UNIVERSITI PUTRA MALAYSIA

SHOOT MULTIPLICATION FROM SHOOT TIP OF F1 HYBRID OKRA (Abelmoschus esculentus) BY DIFFERENT CONCENTRATION OF 6-BENZYLAMINOPURINE (BAP)

NORSAFINA BINTI MOHD AMIROL

FP 2017 6

SHOOT MULTIPLICATION FROM SHOOT TIP OF F1 HYBRID OKRA (*Abelmoschus esculentus*) BY DIFFERENT CONCENTRATION OF 6-BENZYLAMINOPURINE (BAP)

NORSAFINA BINTI MOHD AMIROL

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2016/2017

SHOOT MULTIPLICATION FROM SHOOT TIP OF F1 HYBRID OKRA (*Abelmoschus esculentus*) BY USING DIFFERENT CONCENTRATION OF 6-BENZYLAMINOPURINE (BAP)

BY:

NORSAFINA BINTI MOHD AMIROL

(175157)

This project submitted to Faculty of Agriculture,

Universiti Putra Malaysia,

In fulfillment of the requirement of PRT4999 (Final Year Project),

Bachelor of Horticultural Science.

DEPARTMENT OF AGRICULTURAL TECHNOLOGY

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2016

CERTIFICATION

This project entitled **Shoot Multiplication From Shoot Tip Of F1 Hybrid Okra (*Abelmoschus Esculentus*) By Using Different Concentration Of 6-Benzylaminopurine (BAP**) is prepared by **Norsafina binti Mohd Amirol (175157)** and submitted to the Faculty of Agriculture in partial fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

Student's name, Student's signature,

NORSAFINA BINTI MOHD AMIROL .....

Certified by,

_____

(EN. AZMI BIN ABDUL RASHID)

Project Supervisor,

Department of Agricultural Technology,

Faculty of Agriculture,

Universit Putra Malaysia.

Date : .....

ACKNOWLEDGEMENT

In the name of Allah the Most Graciuous and the Most Merciful, Alhamdulillah, my great thanks goes to Allah SWT whose blessings have enabled me to accomplish my research project successfully. Without an iota of a doubt, everything, and we reiterate, absolutely everything that happens in this universe, without exception, happens with the precise Will and Permission of the Allah Most Majestic, Most Supreme, Most High, Most Exalted.

Firstly, I would like to express my utmost appreciation to all individuals who contributed to this project especially my supervisor, Mr. Azmi bin Abdul Rashid for his continuous guidance, great concern, valuable comments, advises and suggestions from the beginning till the end of this study.

I would like to thank all the academic staff in the Faculty of Agriculture (UPM) for their dedication in delivering knowledge and contribution towards research in the Faculty. Next, a very special thank to postgraduate students and laboratory staffs including Miss Siti Raziah and Mrs. Rohani who gave me advices and encouragement to complete the research project.

Finally, my deepest thank goes to both my parents Mohd Amirol bin Hamid and Saidah binti Inis and siblings for their continuous understanding, support and sacrifice throughout the period of my study. Sincere thanks are also extended to my friends for their support and help during this study.

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Varience
BAP	6-Benzylaminopurine
et al.	Et alia
HCl	Hydrogen Chloride
Kn	Kinetin
MS	Murashige and Skoog
NAA	1-Naphthaleneacetic acid
NH_4	Ammonium
NO_3	Nitrates
NaOH	Sodium Hydroxxide
pH	Hydrogen ion concentration/-log(H+)
PGR	Plant Growth Regulator
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
v/v	Volume per volume
2iP	2-Isopentenyladenine

LIST OF APPENDICES

ABSTRACT

Okra (*Abelmoschus esculentus* L. Moench) belongs to Malvaceae family is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This research was conducted to determine the best 6-benzylaminopurine (BAP) for shoot multiplication from shoot tip explants of F1 hybrid okra (*Abelmoschus esculentus*). Seeds of F1 hybrid okra were germinated on Murashige and Skoog (MS) medium without addition any of plant growth regulator. In this study, shoot tips were excised from the 2 weeks old germinated seedlings and then cultured vertically in MS medium supplemented with different concentration of BAP (0 mg/L, 0.5mg/L, 1.0mg/L, 2.0mg/L, 5.0mg/L and 10.0mg/L). The media containing 2.0mg/L of BAP show the highest shoot multiplication of shoot tip culture of *Abelmoschus esculentus* with 7.6 shoot produced per explant after 5 weeks of culture.

ABSTRAK

Okra (Abelmoschus esculentus L. Moench) tergolong dalam keluarga Malvaceae adalah tanaman sayur-sayuran yang penting dari segi ekonomi dan ditanam di bahagian-bahagian tropika dan sub-tropika di dunia. Kajian ini dijalankan adalah untuk menentukan kepekatan yang terbaik daripada 6-benzylaminopurine (BAP) untuk pendaraban pucuk daripada eksplan hujung pucuk bagi hibrid F1 bendi (Abelmoschus esculentus). Benih bendi hibrid F1 telah dicambajkan pada media Murashige dan Skoog (MS) tanpa penambahan sebarang pengawal penggalak pertumbuhan tumbuhan. Dalam kajian ini, hujung pucuk akan dipotong daripada anak benih yang telah bercambah selama 2 minggu dan kemudian dikulturkan secara menegak dalam MS yang ditambahkan dengan perbezaan kepekatan BAP (0 mg / L, 0.5mg / L, 1.0mg / L, 2.0mg / L, 5.0mg / L dan 10.0mg / L). Media yang mengandungi 2.0mg / L BAP menunjukkan pendaraban pucuk yang tertinggi di dalam pelkulturan hujung pucuk Abelmoschus esculentus dengan 7.6 pucuk yang dihasilkan oleh setiap eksplan selepas 5 minggu dikulturkan.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Scientific classification:	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	*Abelmoschus*
Species	*A.esculentus*

Binomial name: *Abelmoschus esculentus*

Other Names: Kacang Bendi, Qiu kui, Okra, Okura, Okro, Quiabos, Ochro, Quiabo, Gumbo, Quimgombo, Bamieh, Bamya, Quingumbo, Bamia, Ladies Fingers, Bendi, Bhindi, Kopi Arab2

Okra (*Abelmoschus esculentus*) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In India, it ranks number one in its consumption but its original home is Ethiopia and Sudan, the north-eastern African countries. It is one of the oldest cultivated crops and presently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America. It is a tropical to subtropical crop and is sensitive to frost; low temperature, water logging and drought conditions, and the cultivation from different countries have certain adapted distinguishing characteristics specific to the country to which they belong (Naveed *et al.,* 2009).

It is an oligo purpose crop, but it is usually consumed for its green tender fruits as a vegetable in a variety of ways. These fruits are rich in vitamins, calcium, potassium and other mineral matters. The mature okra seed is a good source of oil and protein. It has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition. Its mature fruit and stems contain crude fibre, which is used in the paper industry.

Okra can be to propagated using sexual technique through the production of seeds (Ahmad *et al.,* 2003). Production of plants using seeds will produce plants which might be non-true to type because plants produced at the second filial generation usually segregate. Therefore, micropropagating the plant at the F1 generation are necessary to maintain the true to typeness of the F1 hybrid okra (Ahsan *et al*. 2003).

At present not much has been reported on shoot multiplication from shoot tip culture of *Abelmoschus esculentus* F1 hybrid. Thus, this experiment is carried out with the objective to determine the best BAP concentration in optimizing shoot proliferation from shoot tip explant of F1 hybrid okra.

REFERENCES

Ademoyegun O.T. (2009). Tissue Culture as a Plant Production Technique for Horticultural Crops. Afr. J. Biotechnol. 8(16): 3782-3788.

Ahmad, N. and Anis, M. (2005). *In vitro* Mass Propagation of *Cucumis sativus* L. From Nodal Gegments. Turk. J. Bot 29:237-240.

Ahmad, T., H.U. Rehman, C.M.S. Ahmed and M.H. Leghari. (2003). Effect of Culture Media and Growth Regulators on Micropropagation of Peach Rootstock GF 677. Pak. J. Bot., 35(3): 331-338.

Ahsan, K., Aman, R., Schieber, A., and Carle, R. (2003). Effects of BAP and Nutrient Salt Formulation on Micropropagation of Hardy Hibiscus (*Hibiscus moscheutos* L.). Acta Hortic 630:293–297.

Akbas K.M. (2007). Rapid *In vitro* Propagation of *Cucumis melo*. Plant Cell Tissue Organ Cult. 1991;26(3):167–71.

Akin-Idowu J.M. (2006). Effects of Nutrient Salt Formulations and PGR on Axilliary Shoot Proliferation of Tropical Hibiscus. Hortscience 41:1024.

Andras, C.D., Simandi, B., Orsi, F., Lambrou, C., Tatla, D.M., Domokos, J., & Doleschall. F. (2005). Supercritical Carbon dioxide Extraction of Okra (*Hibiscus esculentus* L.) Seeds. J. Sci. Food Agric., 85: 1415-1419.

Aswath, C.R. and Choudhary, M.L. (2002). Rapid Plant Generation from *Gerbera jamesonii* Bolus Callus Culture. Acta Bot Croat. 61:125-34.

Badji, H., Kaushal, N., Modgil, M. and Sharma, D.R (2003). Effects of Explant Source, Culture Medium: Strength and Growth Regulators on the *In-vitro* Propagation of *Acacia senegal*. Horticulture Science.

Barba, R.C., Zamora, A.B., Malion, A.K. and Linga, C.K. (1978). Sugarcane Tissue Culture Research. Proc. Int. Soc. Sugarcane Technol. 16:1843-1863.

Baskaran and Jayabalan (2005). The Effect of Different Plant Growth Regulators on Shoot Induction of *Andrographis paniculata*. Hormones.

Benniamin, M., Sheena, K., Jothi, G.J, Gray, D.N. and Tabata, M. (2004). Effect of 6 Benzyl Amino Purine (6—Bap) on *In vitro* Shoot Multiplication of *Wrightia religiosa*. International Journal, 5, 36-42.

Calisir, S., & Yildiz, M. U. (2005). A Study on Some Physico-chemical Properties of Turkeyokra (*Hibiscus esculenta*) Seeds. Journal of Food Engineering, 68, 73–78.

Chadha K.L (2002). Hand book of Horticulture, Indian Council of Agricultural Research. Okra., (27) pp 422-427.

Chalupa V (2008). Large Scale Micropropagation of *Quercus robur* L. Using Adenine Type Cytokinins and Thidiazuron to Stimulate Shoot Proliferation. Biol Plantarum 30:414.

Chawla, M (2003). Pathogen and Microbial Contamination Management in Micropropagation, (ed). Dordrecht: Kluwer Academic Publisher.

Chueh, M., Pastuszyn, A., Mounkaila, G., Glew, RS., Millson, M., and Glew, RH. (2001). Plant Regeneration from Stem Nodal Segment of *Clinacanthus nutans*, a Medicinal Plant. *In Vitro* Cellular and Developmental Biology – Plant, 40(1), 115—118.

Das Y.J., Trigiano R.N., May R.A. and Gray, D.J. (2006). Advances in Tissue Culture of *Anacardium occidentale* In: Dallos M.P. (Ed.)- Agricultural Biotechnology, a Focus on the Improvement of Plants.

Dave, M., Chandra, I., Singh, P., Bhattacharya, A., Singh, P. and Javed, S. (2003). Effect of Medium Strength and Hormone Concentration on Regeneration of *Chlorophytum borivillianum* Ssing Nodes Explant.pd.(n.d).

Dobranszki, J. And Teixeira da Silva, J.A. (2010). Micropropagation of Apple- A review. Biotech. Adv. 28(4):462-488.

Douglas G.C. (1989). Micropropagation of Floribunda, Ground Cover and Miniature Roses. Plant Cell Tis. Org. Cult. 19:55-64.

Elkonin and Pakhomova (2000). The Role of Nutrient Availability in Regulating Root Architecture. Curr. Opin. Plant Biol. 6:280—287.

Gaspar, T. (1996). Plant Hormones And Plant Growth Regulators In Plant Tissue Culture. In Vitro Cellular & Developmental Biology – Plant. Volume 32, Issue 4, pp 272-289.

George E.F (2000). Plant Propagation by Tissue Culture. Part 2. The practice, 2nd edn. Exegetics Limited, Edington, pp 1–1361.

George, E.F. and Debergh, P.C. (2008). Micropropagation: Uses and Method. In: George E.F., Hall M.A. and De Klerk G.J. editors. Plant Propagation by Tissue Culture. (3rd ed). Dordrecht. Netherlands: Springer. p. 29-64.

Gopalan. C., Rama Sastri. B.V. and Balasubramanian .S., (2007). Nutritive Value of Indian Foods, published by National Institute of Nutrition (NIN), ICMR.

Gupta, M., Ranalli, P., Bizzari, M., Bordhi, L. and Mari, M. (2000). Effect of Cytokinin Types and their Concentrations on Shoot Proliferation and Hyperhydricity in *In vitro* of Pear Cultivar Shoots. Plant Cell Tis. Org. Cult. 72:261-265.

Hamidullah Itoo M.K. (2013). Effect of BAP and IAA on Shoot Regeneration in Cotyledonary Explants of *Costa rican* Melon Genotypes; Agronomía Costarricense 33 125-131.

Heyl, B.H and Schmulling T.L (2003). Plant Growth Regulators in Plant Tissue Culture and Development, In Plant Develeopment Biotechnology. CRC Press.

Hochreutimer, B.P (1924). Centres of origin for family Malvaceae: Candolla 2:79

Howell , G., Douglas, D., Rutledge, C. and Richardson, D. H. H. (2003). Cytokinin : a Potent Regulator of *In vitro* Plant Morphogenesis. In Vitro Cell Dev-Pl 34:267–275.

Huang F.K., Rout, G.R., Palai, S.K., Pandey, P. and Dos P. (2004). Direct Plant Regeneration of *A. mearnsii* Influence of Explant Source, Age of Explant, Culture Environment, Carbohydrates Nutritional Factors and Hormone Regime, Proc. Nat. Acad. Sci. India, 67(8): 57-66.

Kane H (2003). Problems in Tissue Culture : Culture Contamination. In: Micropropagation: Technology and Application, Derbergh, PC and Zimmerman, RH (Eds), Kluwer Publishers, Dordrecht, pp. 31-44.

Kaushal N.M. (2005). *In vitro* Clonal Multiplication of an Apple Rootstock by Culture of Shoot Apices and Axillary Buds. Ind. Exp. Biol. 43:561-5.

Kenny, J.M. Puglia, D. Santulli, C. Sarasini, F. (2010). Morphological, Thermal and Mechanical Characterization of Okra (*Abelmoschus esculentus*) Fibres as Potential Reinforcement in Polymer Composites. Composites Science and Technology. 70 (1): 116–122.

Kischbaum, D.S. (2004). Direct Adventitious Shoot Formation on Seedling Radicals in Seed Cultures of Strawberry. In: J. Plant Biol.47 (2):160-162.

Kumar, J., Gaspar, T., Kevers, C. and Panel, C. (2005). The Effect of Auxin and Cytokinin on Growth and Development of *Holarrhena antidysenterica* Explants in Tissue Culture, 2005 (November), 2174—2180.

Magyar TM, Tabori R, Lloyd G and Modgil M (2010). Effects of Benzylaminopurine (BAP) on *In vitro* Proliferation of Pineapple (*Ananas cosmseus* (L) Merr). Acta Horticut. (ISHS), 425:242-245.

Martin B.M (1994). Routine Cell Culture. Tissue Culture Techniques: An Introduction. Boston : 29-30

McCoy T.U and Smith J (2006). Organogenesis of Vegetative Shoots from *In vitro* Cultured Flower Buds of *Mammillaria albicoma* (Cartaceae). Plant Cell Tis. Org. Cult. 87:27-32.

McGaw S.D and Burch E (2005). Plant Growth and Development Hormones and Environment, Academic Press, Amsterdam

Mihretu, Y., Wayessa, G., & Adugna, D. (2014). Multivariate Analysis among Okra (*Abelmoschus esculentus* (L.) Moench) Collection in South Western Ethiopia. Journal of Plant Sciences 9(2):43-50.

Murashige T (1974).Tissue Culture as a Plant Production Technique for Horticultural Crops. Afr. J. Biotechnol. 8(16): 3782-3788.

Murashige, T and Skoog,F.(1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cul-tures. Physiologia Plantarum. Vol. 15. No. 3. pp. 473-497.

Naveed, A., Khan, A.A., & Khan, I.A. (2009). Generation Mean Analysis of Water Stress Tolerance in Okra (*Abelmoschus esculentus* L.). Pak. J. Bot., 41: 195-205.

Ndunguru, J., & Rajabu, AC. (2004). Effect of Okra Mosaic Virus Disease on the Above-Ground Morphological Yield Components of Okra in Tanzania. Scienta Horticulturae, 99: 225-235.

Omamor, F., Gurnani, C., Kumar, V., Mukhija. and Dhingra, A. (2007). Problems in Tissue Culture: Culture Contamination. In: Micropropagation: Technology and Application, P.C. Derbergh & R.H. Zimmerman (Eds), 31– 44, Kluwer, Dordrecht.

Oyebanji G, Banu ML, Matthews, R. and Duncan, E.J. (2009). A Method to Reduce Microbial Contamination in *In vitro* culture. Third Caribbean Biotechnology Conference 160-166.

Oyelade, O.J., Ade-Omowaye, B.I.O., and Adeomi, V.F. (2003). Influence of Variety on Protein, Fat Contents and Some Physical Characteristics of Okra Seeds. J. Food Eng., 57: 111-114.

Pattnaik and Chand (1996). Influence of Explant Source, Plant Growth Regulator and Culture Environment on Culture Initiation and Establishment of *Ocimum americanum* and *O. Canum In vitro* J Exp. Bot 48: 951—962; 1996.

Pawar H.K., Seelye J.F., Maddocks D.J., Burge G.K. and Morgan E.R. (1994). Shoot Regeneration from Leaf Discs of *Limonium perigrinum* Using Benzylaminopurine and Kinetin. New Zealand; Journal of Crop and Horticultural Science 22 23-29.

Purkayastha Adkins S, Kamenova I, Achor D, Lewandowski D.J. (2008). *In Vitro* Culture of *Mentha piperita L.* : Roles of Auxin and Cytokinin. Science Horticulture. 83: 139-147.

Qhureshi, Z. (2007). Breeding Investigation in Bhendi (*Abelmoschus esculentus* (L.) Moench). Master Thesis, University of Agriculture Sciences, GKVK, Bangalore.

Rajendrudu K. (2007). *In vitro* Multiplication of *Chrysanthemum sp.* Meristem Tips for Mass Propagation. Hort Sci. 27:15–8.

Robert N. (2010). Plant Tissue Culture,Development and Biotechnology. Boca Raton: CRC Press. p. 186. ISBN 1-4200-8326-0.

Rubatzky, VE., & Yamaguchi, M. (1997). World Vegetables: Principles, Production, and Nutritive values. Chapman and Hall, New York, USA.

Saifullah, M., & Rabbani, MG. (2009). Evaluation and Characterization of Okra (*Abelmoschus esculentus* L. Moench.) Genotypes. SAARC J. Agric. 7: 92-99.

Sathish, D., and Eswar, A. (2013). A Review on: *Abelmoschus esculentus* (Okra). Int. Res J Pharm. App Sci., 2013; 3(4):129-132.

Sathyanarayana, B.N. (2007). Plant Tissue Culture: Practices and New Experimental Protocols. I. K. International. pp. 106

Scoville RE (2006) Effects of Nutrient Salt Formulations and PGR on Axilliary Shoot Proliferation of Tropical Hibiscus. Hortscience 41:1024.

Singh N.M., Ali H.K., Asili R.Y., Javad W.K. and Taghavizadeh Y.F. (2003). The Effect of Media and Plant Growth Regulators on Embryo Culture of Horticultural Crops; Archives Des Sciences 65 426-434

Thorpe T (2007). History of Plant Tissue Culture. J. Mol. Microbial Biotechnol. 37: 169-180.

Van Staden T, George, E.F., Hall, M.A. and De Klerk, G.J. (2008). Effect of Different Concentration of Sucrose and BAP on Shoot Proliferation on MS Strength Basal Media in Potato Cv. Agria. Biology And Environment Proceedings Of The Royal Irish Academy, 3(1), 63—72.

Vasil DCW, Hildebrandt TA (1965). Living Organism Improvement Through Tissue Culture. World J. Microbiol& Biotechnol. 11: 409-415.

West T.P. (2004). Effects of Auxin and Cytokinin Nutrient Salt Formulation on Micropropagation of Hardy Hibiscus (*Hibiscus moscheutos* L.). Acta Hortic 630:293–297.

Zaidah R and Nazri M.S. (2012). The Effect of Different Plant Growth Regulators on Shoot Induction of *Orthosiphon aristatus* Boldingh Seminor on Medicinal Plants.