



**UNIVERSITI PUTRA MALAYSIA**

***MICROPROPAGATION OF SELECTED IMPROVED MATERIALS FROM  
Eucalyptus camaldulensis Dehn. FOR PAPER PRODUCTION  
IN ERBIL, IRAQ***

**NOZAD ALI QADER**

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**By**

**NOZAD ALI QADER**

**Thesis submitted to the School of Graduate Studies, Uiniversiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**February 2015**

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## DEDICATION

**To:**

- \* Our Prophet..... Taught us how to learn**
- \* The spirit of my father..... To his wish and a prayer**
- \* My dear mother ..... symbol of sacrifice and altruism**
- \* My brothers and sisters ..... love and appreciation**
- \* From my wife, who supports me always ..... expensive paper**
- \* Accessories of life and the apple of my eye..... Kazywa and Farzad**

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

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**February 2015**

**Chairman: Professor Nor Aini Ab. Shukor, PhD**

**Faculty: Forestry**

*Eucalyptus camaldulensis* is one of the most popular multipurpose tree species that had been widely planted in Iraq. Eucalyptus plantations are established in Iraq for many purposes including pulp and paper industry. Generally, industrials harvest these trees at 10-12 years old for paper productions. Since the high demand of its pulp and paper increases yearly, the first part of this study was conducted to find out the possibility of shorten rotation cycle from 10-12 years to 5 years, and to conduct plus tree selection as improved material for micropropagation. However, this was followed by developing micropropagation protocol using material selected from the first part using shoot tips and nodal segments for mass production.

In the first part, improved materials were screened through the use of source base on plus tree selection in the field based on good properties. Sixty *E. camaldulensis* trees (thirty from ten-year-old and thirty from five-year-old stand) were randomly selected through stratified sampling from two plantations in Erbil, northern Iraq. The trees were selected based on the growth performance of both qualitative and quantitative characteristics for fibre morphology and quality parameters. In the phase of property selection, sixteen trees with good ranking scores based on growth performance and fibre morphology were selected from these sources. In the final phase, the selected trees were re-examined according to growth performance, fibre morphology, and cumulative characteristics. As a result, four trees (two 5-year-old trees and two 10-year-old trees) with the highest score were chosen as the sources for micropropagation.

In the second part of this study for the micropropagation protocol, surface sterilization protocol of the seeds was established. Therefore, rinsing seeds of four different *E. camaldulensis* (selected plus trees) with commercial Clorox (25%) for 10-30 minutes was found to be effective. Shoot tips and nodal segment explants obtained from seeds of selected genotype of *E. camaldulensis* tree, were cultured on MS medium containing seven concentrations of BAP; control 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5mg L<sup>-1</sup> in a combinations with 0.1 mg L<sup>-1</sup> of NAA and 0.1 mg L<sup>-1</sup> of IBA for each concentration of BAP. Shoot induction was achieved from shoot tips and nodal segment explants through direct organogenesis pathway. Parameters used in this study were the number

of shoots and shoot length per culture. The best result was achieved using shoot tip explants in MS media, supplemented with 3.0 mg L<sup>-1</sup> BAP in combination with 0.1 mg L<sup>-1</sup> NAA. The mean number is 9.10 shoots per explant at 11.83 mm length. For shoot elongation, the best result was achieved using half strength of MS medium, supplemented with 1.0 mg L<sup>-1</sup> BAP, producing 3.31 cm. The elongated shoots were cultured on half-strength of MS medium for *in vitro* rooting. The best result was achieved with medium supplemented with 1.5mg L<sup>-1</sup> of NAA, producing 9.00 roots with 2.63 cm in length. In the hardening and acclimatisation stage, the plantlets were transferred to polybags containing sand, red soil, and peat moss in the ratio 1:1:1, and was kept under the shade at the nursery. However, 70 % of the plantlets survived after 3 weeks of transplanting into the nursery.

In conclusion, based on this study, it was found that there were no significant differences between 5 year-old and 10 year-old trees for paper production. Industrials can harvest Eucalyptus plantations at 5 year-old to reduce the rotation cycle. In addition, a protocol was developed for the micropropagation of superior selected genotype of *E. camaldulensis*.

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

**MIKROPERAMBATAN SUMBER TERPILIH *Eucalyptus camaldulensis*  
Dehn UNTUK PENGHASILAN KERTAS  
DARI ERBIL, IRAQ**

Oleh

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*Eucalyptus camaldulensis* adalah salah satu spesies pokok pelbagai guna yang paling popular ditanam secara meluas di Iraq. Ladang pokok Eucalyptus telah diwujudkan di Iraq untuk pelbagai tujuan termasuk untuk industri pulpa dan kertas. Secara keseluruhannya, pokok ini ditebang pada umur 10-12 tahun bagi pengeluaran kertas. Permintaan bagi pulpa dan kertas semakin meningkat setiap tahun. Kajian ini terdiri daripada dua bahagian utama: pertama kajian dijalankan untuk mengetahui kemungkinan menguraikan kitaran penuaian pada dari 10-12 tahun kepada 5 tahun dan pemilihan pokok plus sebagai bahan terpilih yang terbaik untuk tujuan mikroperambatan. Fasa kedua adalah berkenaan dengan mikroperambatan menggunakan bahan yang terpilih daripada fasa pertama.

Kajian ini dijalankan untuk menghasilkan bahan terbaik yang mungkin daripada genotip *E. camaldulensis* dengan membangunkan teknik mikroperambatan yang sesuai menggunakan eksplan hujung pucuk dan bahagian nod.

Dalam fasa pertama, bahan terbaik telah disaring berdasarkan kegunaan sumber asas pada pemilihan pokok plus di lapangan. Kajian lapangan terdiri daripada mengenal pasti ciri-ciri yang baik. Enam puluh pokok *E. camaldulensis* (tiga puluh pokok dari dirian berusia sepuluh tahun dan tiga puluh pokok dari dirian berusia lima tahun) telah dipilih secara rawak melalui persampelan berlintang dari dua ladang di Erbil, Iraq utara. Pokok dipilih berdasarkan prestasi pertumbuhan kedua-dua ciri kualitatif dan kuantitatif morfologi gentian dan parameter kualiti. Dalam fasa pertama pemilihan ciri berdasarkan prestasi pertumbuhan dan pengelasan morfologi serat, enam belas pokok dalam kelas terbaik telah diskor dan dipilih daripada sumber-sumber ini. Dalam fasa terakhir pemilihan, pokok-pokok yang terpilih dikaji semula berdasarkan prestasi pertumbuhan, serat morfologi dan ciri-ciri kumulatif, empat pokok (dua pokok berusia 5 tahun dan dua pokok berusia 10 tahun) dengan skor parameter baik yang tertinggi telah dipilih sebagai sumber untuk mikroperambatan.

Fasa kedua kajian ini bermula dengan pensterilan biji benih. Pembilasan biji benih empat *E. camaldulensis* yang berbeza (pokok terpilih) dengan Clorox komersial (25%) untuk 10-30 minit didapati berkesan untuk mengurangkan kadar kontaminasi kepada 0%. Eksplan hujung pucuk dan bahagian nod yang diperolehi daripada biji benih genotip terpilih pokok *E. camaldulensis*, telah dikulturkan dalam medium MS yang mengandungi tiga kepekatan sitokinin BAP; kawalan, 1.0, 1.5, 2.0, 2.5, 3.0 dan 3.5mg L<sup>-1</sup> dengan gabungan 0.1 mg L<sup>-1</sup> auksin NAA dan 0.1 mg L<sup>-1</sup> IBA dengan setiap kepekatan sitokinin BAP. Pengaruh pucuk telah dicapai dari eksplan hujung pucuk dan bahagian nod melalui laluan organogenesis langsung. Dua parameter telah digunakan dalam kajian ini iaitu jumlah dan kepanjangan pucuk per eksplan.

Keputusan yang terbaik telah diperolehi dengan eksplan hujung pucuk bagi kedua-dua parameter yang dikaji, dimana rawatan yang ditambah dengan 3.0 mg L<sup>-1</sup> BAP dengan gabungan 0.1 mg L<sup>-1</sup> NAA menghasilkan purata jumlah pucuk sebanyak 9.10 dan purata kepanjangan pucuk 11.83 mm per eksplan. Kadar pemanjangan pucuk pula didapati tertinggi pada rawatan medium MS separuh penuh dengan 1.0 mg L<sup>-1</sup> BAP (3.31 cm). Pucuk yang dipanjangkan kemudian dikulturkan pada medium MS separuh penuh untuk pengakaran *in vitro*. Keputusan terbaik dicapai dalam medium yang ditambah dengan 1.5mg L<sup>-1</sup> NAA dimana ia menghasilkan purata bilangan akar 9.00 dengan kepanjangan sebanyak 2.63 cm. Pada peringkat pengikliman, pindah tanam anak benih yang diakar secara *in vitro* ke dalam polibeg yang mengandungi pasir, tanah merah dan gambut lumut dalam nisbah 1: 1: 1 memberikan peratus madiri tertinggi (70%) selepas 3 minggu dipindahkan di bawah tempat teduh di nurseri.

Kesimpulannya, berdasarkan keputusan daripada kajian ini, didapati bahawa tiada terdapat perbezaan ketara diantara pokok berumur 5 dan 10 tahun untuk pengeluaran kertas. Ini membuktikan bahawa pokok Eucalyptus boleh dituai pada umur 5 tahun. Protokol telah dibangunkan untuk mikroperambatan genotip terpilih *E. camaldulensis* yang unggul untuk penghasilan kertas.



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## LIST OF ABBREVIATIONS

ANOVA	Analyses of variance
a.s.l.	Above sea level
BAP	Benzyaminopurine
°C	degree centigrade
Ca (OCl) <sub>2</sub>	Calcium hypochlorite
dbh	Diameter at breast height
df	Degree of freedom
g	gram
h	hour
HgCl <sub>2</sub>	mercuric chloride
IAA	Indole acetic add
IBA	Indole butyric acid
L	litre
mg L <sup>-1</sup>	Milligram per litre
MS	Murashige & Skoog's medium
MSO	Murashige & Skoog's medium without hormone
NAA	Naphthaleneacetic Acid
NaOCl <sub>2</sub>	Sodium chlorite
SS	Sum of square

## TERMINOLOGY

Acclimatisation	Show change in the physiology of an organism/ plant, as a result of its exposure to a changed environment
Auxins	Plant hormone naturally synthesised (Indole-3-Acetic Acid-IAA) in the apex and transported downward the stem. Also occurred in synthetic form (Naphthalene Acetic Acid-NAA and Indole-3-butyric Acid-IBA), auxins influence cell elongation, cell division, induction of primary vascular tissue, adventitious root formation, senescence, fruit growth, outgrowth of axillary buds and sex expression.
Axillary shoots	Shoot buds formed at the juncture of the leaf and the stem.
Callus	Actively growing relatively undifferentiated tissue, devoid of macroscopic organised structure, normally produced in higher plants in response to wounding or infection but often formed <i>in vitro</i> during the artificial culture of plant tissue.
Culture medium	A mixture of organic and inorganic nutrients used for the cultivation of cells.
Cytokinin	A class of growth regulators chemically and functionally hormone zeatin, cytokinins stimulate cell division, cell and/ or shoot differentiation, lateral bud break etc.
Explant	The tissue taken from a plant or seed and transferred to a culture medium to establish a tissue cultures system or regenerates a plant.
Growth	An irreversible increase in volume or mass associated with the development, it usually involves cell division, expansion, differentiation and morphogenesis.
Induction	Determination and/or initiation of a plant structure, organ or process <i>in vitro</i> as the results of a specific stimulus.
<i>In vitro</i>	A sterile artificial environment typically in glass vessels, in which cultured cells, tissue, organs or whole plants may reside.
Juvenile	A phase in the sexual cycle of a plant characterised by differences in appearance from the adult and which lacks the ability to respond to flower inducing stimuli

Micropropagation	Rapid vegetative propagation of a plant via small pieces of tissue and usually beyond that obtained in nature. The process includes many steps-stock plant cares, explant selection and sterilisation, media manipulation to obtain proliferation, rooting, acclimation, and growing on of liners
Morphogenesis	The development of form or structure in ontogeny or in regeneration
Organogenesis	Initiation of an organ or the production of a plantlet <i>In vitro</i> through the sequential usually non-synchronised initiation of root and shoot structure connected by vascular system.
Plantlet	A tiny plant with a distinct root and shoot system formed via tissue culture either by embryogenesis or organogenesis.
Proliferation	Growth by active division
Regeneration	Laboratory techniques for forming a new plant or organ from cultured cells.
Subculture	The transfer or subculture of cells or organs, with or without dilution, from one culture vessel to another contains fresh medium.
Tissue culture	A general term used to describe the development of tissue in culture under sterile conditions.

## CHAPTER 1

### INTRODUCTION

Forest is a substantial asset to each country's economy. It provides many natural resources such as fuelwood, food, raw materials, and fodder for various kinds of industries. Furthermore, it has numerous advantages and benefits including landscape and recreational opportunities, soil protection, erosion decrease, water purification, oxygen production, carbon dioxide consumption, microclimate regulation, and provision of biological diversity. In the twentieth century, the industrial revolution and high demand on forest products led to heavy deforestation and the reduction of the world total forest area from 50% to 30 % (Kataria et al., 2013). Globally, 48% of the total forest plantation is for industrial purposes; about 26% is for other purposes such as fuel wood, agroforestry, soil, and water conservation; while the remaining 26% is not specified (Yasodha et al., 2004). Therefore, the demand on forest plantation in replacing the natural forest and fulfilling the demand on forest product is increasing (Kröger, 2012).

Recently, because of the high demand on forest products as a result of developing technology, governments and private sectors around the world have focused on mass reforestation with fast growing tree species in order to shorten rotation cycle and fulfil the market demand (FAO, 2012). However, the establishment of new forest plantation is an alternative approach for preserving the existing natural forest and providing forest products such as fuel wood, lumber, paper, timber, and charcoal for humankind (Kataria et al., 2013). Some fast-growing tree species such as Pines, Acacia, and Eucalyptus have been chosen for forest plantation around the world (Borras et al., 2012). Some Eucalyptus species are among those species which have been widely planted around the world for the purpose of supplying the sustainable yield for industrial and agroforestry purposes (Girijashankar, 2012; Kataria et al., 2013; Kawaoka et al., 2006; Kröger, 2012).

There are about 800 species in Eucalyptus genus; and all of these species except three or four, are native to Australia (Kavitha, 2009). This genus is the most widely cultivated forest tree species around the world, and this is due to its adaptability and multipurpose uses (Kröger, 2012). According to Global\_Eucalyptus Map Website (2009), there are more than 20 million ha of Eucalyptus plantation all over the world. According to Nevill et al. (2010), most of the Eucalyptus species are tall, fast growing, and even some of them can grow up to more than 100 m. Thus, this makes it the tallest flowering plant in the world.

One of the most well-known Eucalyptus species is *Eucalyptus camaldulensis* (Dehn.). It is commonly known as the river red gum tree. This species is a multipurpose and fast growing species that belongs to the Myrtaceae family. It is one of the species that has been widely planted around the world for fuel wood, timber, paper, pulp, charcoal, and essential oil (Girijashankar, 2012). This tree is an indigenous species to Australia, which plays an important role in paper production industry in different countries all over the world (Chen et al., 2001; FAO, 2010).

*E. camaldulensis* and several other Eucalyptus species were introduced to Iraq about 50 years ago, and is now widely cultivated and distributed on irrigated land at an altitude of 250 m or more. It is also reported that *E. camaldulensis* can grow well in many parts of Iraq (Sherzad, 2003). It is usually cultivated for paper and pulp, charcoal production, landscape, agroforestry, ornamentals, shade, fuel wood, poles, windbreak, and shelter belts (Poke et al., 2006; Sherzad, 2003). Its plantation is usually productive, but can be hampered by the variable growth in many characteristics especially when regeneration plant is by seeds (Bindumadhava et al., 2011). In addition, most trees produce poor seed yield. It is vital to secure means to the mass propagation of this species not only to produce uniform trees, but also to increase wood productivity (Prakash & Gurumurthi, 2010). In this regard, *in vitro* plant regeneration is a practical approach, as it will result in the production of uniform stocks that have high genetic stability. Thus, the objective of this study is to establish micropropagation techniques using improved materials of *E. camaldulensis* for the establishment of plantation for paper production. To ensure improved productivity, uniform trees, and increased genetic gain in *E. camaldulensis*, tissue culture techniques were employed. The healthy explants in the form of nodal segments and shoot tips selected from seeds of superior trees of two different age groups were used in this study. The healthy explants were selected from two plantations in Erbil, North of Iraq. The tissue culture techniques including shoot induction, multiplication, elongation, and rooting were assessed by adopting the techniques developed by Fazal et al. (2003) and Girijashankar (2012) on *E. camaldulensis* with some modifications.

In this research project, we have attempted to overcome the problems that are related to Eucalyptus propagation and productivity. Thus, this study proposed a new tissue culture method in order to mass propagate improved planting materials for better productivity.

The specific objectives of this research were:

1. To produce improved planting materials based on the selection of plus trees.
2. To develop workable micropropagation techniques for mass production of improved planting materials.
3. To determine the effects of pyramid shape structure on the contamination rate of shoot induction.

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