UNIVERSITI PUTRA MALAYSIA

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

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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

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

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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^{-1} in a combinations with 0.1 mg L^{-1} of NAA and 0.1 mg L^{-1} 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^{-1} BAP in combination with 0.1 mg L^{-1} 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^{-1} 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^{-1} 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*.

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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

NOZAD ALI QADER

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Pengerusi: Profesor Nor Aini Ab Shukor, PhD

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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 keselurahannya, 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 berlintap 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 pengkelasan 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^{-1} dengan gabungan 0.1 mg L^{-1} auksin NAA dan 0.1 mg L^{-1} IBA dengan setiap kepekatan sitokinin BAP. Pengaruhan 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^{-1} BAP dengan gabungan 0.1 mg L^{-1} 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^{-1} 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^{-1} NAA dimana ia menghasilkan purata bilanagn 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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ney.

I certify that a Thesis Examination Committee has met on 25 February 2015 to conduct the final examination of Nozad Ali Qader on his thesis entitled "Micropropagation of Selected Improved Materials from *Eucalyptus camaldulensis* Dehn. for Paper Production in Erbil, Iraq" 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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the other supervisory committee member Prof Maheran Azhar retired from UPM.

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LIST OF ABBREVIATIONS

ANOVA	Analyses of variance
a.s.l.	Above sea level
BAP	Benzyaminopurine
°C	degree centigrade
$Ca(OCl)_2$	Calcium hypochlorite
dbh	Diameter at breast height
df	Degree of freedom
g	gram
h	hour
$HgCl_2$	mercuric chloride
IAA	Indole acetic add
lBA	Indole butyric acid
L	litre
mg L^{-1}	Milligram per litre
MS	Murashige & Skoog's medium
MSO	Murashige & Skoog's medium without hormone
NAA	Naphthaleneacetic Acid
$NaOCl_2$	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 *in vitro* 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 *in vitro* as the results of a specific stimulus.
In vitro	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 *In vitro* 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 ninetieth 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.

REFERENCES

Abdelsamie, M. A. A., Rahman, R. A., & Mustafa, S. (2014). Pyramid shape power as a new halal-compliant food preservation and packaging technique. *Procedia - Social and Behavioral Sciences*. 121: 232–242.

Abdelsamie, M. A. A., Rahman, R. A., Mustafa, S., & Hashim, D. (2013). Effect of packaging shape and storage on the keeping quality of mineral water and a development of water-treatment device. *Journal of Food Processing & Technology*. 4(5): 231.

Abrahamian, P., & Kantharajah, A. (2011). Effect of vitamins on *in vitro* organogenesis of plant. *American Journal of Plant Sciences*. 2: 669–674.

Acosta, M. S., Mastrandrea, C., & Lima, J. T. (2008). Wood Technologies and Uses of Eucalyptus wood from Fast Frown Plantation for Solid Products. In *Proceedings of the 51st International Convention of Society of Wood Science and Technology* (pp. 1–12). Concepción, Chile.

Aggarwal, D., Kumar, A., Sharma, J., & Reddy, M. S. (2012). Factors affecting micropropagation and acclimatization of an elite clone of *Eucalyptus tereticornis* Sm. *In Vitro Cellular & Developmental Biology - Plant*. 48(5): 521–529.

Ajlouni, S., & Puripast, S. (2010). Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. *Food Chemistry*. 119(3): 1000–1005.

Akeng, G. (2000). *Micropropagation of Acacia crassicarpa A. Cunn. Ex Benth*. Master of Science, Universiti Putra Malaysia, pp160.

Al-douri, N. A., & Al-essa, L. Y. (2010). A Survey of plants used in Iraqi traditional medicine. *Jordan Journal of Pharmaceutical Sciences*. 3(2): 100–108.

Arezki, O., Boxus, P., Kevers, C., & Gaspar, T. (2001). Changes in peroxidase activity , and level of phenolic compounds during light-induced plantlet regeneration from *Eucalyptus camaldulensis* Dehn . nodes. *in vitro*. *Plant Growth Regulation*. 33: 215–219.

Arya, I. D., Sudhir, S., & Arya, S. (2009). Micropropagation of superior Eucalyptus hybrids FRI-5 (*Eucalyptus camaldulensis* Dehn x *E* . *tereticornis* Sm) and FRI-14 (*Eucalyptus torelliana* F . V . Muell x *E* . *citriodora* Hook): A commercial multiplication and field evaluation. *African Journal of Biotechnology*. 8(21): 5718–5726.

Barclay, I. (2004). *The Haedy Eucalyptus page*. Retrieved 15 August 2013 from *http://www.angelfire.com/bc/eucalyptus/*.

Benyon, R. G., Marcar, N. E., Crawford, D. F., & Nicholson, A. T. (1999). Growth and water use of *Eucalyptus camaldulensis* and *E . occidentalis* on a saline discharge site near Wellington, NSW, Australia. *Agricultural Water Management*. 39(2-3): 229–244.

Bhat, S., Rao, G., Murthy, K. D., & Bhat, P. G. (2007). Influence of alignment of the pyramid on its beneficial effects. *Indian Journal of Experimental Biology*. 45(5): 455–458.

Bhojwani, S. S., & Razdan, M. K. (1996). *Plant Tissue Culture: Theory and Practice* (p. 767). Amsterdam, The Netherlands: Elsevier B.V.

Bindumadhava, H., Jagdish, T., Mahavishnan, K., Upadhyay, A. P., Mohan, V., & Sharma, N. (2011). Clonal propagation in *Eucalyptus camaldulensis* using minicutting technique. *Current Science*. 101(12): 1578–1585.

Bonney, N. (2003). *What seed is that?* (2nd ed., p. 351). Tantanoola, South Australia.

Borras, S. M., Franco, J., Gómez, S., Kay, C., & Spoor, M. (2012). Land grabbing in Latin America and the Caribbean. *Journal of Peasant Studies*. 39(3-4): 845–872.

Bren, L. J., & Gibbs, N. L. (1986). Relationships between flood frequency, vegetation and topography in a river red gum forest. *Australian Forest Research*. 16: 357–370.

Brooker, M. H., Connors, J. R., Slee, A. V., & Duffy, S. (2002). EUCLID: eucalypts of southern Australia (CD Rom),. CSIRO Publishing, Collingwood.

Brooker, M. H., & Kleining, D. A. (2006). *Field guide to eucalypts: South-eastern Australia*. Melbourne: Bloomings Books Pty Ltd.

Butcher, P. A., Otero, A., McDonald, M. W., & Moran, G. F. (2002). Nuclear RFLP variation in *Eucalyptus camaldulensis* Dehnh . from northern Australia. *Heredity*. 88: 402–412.

Butler, M., & Gartlan, C. (2006). *Corangamite Region Guidelines* . Australia.

Cary, A. J., Liu, W., & Howell, S. H. (1995). Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. *Plant Physiology*. 107: 1075–1082.

Chen, Z. Z., Chang, S., Ho, K., Cheng, Y. C., Tsai, J. B., & Chiang, V. L. (2001). Plant production of transgenic *Eucalyptus camaldulensis* carrying the *Populus tremuloides* cinnamate 4-Hydroxylase Gene. *Taiwan Journal of Forest Science*. 16(4): 249–258.

Chen, Z. Z., Ho, C., Ahn, I. S., & Chiang, V. L. (2007). Eucalyptus. *Methods in Molecular Biology*. 344:125–134.

Close, Dugald, C., & Wilson, Stephen, J. (2002). Provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensi*s seed. *Forest Ecology and Management*. 170(1-3): 299–305.

Das, T., & Mitra, G. C. (1990). Micropropagation of *Eucalyptus tereticornis* Smith. *Plant Cell, Tissue and Organ Culture*. 22(2): 95–103.

Davidson, D. A. (1997). *Shape Power* (p. 161). Arizona, USA: Rivers Publishing.

Desalvo, J. (2003). *The Complete Pyramid Sourcebook* (p. 497).

Dibax, R., Deschamps, C., Bespalhok, J. C., Vieira, L. G., Molinari, H. B., De Campos, M. K., & Quoirin, M. (2010). Organogenesis and agrobacterium tumefaciens-mediated transformation of *Eucalyptus saligna* with P5CS gene. *Biologia Plantarum*. 54(1): 6–12.

Dibax, R., Eisfeld, C. L., Cuquel, F. L., Koehler, H., & Quoirin, M. (2005). Plant regeneration from cotyledonary explants of *Eucalyptus camaldulensis. Scientia Agricola*. 62(4): 406–412.

Ducefa. (1998). *Biochemicals, plant cell and tissue culture catalogue.* (p. 156). The Netherlands: Haarlem.

Dutt, D., & Tyagi, C. H. (2011). Comparison of various Eucalyptus species for their morphological, chemical, pulp and paper making characteristics. *Indian Journal of Chemical Technology*. 18: 145–151.

Edwin, F. G., Hall, A. M., & Klerk, G. D. (2008). *Plant Propagated by tissue Culture 3rd Edittion* (3rd ed., p. 504). Dordrecht, The Netherlands: Springer.

El Moussaouiti, M., Barcha, B., Alves, E., & Francis, R. (2012). Kraft pulping characteristics of three Moroccan Eucalypti part 1. physical and chemical properties of wood and pulps. *BioResources*. 7(2): 1558–1568.

FAO, of the U. N. (2010). Global Forest Resources Assessment 2010 main report.

Fazal, R., Mussarrat, J., & Ihsan, I. (2003). Mass propagation in *Eucalyptys camaldulensis* Dehn. *Asian Journal of Plant Science*. 2(2): 184–187.

Foelkel, C. (2006). *Advance in Eucalyptus Fibre Properties & Paper Products* (p. 6). Brazil. Retrieved from http://www.eucalyptus.com.br/icep03/30Foelkel.text.pdf

Foelkel, C. (2007). *The Eucalyptus Fibres and the Kraft Pulp Quality Requirments for Paper Manufacturing* (p. 42). Brazil: Eucalyptus Online Book & Newsletter.

Foelkel, C. (2009). *Papermaking Properties of Eucalyptus Trees , Woods , and Pulp Fibres*. *Eucalyptus Online Book* (p. 110). Brazil: Eucalyptus Online Book & Newsletter.

Francesco, C., Michela, Z., Elide, F., Mario, T. and Fiorella, L. S. (2003). Cytokinins: new apoptotic inducers in plants. *Planta*. 216(3): 413–21.

Gan, S., & Amasino, R. M. (1995). Inhibition of leaf senescence by autoregulated production of cytokinin. *Science*. 270: 1986–1988.

George, E. F. (1993). *Plant Propagation By Tissue Culture Part 1 . The Technology*. U. K: Exergetics Ltd.

Girijashankar, V. (2012). *In vitro* regeneration of *Eucalyptus camaldulensis. Physiology and Molecular Biology of Plants*. 18(1): 79–87.

Giulini, A., Wang, J., & Jackson, D. (2004). Control of phyllotaxy by the cytokinin-inducible response regulator homologue ABPHYL1. *Nature*. 430: 1031–1034.

Gomes, F., & Canhoto, J. M. (2003). Micropropagation of *Eucalyptus nitens* maiden (Shining gum). *In Vitro Cellular & Developmental Biology - Plant*. 39(3): 316–321.

Goodger, J. Q., Heskes, A. M., King, D. J., Gleadow, R. M., & Woodrow, I. E. (2008). Micropropagation of *Eucalyptus polybractea* selected for key essential oil traits. *Functional Plant Biology*. 35: 247–251.

Gopinath, R. K., Nagaraja, P. A., & Nagendra, H. R. (2008). The effect of pyramids on preservation of milk. *Indian Journal of Traditional Knowledge*. 7(2): 233–236.

Hartmann, H. T., Kester, D. E., & Davies, J. F. (1990). *Plant Propagation Principles and Practices* (5th ed., p. 727). New Jersey: Prentice Hall.

Hirakawa, H., Yasukazu, N., Takakazu, K., Sachiko, I., Hiroe, S., Tomohiko, K., Sato, S. (2011). Survey of the genetic information carried in the genome of *Eucalyptus camaldulensis*. *Plant Biotechnology*. 28(5): 471–480.

Hofman, M., & Anne, J. (2006). *Plant Tissue Culture Engineering*. (S. D. Gupta & Y. Ilbaraki, Eds.) (6th ed., p. 469). Dordrecht, The Netherlands: Springer.

Joshi, I., Bisht, P., Sharma, V. K., & Uniyal, D. P. (2003). *In vitro* clonal propagation of mature Eucalyptus F 1 hybrid (*Eucalyptus tereticornis* Sm . x *E . grandis* Hill ex . Maiden). *Silvae Genetica*. 52(3-4): 110–113.

Kakimoto, T. (2003). Perception and signal transduction of cytokinins. *Annual Review Plant Biology*. 54: 605–627.

Kasmani, J. E., Nemati, M., Samariha, A., Chitsazi, H., Mohammadi, N. S., & Nosrati, H. (2011). Studying the effect of the age in *Eucalyptus camaldulensis* species on wood chemical compounds used in pulping process. *American-Eurasian Journal of Agricultural & Environmental Sciences*. 11(6): 854–856.

Kataria, N., Yadav, K., Kumari, S., & Singh, N. (2013). Micropropagation : An important tool for conserving forest trees. *Pertanika Journal for Tropical and Agricultura Science*. 36(1): 1–26.

Kavitha, K. N. (2009). *Biology and Management of Eucalyptus gall wasp, Leptocybe invasa Fiher & La Salle (Hymenoptera: Eulophidae)*. Unpublished Master dissertation, University of Agricultural Sciences, Dharwad, India.

Kawaoka, A., Nanto, K., Ishii, K., & Ebinuma, H. (2006). Reduction of lignin content by suppression of expression of the LIM domain transcription factor in *Eucalyptus camaldulensis*. *Silvae Genetica*. 55(6): 269–277.

Kiaei, M. and Samariha, A. (2011). Fibre dimensions , physical and mechanical properties of five important hardwood plants. *Indian Journal of Science and Technology*. 4(11): 1460-1463.

Kröger, M. (2012). *Global tree plantation expansion : a review* (p. 24). Netherlands.

Kubo, M., Furuta, K., Demura, T., Fukuda, H., Liu, L., Shibata, D., & Kakimoto, T. (2011). The CKH1/EER4 gene encoding a TAF12-like protein negatively regulates cytokinin sensitivity in *Arabidopsis thaliana*. *Plant & Cell Physiology*. 52(4): 629–637.

Kumar, I. R., Swamy, N. V., & Nagaendra, H., R. (2005). Effect of pyramid on microorganisms. *Indian Jouranl of Traditional Knowledge*. 4(4): 373-379

Kumar, I. R., & Nagendra, H. R. (2011). Pyramids and their shapes effect. *Journal of Arts, Science & Commerce*. 2(2): 195–201.

Kyte, L., & Kleyn, J. (2003). *An Introduction to Micropropagation* (p. 240). Oregon: Timber press.

Le Roux, J. J., & Staden, J. Van. (1991). Micropropagation and tissue culture of Eucalyptus-a review. *Tree Physiology*. 9(4): 435–77.

Lucía, C. V., Díaz-Maroto, M., Emilia, G., & Soledad, P. (2006). Analysis of volatile compounds of Eucalyptus honey by solid phase extraction followed by gas chromatography coupled to mass spectrometry. *European Food Research and Technology*. 224(1): 27–31.

Mahonen, A. P., Bishopp, A., Higuchi, M., Nieminen, K. M., Kinoshita, K., & Tormakangas, K. (2006). Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science*. 311: 94–98.

Map Website http://git-forestry-blog.blogspot.com/2009/09/eucalyptus-global-map-2009-cultivated.html

Matsumoto-Kitano, M., Kusumoto, T., Tarkowski, P., Tsujimura, K. K., Vaclavikova, K., & Miyawaki, K. (2008). Cytokinins are central regulators of cambial activity. *Proceedings of the National Academy of Sciences*. 105: 20027–20031.

Medford, J. I., Horgan, R., El-Sawi, Z., & Klee, H. J. (1989). Alterations of endogenous cytokinins in transgenic plants using a chimeric iso- pentenyl transferase gene. *Plant Cell*. 1: 403–413.

Michael, R. (2013). *Healing Pyramid Energy*. (P. Leach, Ed.) (p. 100). Virginia Beach, USA.: Russ Michael Books.

Mullins, K. V., Llewellyn, D. J., & Hartney, V. J. (1997). Regeneration and transformation of *Eucalyptus camaldulensis*. *Plant Cell Reports*. 16: 787–791.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*. 15: 473–497.

F. A. O. of the U. (2012). *Global Forest Products Facts and Figures* (pp. 1–17).

Nevill, P. G., Bossinger, G., & Ades, P. K. (2010). Phylogeography of the world's tallest angiosperm , *Eucalyptus regnans* : evidence for multiple isolated Quaternary refugia. *Journal of Biogeography*. 37: 179–192.

Nieminen, K., Immanen, J., Laxell, M., Kauppinen, L., Tarkowski, P., & Dolezal, K. (2008). Cytokinin signaling regulates cambial development in poplar. *Proceedings of the National Academy of Sciences*. 105: 20032–20037.

Nordahlia, A. S., Ani, S., Zaidon, A., & Hamami, S. M. (2011). 10-year-old sentang (*Azadirachta excelsa*) planted from rooted cutting and seedlings, 23(2): 222–227.

Nourissier, S., & Monteuuis, O. (2008). *In vitro* rooting of two *Eucalyptus urophylla* X *Eucalyptus grandis* mature clones. *In Vitro Cellular & Developmental Biology - Plant*. 44(4): 263–272.

Oluwafemi, O. A., & Sotannde, O. A. (2007). The relationship between fibre characteristics and pulp-sheet properties of *Leucaena leucocephala* (Lam .) De Wit. *Middle-East Journal of Scientific Research*. 2(2): 63–68.

Ouoimare, A., Philippe, B., Claire, K., & Thomas, G. (2000). Hormonal control of proliferation in meristemic agglomerates of *Eucalyptus camaldulensis* dehn. *In Vitro Cellular & Developmental Biology-Plant*. 36: 398–401.

Peh, T. B., Wong, W. C., Low, C. K., & Khoo, K. C. (1986). The fibre morphology of some Malaysian hardwoods. *The Malaysian Forester*. 49(2): 160–175.

Poke, F. S., Potts, B. M., Vaillancourt, R. E., & Raymond, C. A. (2006). Genetic parameters for lignin , extractives and decay in *Eucalyptus globulus*. *Annals of Forest Science*. 63: 813–821.

Poke, F. S., & Raymond, C. A. (2006). Predicting extractives, lignin, and cellulose contents using near infrared spectroscopy on solid wood in *Eucalyptus globulus*. *Journal of Wood Chemistry and Technology*. 26(2): 187–199.

Prakash, M. G., & Gurumurthi, K. (2010). Effects of type of explant and age, plant growth regulators and medium strength on somatic embryogenesis and plant regeneration in *Eucalyptus camaldulensis*. *Plant Cell, Tissue and Organ Culture*. 100: 13–20.

Rao, R. D., Sujatha, M., & Hemavathi, T. R. (2003). Variation in basic density and anatomical properties of *Eucalyptus tereticornis* clones. *Journal of Tropical Forest Products*. 9(1&2): 59–67.

Roberson, D., Quisen, R., Cleusa, B., & Quoirin, M. (2010). Plant regeneration from cotyledonary explants of *Eucalyptus camaldulensis* Dehn and histological study of organogenesis *in vitro*. *Brazilian Archives of Biology and Thecnology*. 53(2): 311–318.

Sadegh, A. N., & Kiaei, M. (2011). The Within -tree variation in basic density and fibre lenght of the *Eucalyptus camaldulensis* Dehnh wood. *World Applied Sciences Journal*. 13(5): 1042–1046.

Sakakibara, H. (2006). Cytokinins: activity, biosynthesis, and translocation. *Annual Review Plant Biology*. 57: 431– 449.

Shani, E., Yanai, O., & Ori, N. (2006). The role of hormones in shoot apical meristem function. *Current Opinion in Plant Biology*. 9(5): 484 – 489.

Sharma, S. K., & Ramamurthy, V. (2000). Micropropagation of 4-year-old elite *Eucalyptus tereticornis* trees. *Plant Cell Reports*. 19: 511–518.

Sherzad, O. (2003). *The Study of Morphological Variation Phenomenon of Eucalyptus camaldulensis Dehnh. Trees in Erbil City/ Iraq.* Unpublished doctoral dissertation, University of Salahaddin, Erbil.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Expermental Biology*. 11: 118–131.

Smook, G. A. (1982). *Handbook for Pulp and Paper Technologist, Joint Executive Committee of Vocational Education Committees of Pulp and Paper Industry* (p. 359). Montreal.

Sotelo, M., & Monza, J. (2007). Micropropagation of *Eucalyptus maidenii* elite trees. *Agrociencia*. 11: 81– 89.

Stape, J. L., Binkley, D., & Ryan, M. G. (2004). *Eucalyptus production and the supply, use and the efficiency of use of water, light and nitrogen across a geographic gradient in Brazil* (pp. 1–17). Pp: 1-17.

Tanvir, M. A., Siddiqui, M. T., & Shah, A. H. (2002). Growth and price trend of *Eucalyptus camaldulensis* in central Punjab. *International Journal of Agriculture & Biology*. 4(3): 344–346.

Thomas, J. G. (2012). *Power of Pyramid Energy* (1st ed., p. 86). Bengaluru, India: Pyramid Valley International.

Thomas, T. D. (2008). The role of activated charcoal in plant tissue culture. *Biotechnology Advances*. 26(6): 618–631.

Thorpe, T. A. (1994). *Morphogenesis and regeneration*. (T. Vasil, I. K. and Thorpe, Ed.)*Morphogenesis and regeneration* (pp. 17–36). The Netherlands: Kluwer academic Publisher.

Veenin, T., Fujita, M., Nobuchi, T., & Siripatanadilko, S. (2005). Radial variations of anatomical characteristics and specific gravity in *Eucalyptus camaldulensis* clones. *International Associaction of Wood Ananotmists Journal*. 26(3): 353–361.

Watt, M. P., Berjak, P., Makhathini, A., & Blakeway, F. (2003). In vitro field collection techniques for Eucalyptus micropropagation. *Plant Cell, Tissue and Organ Culture*. 75: 233–240.

West, T. P., & Preece, J. E. (2006). Use of acephate, benomyl and alginate encapsulation for eliminating culture mites and fungal contamination from *in vitro* cultures of hardy hibiscus (*Hibiscus moscheutos* L.). *In Vitro Cellular & Developmental Biology - Plant*. 42(3): 301–304.

Whitehouse, A. B., Marks, T. R., & Edwards, G. A. (2002). Control of hyperhydricity in Eucalyptus axillary shoot cultures grown in liquid medium. *Plant Cell, Tissue and Organ Culture*. 71: 245–252.

Woodward, A. J., & Bennett, I. J. (2005). The effect of salt stress and abscisic acid on proline production, chlorophyll content and growth of *in vitro* propagated shoots of *Eucalyptus camaldulensis*. *Plant Cell, Tissue and Organ Culture*. 82(2): 189–200.

Yao, L., Jiang, Y., Singanusong, R., Datta, N., & Raymont, K. (2004). Phenolic acids and abscisic acid in Australian Eucalyptus honeys and their potential for floral authentication. *Food Chemistry*. 8: 169–177.

Yasodha, R., Sumathi, R., & Gurumurthi, K. (2004). Micropropagation for quality propagule production in plantation forestry. *Indian Journal of Biotechnology*. 3: 159–170.

Yavuz S., & Malik, M. A. (1980). The effect of stump height and number of shoots per stump on the stump coppicing power and growth and yield of *Eucalyptus camaldulensis* growth as irrigation plantations in Northern Iraq. *Journal of the Faculty of Forestry, Istanbul University*. 29(1): 1–13.

Yokoi, H., Ishida, Y., Ohtani, H., Tsuge, S., Sonoda, T., & Ona, T. (1999). Characterization of within-tree variation of lignin components in *Eucalyptus camaldulensis* by pyrolysis–gas chromatography. *The Analyst*. 124(5): 669–674.