



UNIVERSITI PUTRA MALAYSIA

***MICROPROPAGATION OF F1 HYBRID BRINJAL
(Solanum melongena L.)***

NURELIA AZIAN BINTI MOHAMAD NAZIR

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MICROPROPAGATION OF F1 HYBRID BRINJAL

(*Solanum melongena* L.)

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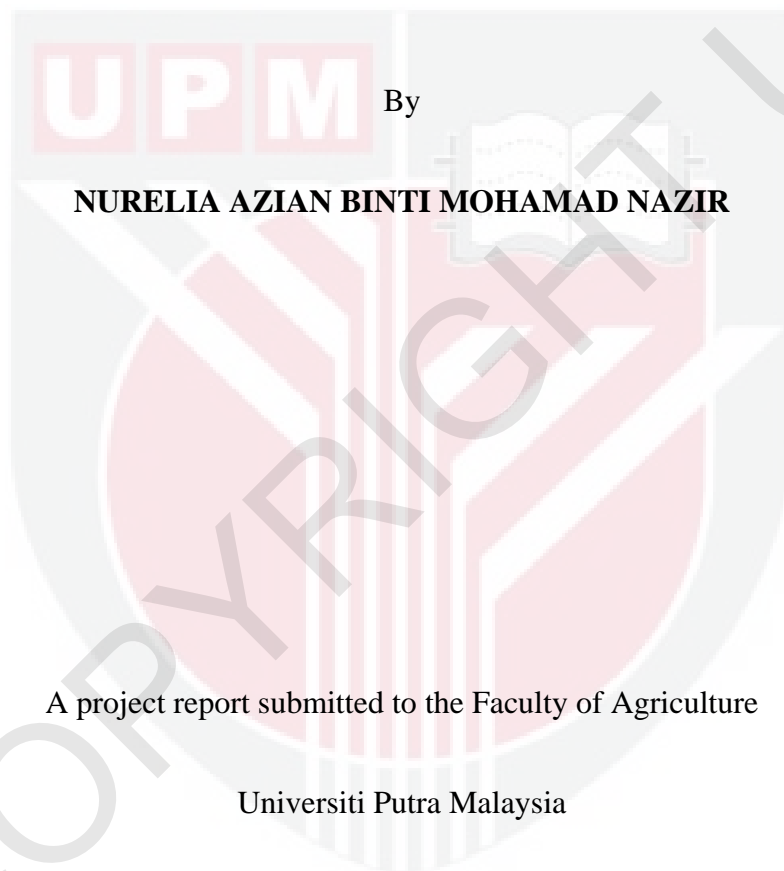
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MICROPROPAGATION OF F1 HYBRID BRINJAL

(Solanum melongena L.)



By

NURELIA AZIAN BINTI MOHAMAD NAZIR

A project report submitted to the Faculty of Agriculture

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CERTIFICATION

This project report entitled Micropropagation of F1 Hybrid Brinjal, prepared by Nurelia Azian Binti Mohamad Nazir (158592) in partial fulfillment of requirement of PRT4999 (Final Year Project) for the award of the Degree of Bachelor of Agricultural Science.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
CERTIFICATION	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF PLATES	ix
LIST OF APPENDICES	xi
LIST OF ABBREVIATIONS	xii
ABSTRACT	xiii
ABSTRAK	xiv
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Micropropagation	4
2.1.1 Stage 0: Preparation for Stock Plant Selection	5
2.1.2 Stage I: Initiation of Culture	5
2.1.3 Stage II: Shoot Multiplication	6
2.1.4 Stage III: Elongation and Root Induction	6

2.1.5 Stage IV: Acclimatization	7
2.2 Media Preparation and Components	7
2.3 Plant Growth Regulator (PGR)	8
2.4 Function of Cytokinins	9
2.5 Source of explants	10
2.6 Shoot Multiplication	11
2.7 Culture conditions	12
3 MATERIALS AND METHOD	13
3.1 Location of Study	13
3.2 Plant Materials	13
3.3 Procedure of Media Preparation	14
3.3.1 Stock Solution Preparation	14
3.3.2 Media Preparation	15
3.4 Seed Sterilization	16
3.5 Aseptic Manipulation Process	17
3.6 Culturing	18
3.7 Experiment	20
3.8 Experimental Design and Data Analysis	21
3.9 Observation and Data Collection	21
4 RESULT AND DISCUSSION	22
4.1 Effect of different BAP or kinetin concentration on explants proliferating shoots after 5 weeks of culture	22
4.2 Effect of different BAP or kinetin concentration on explants proliferating shoots after 3 weeks of subculture	28

5 CONCLUSION	32
BIBLIOGRAPHY	33
APPENDICES	37



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LIST OF TABLES

Tables	Page
Table 1: BAP and kinetin treatments for shoot multiplication of <i>S. melongena</i>	20
Table 2: Effect of different BAP and kinetin concentrations on percentage and mean number of shoots formed per shoot tip explant of F1 hybrid brinjal (<i>S. melongena</i>) after 5 weeks of culture	24
Table 3: Effect of different BAP and kinetin concentrations on percentage and mean number of shoots formed per shoot tip explant of F1 hybrid brinjal (<i>S. melongena</i>) 3 weeks after subculture	29

LIST OF FIGURES

Figures	Page
Figure 1: Mean number of shoots per shoot tip explant of <i>S. melongena</i> for 5 weeks of culture on treatments with various concentrations of BAP and kinetin	27
Figure 2: Mean number of shoots per shoot tip explant of <i>S. melongena</i> 3 weeks after subculture on medium with various concentrations of BAP and kinetin	31

LIST OF PLATES

Plates	Page
Plate 1: Stock solutions	14
Plate 2: Vials arranged on racks in a culture room with the lights and temperature controlled	18
Plate 3: Shoot tip culture on medium at day 0	19
Plate 4: Root formation in medium without Cytokinin (T1) after 1 week of culture	22
Plate 5: New axillary bud on 1.0 mg/L BAP (T2) medium after 2 weeks of culture	23
Plate 6: Growth of callus inhibited axillary shoot formation on 5.0 mg/L BAP (T4) medium after 5 weeks of culture	23
Plate 7: Axillary shoot on 1.0 mg/L kinetin (T5) medium after 2 weeks of culture	25

LIST OF APPENDICES

Appendices	Page
Appendix 1: Murashige and Skoog (1962) basal medium formulation	37
Appendix 2: ANOVA on mean number of shoot formed per explants after 5 weeks of culture	39
Appendix 3: ANOVA on mean number of shoot formed per explants after 3 weeks of subculture	41



LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celcius
ANOVA	Analisis of Variance
BAP	6-benzylaminopurine
Mg/L	Miligram per liter
mL	Mililiter
cm	Centimeter
MS	Murashige and Skoog
pH	Hydrogen ion concentration
NaOH	Sodium Hydroxide
HCl	Hycrochloric acid

ABSTRACT

This study was conducted to determine the best concentration of 6-benzylaminopurine (BAP) or kinetin on shoot multiplication from shoot tip explant of F1 hybrid brinjal (*Solanum melongena*). The shoot tips derived from germinated seedling were used as explants and cultured on half strength Murashige and Skoog (MS) medium supplemented with different BAP or kinetin concentration. This experiment was carried out at the Tissue Culture Laboratory, Department of Agriculture Technology, Universiti Putra Malaysia, Serdang. Seven cytokinin treatments were used in this experiment which were 0.0, 1.0, 2.0, 5.0 mg/L BAP and 1.0, 2.0, 5.0 mg/L kinetin. The experiment was conducted using Completely Randomized Design (CRD) with 10 replications per treatment and 10 explants in each replication. It was observed that the medium containing 1.0 mg/L kinetin induced the highest mean number of shoots per explants (0.91) after 5 weeks of culture but was not significantly different compared with the control treatment and other BAP and kinetin treatments. The highest mean number of shoots (0.92) proliferated after 3 weeks of subculture was on medium supplemented with 1.0 mg/L BAP but also showed no significant difference compared with the control treatment as well as other BAP and kinetin treatments except 1.0 mg/L kinetin which showed a significant reduction on mean number of shoots formed per explant.

ABSTRAK

Kajian ini dijalankan untuk menentukan kepekatan terbaik 6-benzylaminopurine (BAP) atau kinetin pada penggandaan pucuk dari eksplan hujung pucuk F1 terung hibrid (*Solanum melongena*). Hujung pucuk diperolehi daripada anak benih terung hibrid yang tercampah digunakan sebagai eksplan dan dikulturkan pada separuh Murashige dan Skoog (MS) medium yang mengandungi kepekatan BAP atau kinetin yang berbeza. Eksperimen ini telah dijalankan di Makmal Kultur Tisu, Jabatan Teknologi Pertanian, Universiti Putra Malaysia, Serdang. Tujuh rawatan cytokinin digunakan dalam eksperimen ini iaitu 0.0, 1.0, 2.0, 5.0 mg/L BAP dan 1.0, 2.0, 5.0 mg/L kinetin. Eksperimen ini dijalankan dengan menggunakan rekabentuk rawak sempurna (CRD) dengan 10 replikasi setiap rawatan dan 10 eksplan setiap replikasi. Pemerhatian menunjukkan bahawa medium yang mengandungi 1.0 mg/L kinetin mempunyai purata bilangan pucuk tertinggi (0.91) pada setiap eksplan selepas 5 minggu dikultur tetapi ia tidak ada perbezaan yang signifikan berbanding dengan rawatan kawalan dan rawatan BAP atau kinetin lain. Purata bilangan pucuk yang paling tinggi (0.92) selepas 3 minggu subkultur adalah pada medium 1.0 mg/L BAP tetapi juga menunjukkan tidak ada perbezaan yang signifikan berbanding dengan rawatan kawalan serta rawatan BAP dan kinetin lain kecuali 1.0 mg/L kinetin yang menunjukkan pengurangan yang signifikan ke atas purata bilangan pucuk yang terbentuk pada setiap eksplan.

1 INTRODUCTION

Brinjal (*Solanum melongena*) belongs to the Solanaceae family. It is an important solanaceous crop grown as vegetable. This plant is also known as aubergine, eggplant or Guinea squash in different countries. Brinjal probably originated from India but it is now widely cultivated throughout the tropical and subtropical regions. Sihachakr et al., (1993) stated that brinjal has significant economic importance in Asia, Africa, and the subtropics (India, Central America), but is also grown in some warm temperate regions (Mediterranean area, South of the USA). The top producer of brinjal in the world is China producing 27,700,100 metric tons, followed by India, Iran, Egypt and Turkey (FAO, 2011).

Brinjal is one of the non-tuberous species of the nightshade Solanaceae family. It can grow to a height of 1-1.5 m and the plant is normally grown as an annual. The plant has simple leaves and the flowers are single or are borne in cluster. The flower is white to purple, with a five-lobed corolla and yellow stamens. The plant bears fruits that are frequently use in cooking and also can be consumed either fresh, dried or pickled. Brinjal has various fruit shape such as round, ovoid or longish oblong. The colour of brinjal fruit varies from white to yellow, green, purple or dark purple. The fruit length depends on the fruit and the shape ranging between 4-45 cm long and with a thickness of 2-35 cm, and the weight ranging between 15-1500 g (Sekara et al., 2007).

Brinjal has higher calorie, iron, phosphorus, and riboflavin compared with tomato (Ray et al., 2011). According to Khan (1979) the fresh weight of this fruit is composed of 92.7% moisture, 1.4% protein, 1.3% fibre, 0.3% fat, 0.3% minerals, and 4% carbohydrates and vitamins (A and C). Brinjal has been widely used in traditional medicines. For example, tissue extracts of brinjal have been used for treatment of asthma, bronchitis, and cholera. The fruits and leaves of brinjal plant are beneficial in lowering blood cholesterol (Kashyap et al., 2003).

The cultivation of brinjal is mostly by seeds. Usually in the local market non hybrid seeds and hybrid seeds are sold. Hybrid seed has superior characteristic which produces good quality yield. Also, hybrid brinjals are more resistant to pest and disease, especially the fruit borer and wilt disease. However, in small scale farm non hybrid seeds are preferred because they are less expensive than the hybrid seeds.

There are several problems related with the production of F1 hybrid brinjal plant in Malaysia. The price of imported hybrid seeds is expensive. Furthermore, the production of hybrid seeds of brinjal in Malaysia is impossible due to no parent material available for hybridisation. An alternative for this is to micropropagate the F1 hybrid plant using somatic tissues such as using the shoot tip culture. Therefore, *in vitro* propagation is the best option to mass multiply the hybrid variety and maintaining true to typeness of the plant.

Previously, the regeneration of brinjal plants has been reported either directly from shoot tip (Ferdausi et al., 2009), stem, root and leaf (Ray et al., 2011), and hypocotyl and cotyledon (Bardhan et al., 2012). Since very limited report has been made on *in vitro* propagation of brinjal, therefore this experiment is conducted with the objective to determine the best BAP and kinetin concentrations in initiating shoot regeneration from shoot tip explants of F1 hybrid brinjal.



BIBLIOGRAPHY

- Bardhan, S., Sharma, C. and Srivastava, D. (2012). In vitro plant regeneration studies in brinjal. *Journal of Cell and Tissue Research* Vol, 12(2): 3213-3218.
- Cardoza, V. (2007). Tissue culture: The manipulation of plant development. *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*. John Wiley and Sons, Inc., USA. pp. 113-134.
- Cassells, A. C. and Doyle, B. M. (2005). Pathogen and biological contamination management: the road ahead. In Loyola-Vargas, V. M., Vázquez-Flota, F. (Ed.). *Plant Cell Culture Protocols*, Humana Press. New York, USA. pp. 35-50.
- 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. pp. 1-14.
- Dobránszki, J., Teixeira da Silva, and Jaime A. (2010). Micropropagation of apple - A review. *Biotechnology Advances*, 28(4): 462-488.
- Evans, D. E., Coleman, J. O. D. and Kearns, A. (2003). *Plant cell culture*. BIOS Scientific Publishers, New York. pp. 15-16.
- Food and Agriculture Organization of the United Nation. (2011). World Production of Brinjal for 2011. Retrieve 9/9, 2013, from <http://faostat.fao.org/site/339/default.aspx>.
- Ferdausi, A., Nath, U., Das, B. and Alam, M. (2009). *In vitro* regeneration system in brinjal (*Solanum melongena* L.) for stress tolerant somaclone selection. *Journal of the Bangladesh Agricultural University*, 7(2): 253-258.
- Gamborg, O., Murashige, T., Thorpe, T. and Vasil, I. (1976). Plant tissue culture media. *In Vitro Cellular & Developmental Biology-Plant*, 12(7): 473-478.

- Garcia, R., Morán, R., Somonte, D., Zaldúa, Z., López, A. and Mena, C. J. (1999). Sweet potato (*Ipomoea batatas* L.) biotechnology: perspectives and progress. In Altman, A. and Ziv, M. (Eds.). Plant Tissue Culture: Current Status and Opportunities. Plant biotechnology and *in vitro* biology in 21st century. The Netherlands: 143-146.
- George, E. F. and de Klerk, G. J. (2008). The components of plant tissue culture media I: Macro-and micro-nutrients. In George, E. F., Hall, M. A. & De Klerk, G-J. (Eds.), Plant propagation by tissue culture (Third edition ed.). Springer, Netherlands. pp 65-113.
- Hazarika, B. (2003). Acclimatization of tissue-cultured plants. Current Science, 85(12): 1704-1712.
- Hussain, A., Qarshi, I. A., Nazir, H. and Ullah, I. (2012). Plant tissue culture: Current status and opportunities. In Leva, A. and Rinaldi, L. M. R. (Ed.), Recent advances in plant *in vitro* culture. InTech, Croatia. pp. 1-28.
- Husain M. K. and Anis M. (2009). Rapid *in vitro* multiplication of *Melia azedarach* L. (a multipurpose woody tree). Acta Physiologiae Plantarum. 31(4): 765-772.
- Idowu, P., Ibitoye, D., and Ademoyegun, O. (2009). Tissue culture as a plant production technique for horticultural crops. African Journal of Biotechnology, 8(16): 3782-3788.
- Izadpanah, M. and Khosh-Khui, M. (1992). Comparisons of *in vitro* propagation of tomato cultivars. Iran Agric. Res, 8: 37-47.
- Kashyap, V., Vinod Kumar, S., Collonnier, C., Fusari, F., Haicour, R., Rotino, G. L., Sihachakr, D. and Rajam, M. V. (2003). Biotechnology of eggplant. Scientia Horticulturae, 97(1): 1-25.
- Khan, R. (1979). *Solanum melongena* and its ancestral forms. In Hawkes, J. G., Lester, R. N. and Skelding, A. D. (Ed.), The biology and taxonomy of the Solanaceae. Academic Press, London. pp. 629-636.

- Krikorian, A. D. (1995). Hormones in tissue culture and micropropagation. In Davies, P. J., (Ed.), Plant hormones. Kluwer Academic Publishers, Dordrecht. pp. 774-796.
- Marana. J. P., Miglioranza. E. and De Faria. R.T. (2009). *In vitro* establishment of *Jacaratia spinosa* (Aubl.) ADC. Semina-Ciencias Agrarias. 30(2): 271-274.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3): 473-497.
- Pierik, R. L. M. (1989). *In vitro* culture of higher plants. Martinus Nijhoff Publishers, Dordrecht, Netherlands. pp. 1-24.
- Ray, B., Hassan, L. and Nasiruddin, K. (2011). *In vitro* regeneration of brinjal (*Solanum melongena* L.). *Bangladesh Journal of Agricultural Research*, 36(3): 397-406.
- Saini R. and Jaiwal P. K. (2002). Age, position in mother seedling, orientation, and polarity of the epicotyl segments of blackgram (*Vigna mungo* L. Hepper) determines its morphogenic response. *Plant Sci.* 163(1): 101-109.
- Sarker, R., Yesmin, S. and Hoque, M. (2006). Multiple shoot formation in eggplant (*S. melongena* L.). *Plant Tissue Culture and Biotechnology*, 16(1): 53-61.
- Sharma, P. and Rajam, M. V. (1995). Genotype, explants and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). *Journal of Experimental Botany*, 46(1): 135-141.
- Shimizu-Sato, S. and Mori, H. (2001). Control of outgrowth and dormancy in axillary buds. *Plant Physiology*, 127(4): 1405-1413.
- Shivaraj, G. and Rao, S. (2011). Rapid and efficient plant regeneration of eggplant (*Solanum melongena* L.) from cotyledonary leaf explants. *Indian Journal of Biotechnology*, 10: 125-129.

- Sihachakr, D., Chaput, M., Serraf, I., and Ducreux, G. (1993). Regeneration of plants from protoplasts of eggplant (*Solanum melongena* L.). Plant protoplasts and genetic engineering IV. Springer, Berlin. pp. 108-122.
- Singh K. K., and Gurung B. (2009). *In vitro* propagation of *R. maddenii* Hook. f. an endangered Rhododendron species of Sikkim Himalaya. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 37(1): 79-83.
- Sękara, A., Cebula, S. and Kunicki, E. (2007). Cultivated eggplants—origin, breeding objectives and genetic resources, a review. Folia Horti, 19: 97-114.
- Saini, R., Jaiwal, P.K. (2002). Age, position in mother seedling, orientation, and polarity of the epicotyl segments of blackgram (*Vigna mungo* L. Hepper) determines its morphogenic response. Plant Sci. 163(1): 101-109.
- Smith, R. H. (2000). Plant tissue culture: Techniques and experiments. Academic Press, California, USA. pp. 60.
- Thorpe, T., Stasolla, C., Yeung, E. C., de Klerk, G-J., Roberts, A., and George, E. F. (2008). The components of plant tissue culture media II: Organic additions, osmotic and pH effects, and support systems. In George, E. F., Hall, M. A. & De Klerk, G-J. (Ed.), Plant propagation by tissue culture (Third edition ed.). Springer, Netherlands. pp 65-113.
- Tilkat, E., Onay, A., Yildirim, H. and Ayaz, E. (2009). Direct plant regeneration from mature leaf explants of pistachio, *Pistacia vera* L. Scientia Hort. 121(3): 361-365.
- van Staden, J., Zazimalova, E. and George, E. F. (2008). Plant growth regulators II: Cytokinins, their analogues and antagonists. In George, E. F., Hall, M. A. & De Klerk, G-J. (Ed.), Plant propagation by tissue culture (Third edition ed.). Springer, Netherlands. pp 205-226.
- Zheng, W., Xu, X., Dai, H. and Chen, L. (2009). Direct regeneration of plants derived from *in vitro* cultured shoot tips and leaves of three *Lysimachia* species. Scientia Horticulturae, 122(1): 138-141