UNIVERSITI PUTRA MALAYSIA

COMPARISON BETWEEN SOMACLONAL VARIATION IN INTACT PLANTS AND MERICLONES OF TOMATO CV. MT1 AND BETWEEN ANTIOXIDATIVE PROPERTIES OF MATURE AND YOUNG TOMATO FRUITS

SITI NURATIQAH BINTI MAHADI

FS 2015 30

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By

SITI NURATIQAH BINTI MAHADI

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

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

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By

SITI NURATIQAH BINTI MAHADI

July 2015

Chair: Rosimah Binti Nulit, PhD
Faculty: Science

Tomato *Lycopersicon esculentum* is a member of the Solanaceae family. Most of the studies on micropropagation of tomato use stem, hypocotyl, leaf disc and cotyledon as the explant instead of young shoot apical meristem (SAM). Therefore, the first objective of this study was to evaluate the effect of Indole Acetic Acid (IAA) and Kinetin (KIN) on plantlet regeneration from SAM. SAM was isolated from 6-days-old germinating tomato seedling and was then cultured into liquid Murashige and Skoog (MS) medium supplemented with different concentrations of IAA and KIN, respectively, and a combination of both hormones in the ranges of 0.1-0.2 mg/l while liqiud MS medium alone served as control. Results showed that SAM was able to regenerate to an entire plant in all treatments. The highest significant mean in percentage of explant survival shown in treatment 8 (T8) which is liquid MS medium supplemented with combination of 0.05 mg/L IAA + 0.1 mg/L KIN with 66%. T8 was also the best treatment in the *in vitro* regeneration of SAM with an average plant height (4.6 cm), number of leaves (9) and number of roots (7). The second objective was to study the variation on morphology, anatomy and primary metabolites content between tomato intact plant and mericlones. Our findings indicated that the morphology, histology and contents of primary metabolites of all mericlones were almost similar with intact plants; t-test ($p>0.05$). Both intact plant and mericlone has similar odd pinnate leaf, acute leaf tip, oblique leaf base, serrate leaf margin and reticulate vein. Intact plant has higher total chlorophyll and total soluble protein content but lower in total carbohydrate content compared to mericlones. However, no significant difference observed ($p>0.05$) excluding total chlorophyll content. Therefore, it was concluded that SAM serves as a suitable explant that can regenerate true mericlones and combination hormone of 0.05 mg/L IAA + 0.1 mg/L KIN gave the best response in the growth development of the mericlones. The third objective of this study was to compare the antioxidant properties and

antioxidant activity between mature and young fruits of tomato plant cv. MT1 that was grown in conventional propagation. Total carotenoid, flavonoid and phenolic content of mature and young tomato fruit were measured. 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical scavenging activity and reducing power analysis were employed to measure antioxidant activity of both mature and young fruits. Fruit sample (200 mg) was extracted and diluted into a series of concentration (0.5, 1, 2, 5, and 10 mg/ml). Mature fruit was found to have significantly ($p<0.05$) higher total carotenoid, total flavonoid and total phenolic content, and higher antioxidant activity. In addition, mature fruit has lower IC_{50} value when compared to young fruit with 6.00 ± 0.03 and 8.86 ± 0.03 µg/ml respectively. Hence, it was concluded that mature tomato fruit of tomato plant cv. MT1 showed higher contents of antioxidant properties and higher antioxidant activity in comparison to the young tomato fruit of tomato plant cv. MT1.

Keywords: *Lycopersicon esculentum*, shoot apical meristem, IAA, Kinetin, plantlet, antioxidant activity.

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

PERBANDINGAN ANTARA VARIASI SOMAKLONAL PADA POKOK TOMATO CV.MT1 DAN KLONNYA SERTA ANTARA PROFIL ANTIOKSIDA PADA BUAH TOMATO MATANG DAN MUDA

Oleh

SITI NURATIQAH BINTI MAHADI

Julai 2015

Pengerusi: Rosimah Binti Nulit, PhD
Fakulti: Sains

Kajian terdahulu menggunakan daun, ruas dan batang sebagai eksplan untuk mikropropagasi tomato. Oleh itu, kajian ini dijalankan bertujuan untuk mengkaji kesan penggunaan Indol asetik asid (IAA) dan Kinetin (KIN) terhadap penghasilan anak pokok daripada pucuk apikal meristem. Pucuk apikal meristem yang digunakan sebagai eksplan telah dipencilkan daripada anak benih yang berusia 6 hari dan kemudiannya dikultur di dalam media Murashige dan Skoog (MS) yang dibekalkan dengan hormon IAA dan KIN dan gabungan kedua-dua hormon dalam julat kepekatan ialah 0.1 - 0.2 mg / l dan media MS sebagai kawalan. Kajian ini mendapati bahawa tisu pucuk apikal meristem berupaya membesar, berkembang dan membentuk satu pokok tomato dalam semua rawatan termasuk kawalan. Purata tertinggi bagi peratus pokok klon hidup ditunjukkan pada rawatan media MS cecair yang dibekalkan dengan 0.05 mg/L IAA + 0.1 mg/L KIN. Rawatan ini juga merupakan rawatan terbaik untuk perkembangan anak pokok klon dengan purata ketinggian 4.6 sm, jumlah bilangan daun yang terbentuk adalah 9 dan akar adalah 7. Objektif kedua kajian ialah untuk membanding variasi somaklon antara pokok tomato dan klon. Kajian ini mendapati morfologi, histologi dan kandungan metabolit primer semua klon pokok tomato yang dihasilkan adalah serupa dengan pokok induk. Pokok induk dan pokok klon mempunyai jenis daun pinat ganjil, hujung daun meruncing, pangkal daun serong, margin daun bergigi dan urat daun jejala. Pokok induk mempunyai kandungan jumlah klorofil dan jumlah protin larut yang lebih tinggi tetapi lebih rendah dalam jumlah karbohidrat berbanding pokok klon. Walau bagaimanapun, tiada perbezaan signifikan ($p>0.05$) kecuali kandungan jumlah klorofil. Kesimpulannya, pucuk apikal meristem tomato berpotensi sebagai eksplan untuk regenerasi semula pokok tomato dan kombinasi hormon pada kepekatan 0.05 mg/L IAA + 0.1 mg/L KIN memberi kesan terbaik pada perkembangan anak pokok klon. Objektif kajian yang ketiga ialah membanding profil dan aktiviti antioksida antara buah muda dan buah matang tomato cv. MT1. Jumlah kandungan karotenoid, flavonoid dan fenolik

bagi buah muda dan buah matang tomato telah diukur. Analisis aktiviti radikal 2,2-difenil-1-pikrilhidrazil (DPPH) dan kuasa penurunan telah digunakan untuk mengukur aktiviti antioksida antara buah tomato matang dan buah tomato muda. Sampel buah (200 mg) telah diekstrak dan satu siri kepekatan iaitu 0.5, 1, 2, 5, dan 10 mg/ml disediakan. Kajian mendapati buah tomato matang mempunyai kandungan jumlah karotenoid, jumlah flavonoid, dan jumlah fenolik yang lebih tinggi dengan ketara berbanding buah tomato muda. Buah tomato matang juga menunjukkan aktiviti antioksida yang lebih tinggi dengan ketara. Buah tomato matang mempunyai nilai IC_{50} yang lebih rendah berbanding buah tomato muda dengan nilai 6.00 ± 0.03 dan 8.86 ± 0.03 µg/ml masing-masing. Kesimpulannya, buah tomato matang bagi tomato cv. MT1 mempunyai kandungan profil antioksida yang lebih tinggi dan menunjukkan aktiviti antioksida yang lebih tinggi berbanding buah muda tomato cv. MT1.

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I certify that a Thesis Examination Committee has met on 31 July 2015 to conduct the final examination of Siti Nuratiqah Binti Mahadi on her thesis entitled "Comparison between Somaclonal Variation in Intact Plants and Mericlones of Tomato cv. MT1, and between Antioxidative Properties of Mature and Young Tomato Fruits" 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.

Members of the Thesis Examination Committee were as follows:

Shamarina bt Shohaimi, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Yap Chee Kong, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Jualang Azlan Gansau, PhD
Associate Professor
Universiti Malaysia Sabah
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Rosimah Binti Nulit, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Meenakshii a/p Nallappan, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

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Supervisory
Committee: Rosimah binti Nulit

Signature: _____

Name of
Member of
Supervisory
Committee: Meenakshii a/p Nallapan

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LIST OF ABBREVIATIONS

%	Percentage
ºC	Degree Celcius
µl	Microliter
µm	Micrometer
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
BSA	Bosine serum albumin
Cm	Centimeter
cv.	Cultivar
DPPH	2,2-diphenyl-1-picrylhidrazyl
DPX	Diputal petroleum xylene
DTT	Dithiothreital
EDTA	Ethylene diamine tetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
G	Gram
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
IAA	Indole-3-acetic acid
KIN	Kinetin
KOH	Potassium hydroxide
L	Liter
Mg	Milligram
mg/l	Milligram per liter
mg/ml	Milligram per milliliter

$MgCl_2$	Magnesium chloride
Ml	Milliliter
mM	Milimolar
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
UV	Ultraviolet
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In vitro regeneration is one of the technologies used to generate plant clones (Hussain *et al.,* 2012). As early as 1890, this technique was used as an alternative from traditional propagation. A part from a plant such as cell, tissue or organ was used and cultured in a high nutrient artificial media to generate a new whole plant (George *et al*., 2008). *In vitro* regeneration was introduced as a method that can produce virus free and genetically identical mericlones. Apart from that, this technique was widely used in agricultural industry for a bulk plant production (Thorpe, 2013). *In vitro* regeneration is also used for conservation purposes (Maryam *et al.,* 2014).

However, *in vitro* regeneration is challenged by somaclonal variation. Somaclonal variation is a phenotypic and DNA variation occurs in the plant clones produced after repeated subculture (Kaepplar *et al.,* 2000). The mericlones produced can vary in terms of plant length, level resistance to diseases, and also fruit quality. Even though somaclonal variation gives benefit in genetic improvement such as increased resistance to disease, it can lead to the regeneration of genetically non-identical mericlones. In addition, undesirable features such as reduced growth rate and fertility level can occur in some variants. But for conservation purposes, it is crucial to keep the same genetic identity among the mericlones and between the mericlone and mother plant. Maintaining high genetic fidelity can be useful for conservation purposes (He *et al.,* 2011).

The tomato (*Lycopersicon esculentum*) plant has been selected as the plant of interest in this study. Tomato is widely known as a vegetable rich in medicinal and nutritional values (Silva *et al.,* 2008). Tomato is the major source of lycopene which is the most effective natural antioxidant (Rao *et al.,* 2003). Moreover, tomato is rich in vitamin A, C and fiber (Rao and Agarwal, 2000). It can be eaten raw or cooked. Tomato juice, tomato paste and tomato ketchup are some of its commercial products which are widely used the world over. As it is very popular and valuable vegetable, it is important to be conserved finely and maintained its genetic fidelity. Furthermore, through *in vitro* regeneration technique, virus free tomato mericlones can be produced (Thorpe, 2013).

In this study, tomato cultivar MT1 was used as the plant of interest. Tomato MT1 and MT11 types are lowland tomatoes. Therefore, it is favorable since the study was conducted in lowland area. Both cultivars were developed by Malaysian Agricultural Research and Development Institute (MARDI). Tomato MT1 was developed through crossing the CL555-10 lines with the local white variety (Behboodian *et al.,* 2012).

1.2 Problem Statement and Objective of Study

In this study, shoot apical meristem (SAM) of 6 days seedling had been used as explants. Explant is a term referring to the part of mother plant taken to be cultured into the artificial media. To date, *in vitro* regeneration of tomato plant using SAM are yet to be reported in Malaysia. Instead of using SAM as the explant, previous studies used stem (Sheeja *et al.,* 2004), hypocotyl and leaf disc (Sheeja *et al.,* 2004; Devi *et al.,* 2008; Chaudry *et al.,* 2010), and cotyledon (Liza *et al.,* 2013). SAM possesses a high degree of totipotency and has an ability to minimize somaclonal variation (Valizadeh *et al.,* 2007). In addition, SAM is juvenile and this may decrease the potential of having virus infected plantlet. According to Sharma *et al.* (2007), explants selected from highly differentiated tissues have high tendency to produce variation. According to Sahjiram *et al.* (2003), the use of meristematic tissue as explant may reduce the possibility of variation. Therefore, SAM of tomato was selected as the targeted explant.

Subsequent to the above study, a comparative analysis in terms of morphology, anatomy and physiology will be carried out on both tomato intact plant and SAM derived plantlet. This is to verify if the SAM derived plantlet produced is identical to intact plant. Some of the biochemical contents were measured and compared. In this study, total chlorophyll, total carbohydrate, and total protein of both tomato intact plant and SAM derived plantlet leaves were the investigated parameters for biochemical content measurement.

Antioxidant properties of mature tomato fruit and lycopene content have been extensively studied (Wang *et al.,* 1996; George *et al.,* 2004). This might be due to the popularity of this fruit worldwide and also the medicinal value of tomato fruit because of lycopene (Kelkel *et al.,* 2011). There was no study found on the antioxidant property and antioxidant activity of tomato fruit of tomato plant cv. MT1. Hence, this study will also measure some antioxidant activity on the mature and young fruits of tomato plant cv. MT1 that was grown in conventional setting. In a nut shell, this comparative study can provide experimental data on the similarities between tomato plant and tomato plantlet

produced, and also to the variance between the mature and young fruits of tomato plant cv. MT1 in terms of antioxidant properties.

This study was expected to produce a true to type tomato mericlones as SAM serves as the explant. The plantlets produced will be similar to the tomato intact plant in terms of morphology, anatomy and primary metabolites contents. If the young SAM of tomato seedling is capable to regenerate tomato mericlones, this protocol can be applied for conservation of tomato plant and other related plants under Solanaceae family. For the comparative study on antioxidant properties and antioxidant activity, the mature fruit was expected to show higher antioxidant properties and antioxidant activity as compared to the young fruit of tomato plant cv. MT1. This is due to the preference of having ripened tomato fruit as a sample in the other previous studies done (Wang *et al.,* 1996; George *et al.,* 2004; Nour *et al.,* 2014). In order to reach all of these expected results, this study has come out with three main objectives as listed below:-

1. To evaluate the effect of IAA and kinetin on plantlet regeneration from shoot apical meristem (SAM).
2. To study the variation on morphology, anatomy and primary metabolites contents of tomato intact plants and mericlones.
3. To compare antioxidant properties and antioxidant activity on mature fruit and young fruit of tomato plant cv. MT1 in terms of 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical scavenging activity and reducing power analysis.

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