

UNIVERSITI PUTRA MALAYSIA

PROLIFERATING AXILLARY BUD FORMATION FROM SHOOT TIP EXPLANTS OF F1 HYBRID CUCUMBER (*Cucumis sativus* L.) WITH SUPPLEMENTED OF BAP AND KINETIN

NUR ILANI MOHD NAJIB

FP 2015 166

PROLIFERATING AXILLARY BUD FORMATION FROM SHOOT TIP EXPLANTS OF F1 HYBRID CUCUMBER (*Cucumis sativus* L.) WITH SUPPLEMENTED OF BAP AND KINETIN.



BY

NUR ILANI BINTI MOHD NAJIB

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

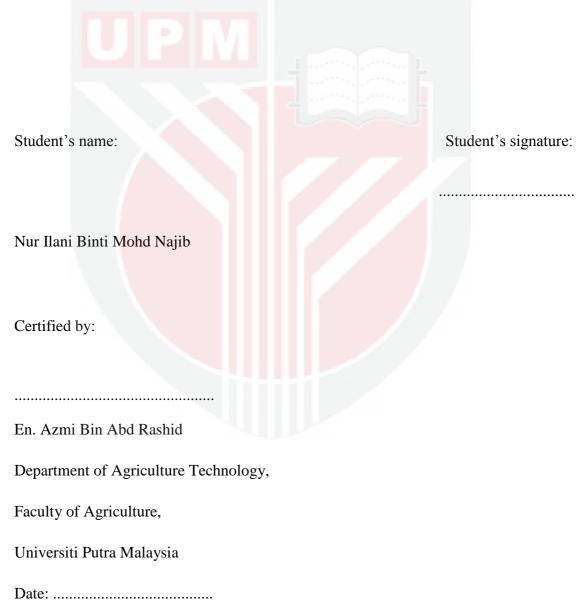
Faculty of Agriculture

Universiti Putra Malaysia

2014/2015

CERTIFICATION

This project report entitled "**Proliferating Axillary Bud Formation From Shoot Tip Explants of F1 Hybrid Cucumber** (*Cucumis sativus* L.) With Supplemented of BAP and Kinetin" is prepared by Nur Ilani Binti Mohd Najib and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor Horticultural Science.



ACKNOWLEDGEMENT

First of all, I would like to express my never ending gratitude to my supervisor, En. Azmi Abdul Rashid for his dedication, support and guidance in helping me complete this research. He helped me a lot and without his guidance, I would not be able to complete this research.

UPM

Not forgetting people that were involved in this research directly or indirectly, postgraduate students and lab staffs including Miss Nurul Husna, Miss Siti Raziah and Mrs. Rohani, without their help I would go nowhere. Besides that, my friends who are always there to help me whenever I needed them.

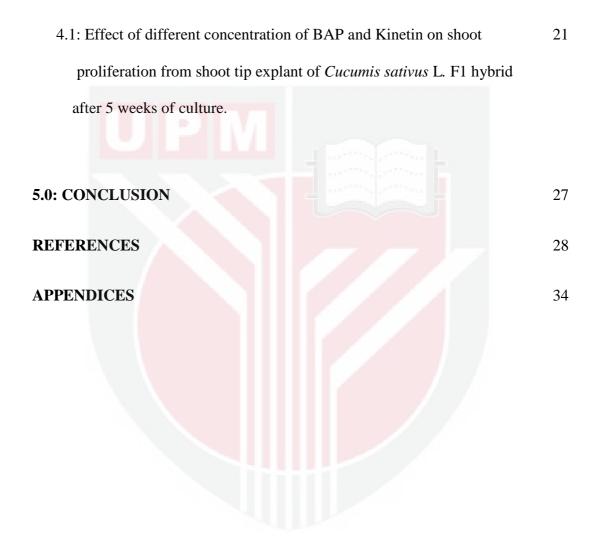
Last but not least, I would like to thank my beloved parents and siblings who are always there to support and gave their words of encouragement to me. The only thing I can say is, without their support, this research could not be completed.

TABLE OF CONTENTS

Content	Page
Acknowledgment	i
Table of Contents	ii
List of Tables	V
List of Plates	vi
List of Appendices	vii
List of Abbreviations	viii
Abstract	ix
Abstrak	X
1.0: INTRODUCTION	
1.1 Introduction	1
2.0: LITERATURE REVIEW	
2.1 Plant micropropagation	4
2.2 Stages of micropropagation	5
2.3 Factors affecting shoot proliferation	

2.3.1 Explants	7
2.3.2 Plant growth regulators	8
2.3.3 Culture medium	10
2.4 Problems occurring in tissue culture	
2.4.1 Culture microbial contamination.	11
3.0: MATERIALS AND METHODS	
3.1 Study location	12
3.2 Plant materials	12
3.3 Medium preparation for seed germination	12
3.4 Medium preparation for shoot tip culture	14
3.5 Aseptic manipulation	15
3.6 Seeds sterilisation and culture	16
3.7 Shoot tip explant inoculation	18
3.8 Explant incubation	19
3.9 Experimental treatment	19
3.10 Data collection and parameters	20

4.0: RESULTS AND DISCUSSION



LIST OF TABLES

Lists	Pages
Table 1 : BAP and Kinetin treatment used for shoot multiplication	19
Table 2: Effect of different concentration of BAP and Kinetin	25
on percentage (%) and mean number of shoot formed	
per shoot tip explants of F1 hybrid cucumber (<i>Cucumis sativus</i> L.)	
after 5 weeks of culture.	
Table 3: The volume of BAP and Kinetin required for each treatment.	34

LIST OF PLATES

Plates	Pages
Plate 1: Various stock solutions of micronutrient, micronutrient, vitamin,	13
and ferum chelate used for full MS medium preparation.	
Plate 2: Seed of cucumber (<i>Cucumis sativus</i> L.) cultured in vial	17
containing full MS medium.	
Plate 3: Shoot tip cultured vertically in jam jar containing full MS	18
medium supplemented with BAP or Kinetin.	
Plate 4: Swelling and slight callus formation at the base of	22
the explant after 1 week of culture.	
Plate 5: Shoot tip explant elongation with axillary bud proliferation	22
without callus formation at the base of the explant after 1 week	
of culture.	
Plate 6: Good axillary bud proliferation on medium with	23
2.5 mg/L BAP after 5 weeks of culture.	
Plate 7: Good axillary bud proliferation on medium with	23
1.0 mg/L kinetin after 5 weeks of culture.	

LIST OF APPENDICES

Appendices		Pages
А	The volume of BAP and Kinetin required for each treatment.	34
В	Analysis of variance table (ANOVA).	35
С	Tukey's Studentized Range (HSD) Test for y.	36
D	Murashige & Skoog Medium (MS) (1962).	37

LIST OF ABBREVIATIONS

The following abbreviations were used in the text.

BAP	6-benzylaminopurine
Kin	Kinetin
Mg/L	Miligram per liter
%	Percentage
°C	Degree Celcius
L	Liter
MS	Murashige and Skoog
MS	Mean of square
SS	Sum of square
F	Degree of freedom
рН	Hydrogen ion concentration -log (H)
RCBD	Randomized Complete Block Design
ANOVA	Analysis of variance

ABSTRACT

This study was conducted to determine the best 6-benzylaminopurine (BAP) or Kinetin (Kin) in proliferating axillary bud formation from shoot tip explants of F1 hybrid cucumber (*Cucumis sativus* L.). Seeds of F1 hybrid cucumber were germinated on full Murashige and Skoog (MS) medium without the addition of plant growth regulator. In this experiment, the shoot tip explants excised from 14 day-old germinated seedlings were used. This explants were cultured on full MS medium supplemented with different BAP and Kinetin concentrations (0.0, 0.5, 1.0, 2.5 and 5.0 mg/L). This experiment was conducted using Randomized Complete Block Design (RCBD) with 10 replications per treatment. All BAP and Kinetin treatments including the control treatment showed 100% of explant proliferating axillary bud formation. Analysis of variance showed no significant difference between the control treatment and all other BAP and Kinetin treatments on mean number of shoots formed per explant.

ABSTRAK

Kajian ini telah dijalankan bagi menentukan yang manakah terbaik di antara 6-benzylaminopurin (BAP) atau Kinetin (Kin) dalam pembiakan tunas aksil dari hujung pucuk bagi hibrid F1 timun (Cucumis sativus L.). Benih timun bagi F1 hibrid akan dicambahkan di dalam media penuh MS (Murashige and Skoog) tanpa penambahan pengawal penggalak pertumbuhan tumbuhan. Di dalam eksperimen ini, hujung pucuk eksplan yang dipotong akan digunakan selepas 14 hari benih timun bercambah. Melalui kajian ini, eksplan akan dicambahkan dalam media penuh MS yang ditambahkan dengan perbezaan kepekatan daripada BAP dan Kinetin (0.0, 0.5, 1.0, 2.5 and 5.0 mg/L). Kajian ini dijalankan dengan menggunakan Rekabentuk Blok Penuh Rawak bersama 10 replikasi bagi setiap rawatan. Kesemua rawatan BAP dan Kinetin termasuklah rawatan kawalan menunjukkan 100% eksplan menghasilkan pembentukan tunas aksil. Analisis varians menunjukkan tiada perbezaan yang signifikan di antara rawatan kawalan dan kesemua rawatan BAP dan Kinetin pada min bilangan pucuk yang terbentuk setiap eksplan.

CHAPTER 1

INTRODUCTION

1.1 Introduction



In Malaysia, cucumber is called *timun* and the scientific name of cucumber is *Cucumis sativus* L. This species belongs to the Cucurbitaceae family. This crop can be grown in the tropic, subtropic and milder temperate zones of both hemispheres.

In India, cucumber is a plant which plays an important role in the horticultural field because it is cultivated as a vegetable. It has several uses such as in pickle production and for the preparation of traditional Indian medicines (Sangeetha and Venkatachalam, 2013) besides being taken fresh as salad.

This crop has antioxidant and detoxification property and contains high mineral content such as phosphorus and potassium. It also has high oxalic acid content (Milner, 2000: Chu et al., 2000). The seeds can be used in diuretic treatment and in making tonic drink (Pandey, 2000).

Botanically, cucumber (*Cucumis sativus* L.) is a type of vegetable with creeping vines. This plant also has leaves which are simple and arranged in an alternate manner. The leaves are deeply cordate with 3-5 lobed on its surfaces with a hairy denticulate margin. The flowers of cucumber are yellow in color. The male flowers are clustered, bearing anther with cohering and connective crush while the female flowers are solitary, thick and is covered by hairs. Its ovary is large and significant (Warrier, 1994).

Cucumber is usually propagated using sexual approach through the production of seeds (Ahmad and Anis, 2005). 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 plants at the F1 generation are necessary to maintain true to typeness of the F1 hybrid cucumber (George et al., 2008). At present not much has been reported on induction of axillary budding from shoot tip culture of *Cucumis sativus* L. F1 hybrid. Therefore, this experiment was conducted with the following objective:

1. To determine the most suitable concentration of BAP or kinetin in proliferating axillary budding from shoot tip culture of *Cucumis sativus* L.



REFERENCES

- Ahmad, N. and Anis, M. (2005). In vitro mass propagation of Cucumis sativus L. from nodal segments. Turk. J. Bot. 29:237–240.
- Aitken-Christie J., Kozai, T. and Smith M. A. L. (1995) Automation and environmental control in plant tissue culture. (eds). Dordrecht. Kluwer Academic Publishers. p 574.
- Ajithkumar, D. and Seeni, S. (1998). Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., medicinal tree Plant Cell Rep. 17:422-426.
- Aswath, C. R. and Choudhary, M. L. (2002). Rapid plant regeneration from *Gerbera jamesonii Bolus* callus cultures. Acta Bot Croat. 61:125–34.
- 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.
- **Bhojwani, S. S. and Dantu, P. K.** (2013). Plant tissue culture: An introductory text. New York, Dordrecht, London. Springer. pp 29-34.
- Campbell, P.R., Hugh, T.B., Coleman, M.J., Munro, V.F. and Hart, L.A. (1984). A comparison of surface particle contamination of powdered and non-powdered surgical gloves. Aust. N.Z. J. Surg. 54:559-563.

- Cardarelli, M., Borgognone, D. and Colla, G. (2010). *In vitro* propagation of *Abregonia denegerII* fri Č. (Cactaceae). Prop. Orn. Plants. 10:29–36.
- **Cassells, A. C.** (1997). Pathogen and microbial contamination management in micropropagation, (ed). Dordrecht: Kluwer Academic Publishers.
- Chu, Y. F., Sun, J., Wu, X. Z. and Liu, R. H. (2002). Antioxidant and anti proliferative activities of common vegetables. J. Agric. Food Chem. 50, 6910–6916.
- **Dobránszki, J. and Teixeira da Silva, J. A.** (2010). Micropropagation of apple- A review. Biotech. Adv. 28(4):462-488.
- Douglas, G. C., Rutledge, C. B., Casey, A. D. and Richardson, D. H. S. (1989) Micropropagation of floribunda, ground cover and miniature roses. Plant Cell Tis. Org. Cult. 19:55-64.
- Garcı'a-Rubio, O. and Malda-Barrera, G. (2010). Micropropagation and reintroduction of the endemic *Mammillaria mathildae* (Cactaceae) to its natural habitat. HortSci. 45(6):934–938.
- Garcia-Saucedo, P. A., Maribel, V. M., Maria, E. V., Andre's, C. H. and Octavio,
 P. L. (2005) Regeneration of three *Opuntia* genotypes used as human food. Plant
 Cell Tis. Org. Cult. 80:215–219.

- George, E. F. and Debergh, P. C. (2008). Micropropagation: uses and methods. 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.
- **George, E. F., Hall, M. A. and De Klerk, G. J.** (2008). Plant propagation by tissue culture (Eds), (3rd ed). Dordrecht. Netherlands: Springer. P403-406.
- Gurnani, C., Kumar, V., Mukhija, S., Dhingra, A., Rajpurohit, S. and Narula, P. In vitro regeneration of brahmi (*Bacopa monneiri* (L.) penn.) - a threatened medicinal plant. J. Sci. 8:97-99.
- Kadota, M. and Niimi, Y. (2003). Effect of cytokinin types and their concentrations on shoot proliferation and hyperhydricity in *in vitro* pear cultivar shoots. Plant Cell Tis. Org. Cult. 72:261-265.
- Kalyani, B. G., and Rao, S. (2013). Effect of plant growth regulators on *in vitro* organogenesis in cultivated tomato (*Lycopersicon esculentum Mill.*) Society App. Sci. 4(4):591-596.
- Kaushal, N., Modgil, M., Thakur, M. and Sharma, D. R. (2005) *In vitro* clonal multiplication of an apple rootstock by culture of shoot apices and axillary buds. Ind. J. Exp. Biol. 43:561–5.
- Khattab, S., El Sherif, F., El-Garhy, H. A., Ahmed, S., and Ibrahim, A. (2014). Genetic and phytochemical analysis of the *in vitro* regenerated *Pilosocereus robinii* by ISSR, SDS–PAGE and HPLC. Gene. 533(1):313-321.

- Lloyd, G. and McCown, B. (1980). Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Comb. Proc. Int. Plant Prop. Soc. 30:421-427.
- Lopez-Bucio, J., Cruz-Ramirez, A. and Herreraestrella, L. (2003). The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6:280-287.
- Lu, M. (2005). Micropropagation of V*itis thunbergii* sieb. et zucc., A medicinal herb, through high-frequency shoot tip culture. Sci. Hort., 107(1):64-69.
- Milner, J.A. (2000). Functional foods: The US Perspective. Am. J. Clin. Nutr. 71:16545–16595.
- Modgil, M., Sharma, D. R. and Bhardwaj, S. V. (1999). Micropropagation of apple cv. tydeman early worcester. Sci. Hort. 81:179–88.
- Mostafa, G. G. and Abou Al-Hamd, M. F. (2011). Effect of gibberellic acid and indole-3 acetic acid on improving growth and accumulation of phytochemical composition in *Balanites aegyptica* plants. Amer. J. Plant Phy. 6:36–43.
- **Murashige, T. Skoog, F.** (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phy. Plant 15:431-497.

Oana, C. T., Marcela, F. and Maria, P. (2008). Considerations regarding the effects of growth regulators over the *in vitro* morphogenetic reaction at *Origanum vulgare* L. J. Plant Dev. 15:133-138.

Pandey, B. P. (2000). Economic botany. pp 76-77. New Delhi: S. Chand & Co. Ltd.

- Ranalli, P. Bizzari, M. Bordhi, L. and Mari, M. (1994). Genotypic influence on *in vitro* induction dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum L.*). Ann. Appl. Biol., 125: 161-172.
- **Razdan, M. K.** (2005), Introduction to plant tissue culture. 2nd (ed). Science Publishers, Inc. Enfield (NH), USA.
- Rout, G. R. (1991). Studies on *in vitro* propagation of rose cultivars. PhD thesis, Utkal Uni. Ind.
- Sangeetha, P. and Venkatachalam, P. (2013). Induction of shoot organogenesis and *in vitro* flowering from shoot tip explants of cucumber (*Cucumis sativus* L. Cv. "Green long"). In Vitro Cell. Dev. Biol. 50:242-248.
- Schmulling, T. (2004). Cytokinin in encyclopedia of biological chemistry. Academic Press/Elsevier Science.

- **Trigiano, R. N. and Gray, D. J.** (2005) Plant growth regulators in plant tissue culture and development, In Plant Development Biotechnology. CRC Press.
- Warrier, P. K. (1994). Indian medicinal plants: A compendium of 500 Species. Chennai: Press Orient Longman.
- Wyka, P. T., Hamerska, M. and Wrablewska, M. (2006). Organogenesis of vegetative shoots from *in vitro* cultured flower buds of *Mammillaria albicoma* (Cactaceae). Plant Cell Tis. Org. Cult. 87:27–32.

