



UNIVERSITI PUTRA MALAYSIA

***EFFECT OF DIFFERENT CONCENTRATION OF KINETIN ON AXILLARY
SHOOT PROLIFERATION OF F1 HYBRID GREEN OKRA
(Abelmoschus esculentus L.)***

ANNA ARINA BT AB. HALIM

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BY:

ANNA ARINA BT AB. HALIM

(155951)

A project report submitted to the Faculty of Agriculture

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In fulfilment of the requirement for PRT 4999 (Project)

For the award of the Degree of Bachelor of Agriculture Science

DEPARTMENT OF AGRICULTURE TECHNOLOGY

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2012/2013

CERTIFICATION FORM

This project paper entitled The Effect of Different Concentration of Kinetin on Axillary Shoot Proliferation of F1 Hybrid Green Okra (*Abelmoschus esculentus L.*), prepared by Anna Arinabt Ab. Halim (155951) in partial fulfilment of the requirement of PRT 4999 (Project) for the award of the Degree of Bachelor of Agriculture Science.

Submitted by,

ANNA ARINA BT AB.HALIM

Signature,

.....

Certified by,

.....

En.Azmi b. Abdul Rashid
Project Supervisor,
Department of Agriculture Technology
Faculty of Agriculture
University Putra Malaysia
Serdang, Selangor.

Date :



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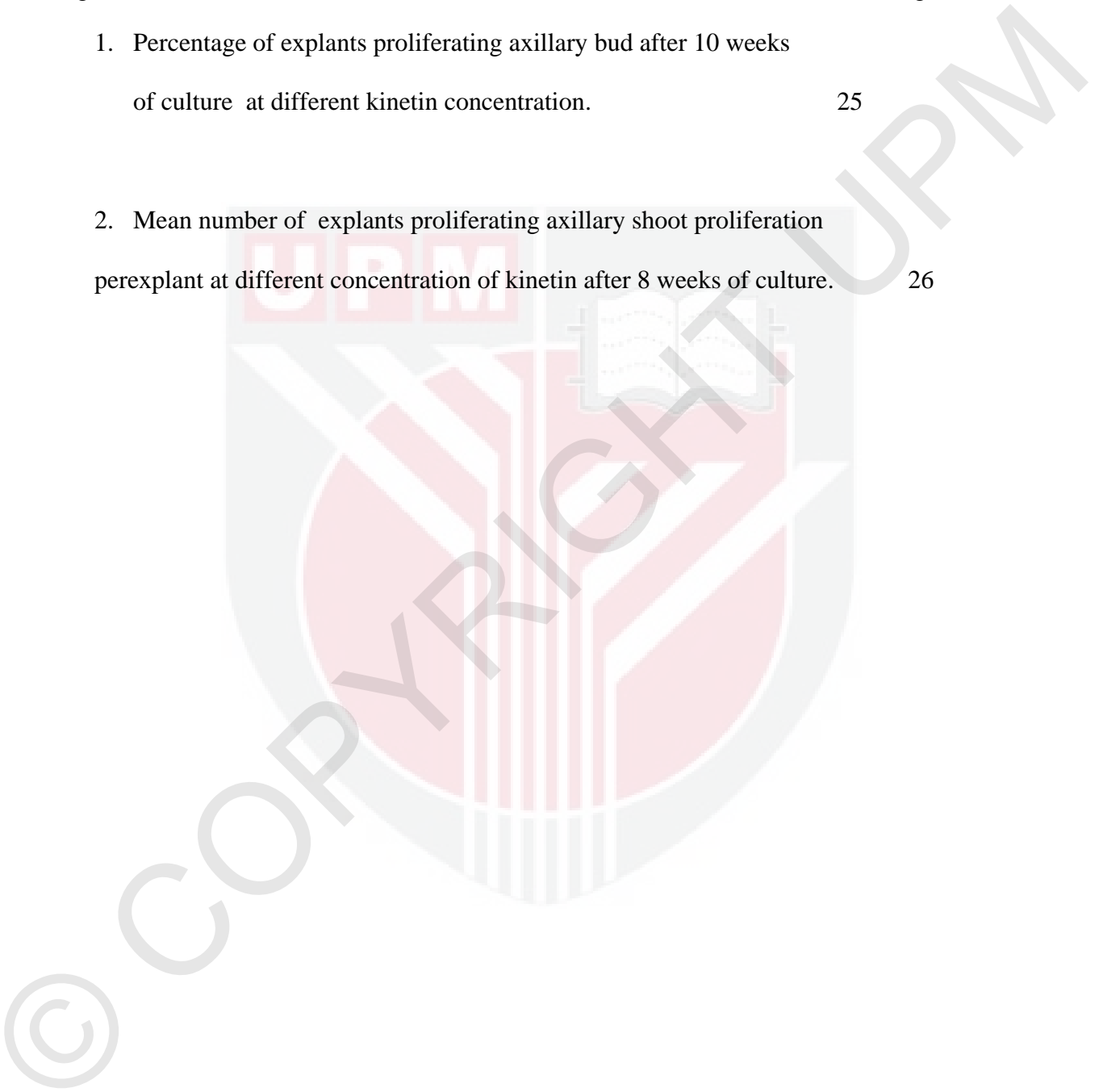
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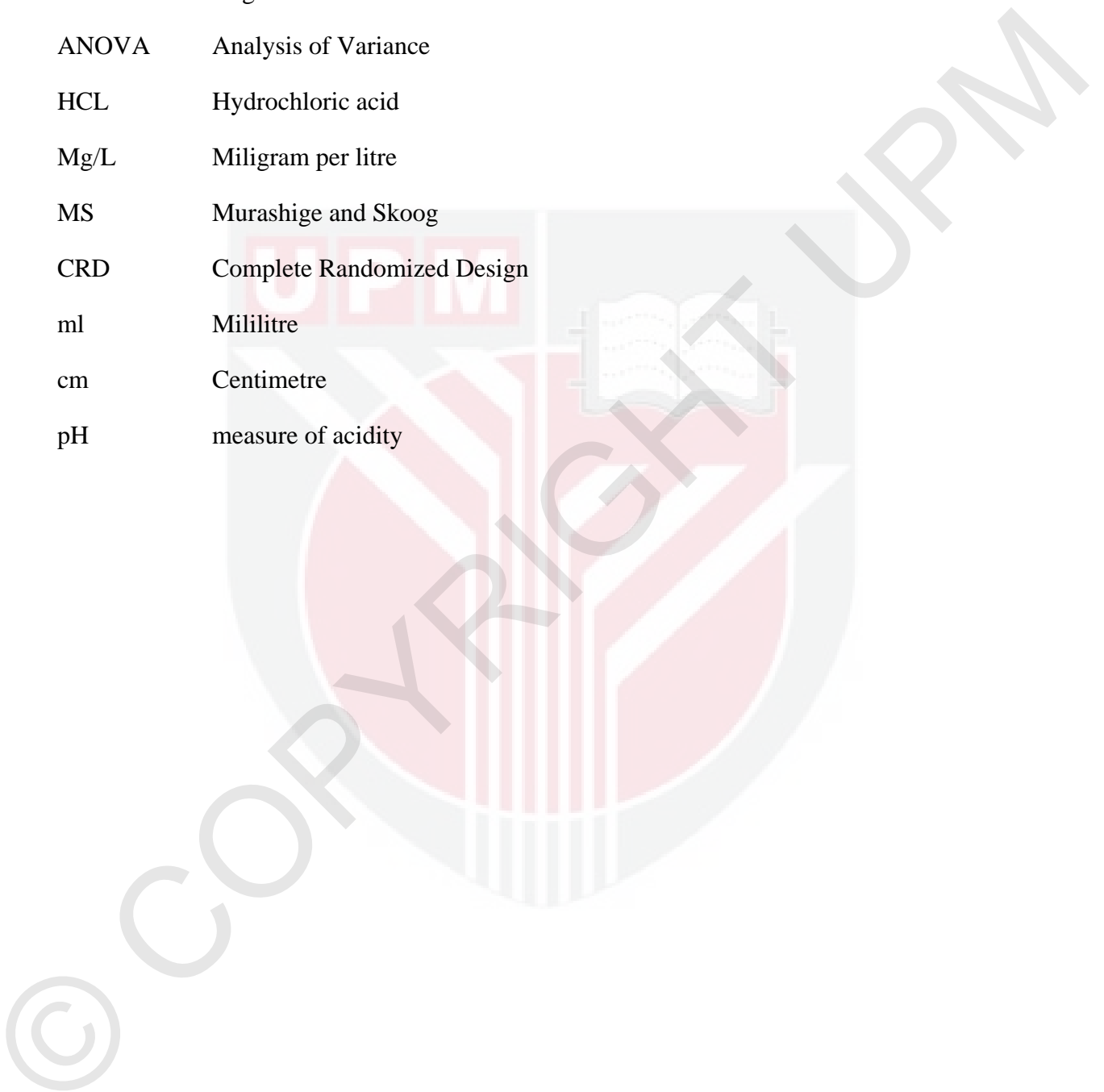
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LIST OF ABBREVIATION

| | |
|-------|----------------------------|
| % | Percentage |
| °C | Degree Celcius |
| ANOVA | Analysis of Variance |
| HCL | Hydrochloric acid |
| Mg/L | Miligram per litre |
| MS | Murashige and Skoog |
| CRD | Complete Randomized Design |
| ml | Mililitre |
| cm | Centimetre |
| pH | measure of acidity |



ABSTRACT

Abelmoschus esculentus L., belongs to the Malvaceae family is one of the important vegetable crop in the world. This study was carried out at the Tissue Culture Laboratory, Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The aim of the experiment is to determine the effect of different concentration of kinetin on axillary shoot proliferation of F1 hybrid green okra. The experiment was designed using Randomized Complete Block Design (RCBD). In the experiment, shoot tips of the germinated F1 hybrid green okra were cultured in medium containing 5 different concentration of kinetin (0.0, 1.0, 2.5, 5.0 and 10.0 mg/L). It was observed that a high percentage of explants regenerating shoot occurred ranging from 60% to 90% within the kinetin concentration tested. However, no significant difference was observed between all the kinetin treatment tested compared with the control treatment.

ABSTRAK

Abelmoschus esculentus L., tergolong dalam famili Malvaceae dan ia merupakan salah satu tanaman sayuran yang terpenting di dunia. Kajian ini telah dijalankan di Makmal Kultur Tisu, Jabatan Teknologi Pertanian, Fakulti Pertanian, Universiti Putra Malaysia, Serdang, Selangor. Tujuan utama kajian ini dijalankan adalah untuk mengkaji kesan perbezaan kepekatan kinetin terhadap penggandaan pucuk bagitanaman F1 hibrid bendihijau. Reka bentuk eksperimen bagi kajian ini ialah Reka bentuk Penuh Rawak Berblok (RCBD). Dalam kajian ini, mercupucuk F1 hibrid bendihijau daripada pencambahan biji benih dikultur di dalam media yang mempunyai 5 kepekatan hormone kinetin yang berbeza (0.0, 1.0, 2.5, 5.0, dan 10.0 mg/L). Dari kajian tersebut, didapati peratus yang tinggi eksplan regenerasi tunas diperolehi pada kadar 60% hingga 90% pada kepekatan kinetin yang diuji. Walaubagaimanapun tiada perbezaan beerti diperolehi antara kepekatan kinetin yang diuji berbanding rawatan kawalan.

CHAPTER 1

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.)) is an important vegetable crop in the world. It is also known as lady's fingers and some people called it as bhindi (Hindi) or gumbo (Spanish). Okra is a flowering plant that belongs to the Malvaceae family. Its origin is from the Ethiopian region of Africa. However, it is now widely cultivated in other parts of Africa like Sudan and Egypt and also in other parts of the world like Malaysia (Singh et al, 2004)

In Malaysia, okra is widely used in cooking either as modern style or traditional style food. The product from the plant is mucilaginous and contains soluble fibre. Besides that, it also contains high nutrient composition. The plant provides high fibre, vitamin C and folate. Therefore, it is also widely used in health products. Okra is also known for being high in antioxidants and a good source of calcium and potassium (<http://en.wikipedia.org/Okra>)

Okra can be grown as a warm season crop throughout the tropics. It is a short day plant which requires a minimum day temperature of 25°C. It is grown as an annual crop but sometimes as a perennial crop according to the requirement. This plant is a flowering plant which can grow up to 2 meters tall. It is also slightly tolerant to acid conditions but grows best at pH between 6.0 to 6.8 (Singh et al 2004)

Worldwide production of okra as fruit vegetables is estimated at six million tonnes per year. In West Africa, okra production is estimated at 500 000 to 600 000 tonnes per year. The large amount of the production shows the high demand of this plant (Burkill,1997)

The cultivation of okra is mostly by seed. The seeds that are widely available in the market is the non hybrid type. Hybrid seed are very expensive and rarely available. Production of this plant using sexual approach will result in segregation of the genotype at the F₂ generation. Therefore, asexual propagation via *in vitro* approach will result in production of uniform and true to type planting materials of the F₁ hybrid plant of okra. Currently not much has been reported on *in vitro* cultured of okra.

Therefore, this experiment is conducted with the objective to determine the best kinetin concentration on shoot regeneration from shoot tip explants of F₁ hybrid plant of green okra.

REFERENCES

Ariyo OJ (1990). Variation and heritability of fifteen characters on okra (*Abelmoschus esculentus*(L.) Moench). Trop. Agric. 67:213-216.

Beyl. C.A, (2005), Getting started with Tissue Culture: Media Preparation, Sterile technique, and Laboratory Equipment. In: Trigiano, R.N and Gray J.D. (Ed.) Plant Development and Biotechnology, CRC Press, Boca Raton, Washington, pp.19-37

Bonga, J.M, and Aderkas, P.V. 1992. In Vitro Culture of Forest. Kluwer academic Publisher, Dordrecht, Bohnson, London. pp 12-17

Burkill, H.M.(1997). The useful plant of West Tropical Africa. 2nd edition. Vol 4, families M-R, Royal Botanic Garden, Kew. United Kingdom. pp 969.

Caponetti, J.D, Gray J.D, and Trigiano R.N. (2005). History of Plant Tissue Culture and Cell Culture. In: Trigiano R.N and Gray J.D. (Ed.). Plant Development and Biotechnology, CRC Press, Boca Raton, Washington. Pp 9.

Campbell, R. 1985. Plant Microbiology. Arnold, London. pp 191

Cassel, A.C. 1990. Problem in Tissue Culture Contamination. In: Debergh, P.C. and Y.P.S.(Ed.) Biotechnology in Agriculture and Forestry , Springer-Verlag,Berlin.

Chandra R. and Mishra M. (2003).Micropropagation of Horticulture Crops. 1stedition.International Book distributing.Co, Charbagh,India.

Collin, H.A., and Edwards, S. (1998). Plant Cell Culture, Bios Scientific publisher, Oxford, UK.Pp 11-37.

Debergh P.C &Maena L.J. (1981) A scheme for the commercial propagation of ornamental plants by tissue culture. Sci.Hortic. 14,335-345'

Debergh,P.C., and Read, P.E.(1991). Micropropagation. In: Debergh P.C., and Zimmerman, R.H (Ed.) Micropropagation Technology and application. Kluwer academic Publisher, Dordrecht, Boston, London. pp.1-14

Debergh,P.C.(1987). Improving micropropagation. IAPTC News1.51: 2-10.

Dodds,J.H., and Roberts,L.W. (1995). Experiments in Plants Tissue Culture third edition.Cambridge University Press, Cambridge, New York.Pp 19-66

Duzyaman, E. (1997). Okra: Botany and Horticulture. In J. Janick, *Horticulture review* (pp. 42-49). John Wiley & sons.

Edet, G.E and Etim,N.A. (2010). ECONOMIC ANALYSIS OF OKRA PRODUCTION: A CASE OF IVO LOCAL GOVERNMENT AREA OF EBONYI STATE. *Nigerian Journal of Agriculture* , 1-5.

Fasheum, A.(1988). Soil temperature management for optimal seedling emergence in *A. Esculentus* and *C. Olitorius*. *Int. Agrophysics*, 4:333-338

Gaba, V.P, (2005). Plant Growth Regulators in Plant Tissue Culture and Development..In: Trigiano, R.N and gray J.D. (Ed.) *Plant Development and Biotechnology*, CRC Press,Boca Raton, Washington,pp.87-100

George E.F and Debergh P.C.(2008). Micropropagation:Uses and Method. In:George E.F, Hall M.A and De Klerk G. (Ed.) *Plant Propagation by Tissue Culture*.3rd edition.Volume 1.Springer. Dordrecht. The Netherland.pp.29-60

George, E.F. (1993). *Plant Propagation by Tissue Culture, Part 1: The Technology*.Exegetics, Ltd., London.

George R.A.T. (2009).*Vegetables Seed Production*.3rd edition.CABI,Cambridge,USA.

[http:// www.natural-medicinal-herbs.com/herbs/kinetin.htm](http://www.natural-medicinal-herbs.com/herbs/kinetin.htm), 11 March 2013

Hamon, S and D.H.VanSloten, 1989.Characterisation and evaluation of okra.p.173-196. IN; A.H.D. Brown, O.H.Frankel, D.R.Marshall, and J.T.William (eds)., The use of plant genetic resources. Cambridge Univ.Press, Cambridge.

Hartman, H.T, Kester, D.E, Geneve,R.L and Davices, F.T (2001).Problem in Tissue Culture In: Hartman and Kesters (Ed.) Hartman and Kester's Plant Propagation Principle,7th edition. Prentice Hall.

Hesar A.A, Kaviani B., Tarang T, and Zanjani S.H. (2011). Effect of differeny concentration of kinetin in regeneration of ten weeks (*Matthiola incana*). *Plant omics journal* , 236-238.

Hudges,K.W.(1981). Ornamental species. In: George, B.V (ed) Cloning Agriculture Plants Via In Vitro technique. CRC Press, Boca Raton, F1, pp.5-3

Hussey, G.(1983). In vitro propagation of horticultural and agricultural crop. In: Mantell, S.H. and smith, H.,(ed) Plant Biotechnology. Cambridge University Press, Cambridge. Pp 111-138

Hyperhydricity.<http://en.wikipedia.org/wiki/Hyperhydricity>. 8 March 2013

Jain S.M, Ochatt SJ (2010). Protocols for *in vitro* propagation of ornamental plants. Springer protocols. Hamana press.

Joshi A.B, gadwall V.R., and Hardas M.W., (1974). Okra, *Abelmoschus esculentus* (Malvaceae), In Hutchinson J.B (ed.). Evolutionary studies in world Crops. Diversity and change in the Indian subcontinent. Cambridge 99-105.

J.N. Buah, E. Danso, K.J. Taah, E.A. abole, E.A. bediako, J. asiedu and R. Baidoo. (2010). The Effect of Different Concentrations Cytokinins on the *in vitro* Multiplication of Plantain (*Musa* sp). *Biotechnology*, 1-5.

Kane, M.E, (2005). Shoot Culture Procedures. In: Trigiano, R.N and gray J.D. (ed) Plant Development and Biotechnology, CRC Press, Boca Raton, Washington, pp. 145-158.

Kurtz, S., R.D. Hartmann, and I.Y.E. Chu. (1991). Current methods of commercial micropropagation. In: Scale Up and Automation in Plant Propagation, Volume 8: Cell Culture and Somatic Cell Genetics of Plants (ed.) I.K. Vasil. Academic Press, San Diego. 7-

Kyte, L. and Kleyn, J. (2003). An introduction to Micropropagation. Timber Press, Oregon. 240pp

Leopald, AC. (1987). Contemplation on Hormone as Biological Regulator. In: Hoad G.V., Lenton, J.R., Jackson, M.B., and Atkon, R.K. (Ed.). Hormone Action in Plant Development, A critical Paaraisal ,Butterworths, London. pp 3-15.

Martin, F.W., and A.M. Rhodes.(1983). Seed characteristics of okra and related *Abelmochusspecies* .QualitasPlanntarum, Plant Foods Human Nutr. 33:41-49

M.Genesan, R.Chandrasekar, B.D.Ranjitha Kumari and N.Jayabalan. (2007). Somatic embryogenesis and plant regeneration of *Abelmochus esculentus* through suspension culture. *Biology plantarium* , 414-418

Michael E.K. (2005). Shoot Culture Procedure, In: Debergh P.C., and Zimmerman, R.H (ed.) Micropropagation Technology and Application. Kluwer Academic Publisher, Dordrecht, Boston, London.pp.1-14

Miller C.O, Ortiz J, and Jua'rez J.(1985). Aberrant citrus plant obtained by somatic embryogenesis of nucellus cultured in vitro. Hortscience 20:214-215

More, D.C., and U.D.Vibhute.(1983). Inheritance of pigmentation in okra. J. Maharashtra Agr. Univ. 8: 137-140

M.T Rahman, M.J Hossain and M. Khalekuzzaman. (2008). In Vitro Indirect Plantlet regeneration From Hypocotyl segments and Cotyledonary explant Derived Calli in Lady's Finger(*Abelmoschus esculentus* L. Monech). *J.bio-sci* , 50-57.

Murashige,T.(1974).Plant Propagation through Tissue Culture. *Annu.Rev.Plant.Physiol.*25:135-66.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays tobacco tissue culture. *Physiol Plant* 15: 473-497

Muruges, C. Subathra Devi, V. MohanaSrinivasan, N. Mahesh and S. Vijayalakshmi School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur-613 402, Tamilnadu. Bacterial and Fungal Contamination in Plant Tissueculture Laboratory: A Guide to Recognition Andmanagements. <http://www.aseanbiotechnology.info>. 8 March 2013.

Okra: <http://en.wikipedia.org/Okra> 19 February 2013

Possingham, J.V.(1990) .Under-exploited wild species that have potential for horticulture. *Adv. Hort. Sci.* 4: 49-55

Purohit S.S. (1998). *Agricultural Boitechnology*. Agro Botanica, Bikaner, India.

Purseglove J.W (1968). Tropical Crops Dicotyledons. Longman Group limited.pp.368-370

Quagliotti,L., and A. Lotito. (1989).Un ortaggiomuovo per I'Italia: I'okra.InformatoreAgrario 45:59-65

Read, P.E.1988. stock plants influence micropropagation success. Acta. Hort. 226:41-52.

Rao,S.R.S.(1991). Structure and distribution of plant trichomes in relation to taxonomy : Hibiscus L. feddesRepertorium 102:335-334

Rout, G.R., Samantaray, S., and Das, P.(2000). *In vitro* manipulation and propagation of medical plants.Biotechnol. Adv. 18:19-120

Sanjeet Kumar, Sokona Dagnoko, adamou Haougui, Alain Ratnadass, Dov Pasternak and Christophe Kouame. (2010). Okra (abelmoschus spp) in west and Central Africa: Potential and progress on its improve. *African Journal of Agricultural research* .3

Siemonsma,J.S. (1982b). West African Okra.morphological and cytological indications for the existence of natural amphidiploids of *Abelmoschus esculentus* (L.) Moench and *A.manihot* (L.)Medikus, *Euphytica* 31: 241-242,

Singh, P.K., Dasgupta, S.K. and Tripathi, S.K.(2004). Hybrid Vegetables Development. Volume 6. Food Product Press. New York.

Skoog, F and C. Tsui. 1951. Growth substances and the formation of buds in plant tissue. In: Plant Growth Substances. (Ed). F. Skoog. University of Wisconsin Press, Madison, WI, 23-285

Skoog, F and C. O Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11:118-131

Stages of Micropropagation. <http://theagricos.com/tissue-culture/micropropagation/stages-of-micropropagation/> 28 February 2013.

Sutter, E. And Langhans, R.W.(1979). Epicuticular wax formation on carnation plantlets regenerated from shoot tip cultures. J. Amer Soc. Hortic. Sci. 104: 493-496

Tindall, H.D (1983). Vegetables in the Tropics. MACMILLAN EDUCATION L.T.D, Hampshire, London.

Torres, K.C.(1989). Tissue Culture techniques for Horticultural Crops. New York: Van Nostrand Reinhold.

Trigianno, R.N. and Gray, D.N.2011. Plant Tissue Culture Development and Biotechnology.

CRC Press,Boca Raton. Fl.pp 289-230.



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