ion concentration

SAM	shoot apical meristem
SE	standard error
t	time
v/v	volume per volume

CHAPTER 1

INTRODUCTION

1.1 General Introduction

One of the most significant content of cell engineering is the tissue culture, which is the *in vitro* culture of cells, tissues or organs. Plant tissue culture has undergone an interesting development because it provides knowledge about totipotency, cell nutrition, cell division, differentiation, cell preservation, metabolism, radiobiology and mutations (Ganapathi *et al.*, 2004).

History has proved that by tissue culture technology, new variety of plantlets has been produced. Leva *et al.* (2012) also mentioned that somaclonal variation provides a valuable source of genetic variation for the plant improvement by selecting the novel variants that show resistance to disease, higher yield or improved quality. Yet, *in vitro* regeneration has an advantage over conventional propagation to produce disease free plants at high multiplication rate. According to Ganapathi *et al.* (2004), this technology also shows high potential to produce many biologically active compounds, such as alkaloids, phenolics, vitamins, steroids and other useful chemicals.

One of the advantages of the *in vitro* tissue culture is for the conservation of endangered plant species. Tissue culture technology has been successful to conserve endangered species (Sudhersan *et al*., 2003). Thus, tissue culture technique can be used to replace the conventional method in order to produce plants that have been considered as rare and endangered. In order to conserve the endangered species, it is necessary to produce true-to-type plantlets and to eliminate somaclonal variation of plantlets. Therefore, the plantlets must have similar characteristics in the morphology, anatomy and physiology, as well as the genetics. By manipulation or optimizing in tissue culture technique, the characteristics of plantlets can be retained.

Clone is genetically identical copy of an organism produced by asexual reproduction while the term somaclone refer to plants produced from *in vitro* culture and somaclonal variation refer to the genetic variation among that plants (Leva *et al.*, 2012). This somaclonal variation usually results in off-types that reduce the commercial value of the regenerated plants (Chuang *et al*., 2009). Factors that caused somaclonal variation are changes in chromose number (polyploidy or aneuploidy), chromosome damage (insertions, deletions, mutations, translocation) or changes in methylation of chromatin (Biswas *et al.*, 2009). According to Bordallo *et al.* (2004), four critical variables for somaclonal variation are genotype, explant origin, cultivation period and culture condition. The conditions of *in vitro* culture can be mutagenic and regenerated plants derived from calli, organ cultures, somatic embryos and protoplast sometimes can lead to phenotypic and genotypic variation (Orbovic *et al*., 2008). Because the goal of plant

conservation is to obtain clonal identity, it is necessary to control the somaclonal variation (Bordallo *et al.*, 2004).

1.2 Problem Statements, Justification and Objectives of Study

In vitro regeneration through tissue culture is one of the methods for the conservation of endangered plant species (Lynch, 2002). However, tissue culture can cause somaclonal variation in the plantlets produced. The sources of explant for tissue culture such as stem, root, shoot or leaf, the application of exogenous plant growth regulators and the culture condition are the factors that determine the existence of somaclonal variation of the plantlets. The sources of explant are considered as the main factor for somaclonal variation because explant tissue can affect the frequency and nature of somaclonal variation (Leva *et al*., 2012). Explants may present dissimilar regeneration rates, so the selection procedures can differ among different explant types (Bordallo *et al.*, 2004). Therefore, to minimize the variation, suitable explants must be selected for the *in vitro* regeneration. According to Leva *et al.* (2012), the use of meristematic tissues as starting materials for tissue culture can reduce the possibility of variation. In the present study, shoot apical meristem (SAM) tissue was selected as explant due to its lower tendency to generate somaclonal variation as it avoids the formation of callus (Duncan 1997).

In the present study, tobacco plant was chosen because it has been considered as the model plants in the field of tissue culture (Ganapathi *et al.*, 2004). Previous studies on *in vitro* regeneration of tobacco have used several different types of explants, such as shoots (Sun and Kang, 2003), young leaf (Yang *et al*., 1995) and stem (Smith and Murashige, 1970; da Silva, 2005). There are reports published on the use of SAM in other plant species such as chrysanthemum (Kumar *et al*., 2009), red pepper (Peddaboina *et al*., 2006), rice (Duong *et al*., 2000; Silvarajan *et al*., 2012) and lily (Chen *et al*., 2011). However, there are no reports on using SAM of tobacco's germinating seed as explants in *in vitro* tissue culture so far.

SAM used in this study was obtained from the plumule of germinated tobacco seedlings. This is because the response of plumules to *in vitro* culture conditions is better than that of other tissues (Fernando *et al*., 2004). The roots also develop at a high rate and in a large numbers from plumules as a response to the production of endogenous auxin by the plumules (Vesperinas, 1998). According to Perez-Nunez *et al*. (2006), the use of plumule explants, consisting of the shoot meristem and surrounded by leaf primordia, has allowed the development of a reproducible micropropagation protocol. Seed was not used for starting material for the *in vitro* regeneration of tobacco because the objective of the present study is to use SAM as the explants, not seeds.

Tobacco is well known to be harmful to human health and environment. However, a few studies reported that it also have medicinal values and insecticide properties. There

are also reports published on the antioxidant properties of tobacco plant. In the present study, the secondary metabolites (antioxidant properties) and antioxidant activities between tobacco plant and its plantlet were evaluated and compared. The purpose of the comparison is to detect whether the variation among the plantlets exists in term of secondary metabolites and antioxidant activities. Therefore, the objectives of this study are:

1. To regenerate entire tobacco plantlets *in vitro* by using shoot apical meristem (SAM),
2. To compare the morphology, anatomy and primary metabolites between tobacco plant and its plantlet and
3. To study the secondary metabolites and anti-oxidative profiling of tobacco plant and its plantlet.

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