

# **UNIVERSITI PUTRA MALAYSIA**

THE GROWTH OF TYPHONIUM FLAGELLIFORME TREATED WITH DIFFERENT LEVELS OF THIDIAZURON AND N6– BENZYLAMINOPURINE

VAJIDAH SUNOTO @ HJ. FAISAL

FP 2012 93

# THE GROWTH OF *TYPHONIUM FLAGELLIFORME* TREATED WITH DIFFERENT LEVELS OF THIDIAZURON AND N<sup>6</sup>– BENZYLAMINOPURINE



VAJIDAH SUNOTO @ HJ. FAISAL



## FACULTY OF AGRICULTURE

# UNIVERSITI PUTRA MALAYSIA

### SERDANG, SELANGOR

2011/2012

# THE GROWTH OF *TYPHONIUM FLAGELLIFORME* TREATED WITH DIFFERENT LEVELS OF THIDIAZURON AND N<sup>6</sup>– BENZYLAMINOPURINE



BY

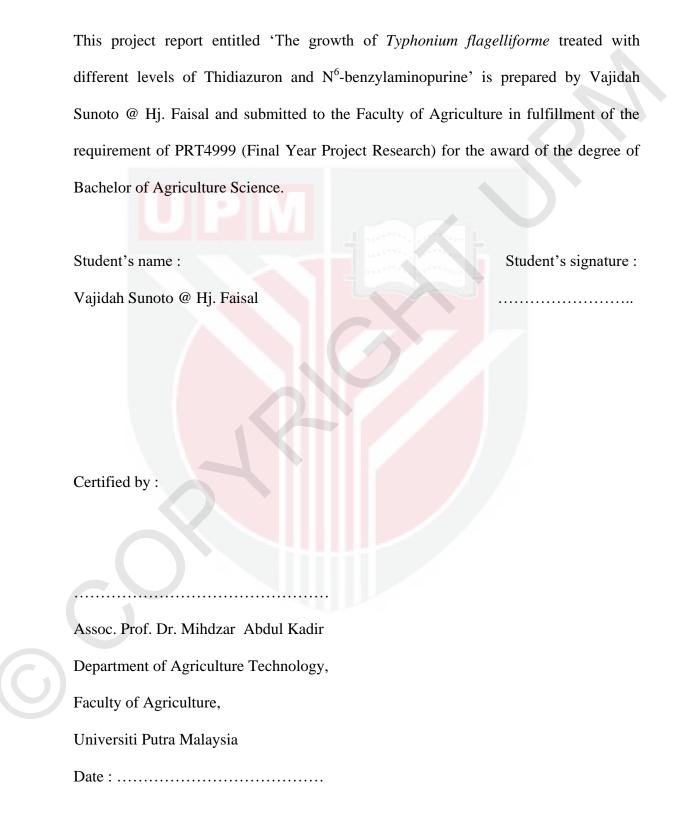
### VAJIDAH SUNOTO @ HJ. FAISAL

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 Agricultural Science

> FACULTY OF AGRICULTURE UNIVERSITI PUTRA MALAYSIA

> > 2011/2012

### CERTIFICATION



#### ACKNOWLEDGEMENT

In the name of Allah, The Most Gracious and The Most Merciful.

First and foremost all praises and thanks to Allah s.w.t who gave me the strength and easiness to accomplish my project as the requirement of PRT499A for the Bachelor in Agriculture Science degree with full of success. I gratefully acknowledge especially to my dearest supervisor, Dr. Mihdzar Abdul Kadir, for kindly providing the guidance, information and encouragement, to help me completed the thesis, who provided me invaluable critical comments and suggestions when the projects was carried out. His effort, sincere approach to motivate, advice and patience is highly appreciated.

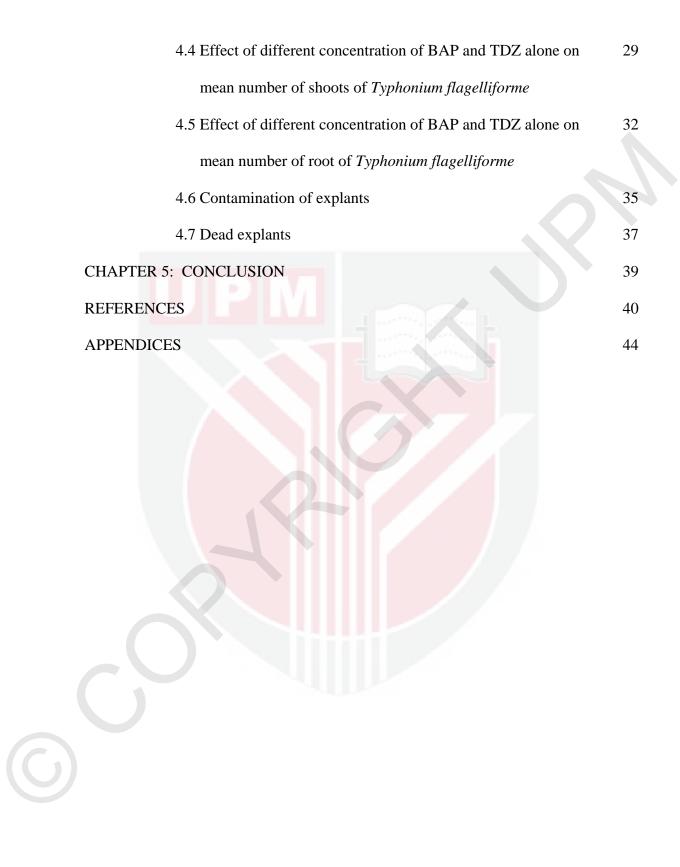
Further, I also wish to acknowledge the Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia and staff of the Cell Biology and Genetic Laboratory, Ms. Siti Raziah Rosli and Mr. Sharpudin Md. Nor for their guidance and assistance, in completing the project.

Finally special thanks to my beloved mother, Madam Mariyam binti Sulaiman, sisters, Taufikah and Xiammun Nur, and course mates, Siti Nurkhairun Nisa and Ruzaini who encouraged and provided me constant inspiration during the completion of the project. Without their moral supports and prayers, I would not have managed to complete this project successfully.

# TABLE OF CONTENTS

Contents	Page
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS	ii
LIST OF ABBREVIATIONS	v
LIST OF FIGURES	vi
LIST OF TABLES	vii
LIST OF APPENDICES	viii
ABSTRACT	1
ABSTRAK	2
CHAPTER 1: INTRODUCTION	3
1.1 Introduction	3
1.2 Justification and problem statement	4
1.3 Objectives	5
1.4 Hypothesis	5
CHAPTER 2: LITERATURE REVIEW	6
2.1 Typhonium flagelliforme	6
2.2 In-vitro culture	7
2.3 Sterilization technique	8
2.3.1 Equipment sterilization	8
2.3.2 Sterilization of explant	8
2.4 Multiplication	9

2.5 Plant growth regulators	9
2.5.1 Cytokinins	10
2.5.1.1 Benzylaminopurine (BAP)	11
2.5.1.2 Thidiazuron (TDZ)	11
2.5.2 Auxin	12
2.6 Medium	13
CHAPTER 3: MATERIALS AND METHODS	14
3.1 Study location	14
3.2 Source of explants	14
3.3 New Dogashima Medium preparation	15
3.4 Sterilization	17
3.4.1 Sterilization of culture equipments	17
3.4.2 Explants sterilization	18
3.5 Experiment	21
3.6 Observations and parameters recorded	22
3.7 Experimental design and statistical analysis	22
CHAPTER 4: RESULTS AND DISCUSSIONS	23
4.1 Effect of different concentration of BAP and TDZ in NDM	26
medium and the growth of <i>Typhonium flagelliforme</i>	
4.2 Effect of different concentration of BAP and TDZ alone on	27
mean fresh weight of <i>Typhonium flagelliforme</i>	
4.3 Effect of different concentration of BAP and TDZ alone on	28
mean height Typhonium flagelliforme	



# LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BAP	N <sup>6</sup> – Benzylaminopurine
cm	Centimeter
CRD	Complete Randomized Design
DMRT	Duncan Multiple Range Test
g	Gram
HCl	Hydrochloric acid
mg/L	Milligram per litre
ml	Milliliter
MS	Murashige and Skoog Medium
NAA	Naphthalene Acetic Acid
NaOH	Sodium hydroxide
NDM	New Dogashima Medium
ns	No significant difference at $p \le 0.05$
рН	Acidic scale
p.s.i	Pressure square inch
TDZ	Thidiazuron
°C	Degree Celcius
*	Significant different at $p \le 0.05$
**	Significant difference $p \le 0.01$
%	Percent

### LIST OF FIGURES

Figure	Title	Page
1	Typhonium flagelliforme	3
2	Prepation of substrate for <i>T. flagelliforme</i> growth	14
3	Medium cooled, hardened after finish autoclaved and	16
	ready to be used for culture	
4	Several equipments used during culturing process	17
5	A series of explants sterilization before culture	19
6	Shoot formation on different treatment (T) at 2 <sup>nd</sup>	30
	week of subculture	
7	Shoot formation on different treatment (T) at 4 <sup>th</sup>	30
	week of subculture	
8	Shoot formation on different treatment (T) at 6 <sup>th</sup>	31
	week of subculture	
9	Root formed at 2 <sup>nd</sup> week of subculture	33
10	Root formed at 4 <sup>th</sup> week of subculture	33
11	More root formed at 6 <sup>th</sup> week of subculture at	34
	different levels of treatments.	
12	Various contamination on medium culture growth	36
13	Dead of explants	38

# LIST OF TABLES

r

C

Гable	Title	Page
1	Various levels of concentration of BAP and TDZ	21
	with 0.1 mg/L NAA in NDM medium	21
2	Mean for all different levels of treatments of BAP	
	(T1 – T6) and TDZ (T7 – T12) based on each	25
	parameter between different weeks of observation.	
3	Percentage of contamination (%)	35
4	Percentage of dead explants (%)	36

# LIST OF APPENDICES

Appendix	Title	Page
1	Preparation of stock solution for hormones	44
2	New Dogashima Medium	45
3	Results by week	47
4	Result ANOVA of <i>Typhonium flagelliforme</i> at week 2	48
5	Result ANOVA of <i>Typhonium flagelliforme</i> at week 4	54
6	Result ANOVA of <i>Typhonium flagelliforme</i> at week 6	60

### ABSTRACT

Since it has known as herbal therapies for cancer treatment, the demand for *Typhonium flagelliforme* plant is high. Shortage of high quality planting materials has limited the supply of this plant. Through *in vitro* technique, mass multiplication of rodent tuber has been achieved. Thus a study was conducted at Cell Biology and Genetics Laboratory, Department of Agriculture Technology, Faculty of Agriculture. An experiment was conducted using two different cytokinins, BAP and TDZ in New Dogashima Medium at various levels of concentrations for this study. The objectives of this study are to study the effect of different concentrations of BAP and TDZ on the multiplication of T. flagelliforme on modified NDM medium and to determine the best concentration of hormones used for mass propagation of T. flagelliforme. The explant used was very small portion of tuber. A series of explant sterilizations were conducted before undergo culturing process. Twelve treatments used are BAP and TDZ with 0, 0.25, 0.5, 1, 3, and 5 mg/L respectively, added with 0.1 mg/L NAA to induce root growth for complete growth of individual plant. The explants were subcultured onto similar fresh media every 2 weeks. Parameters that were recorded include fresh weight of culture (g), explant height (cm), number of shoot(s) and root(s) per explant. Percent of contamination and dead of explant were also recorded. This study was conducted using Complete Randomized Design (CRD) and data were analyzed using the Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was employed for comparison between means. After several weeks of culture, the results showed that there were significant differences on the growth of explants among all the treatments.

1

#### ABSTRAK

Semenjak terkenal sebagai tumbuhan herba yang mempunyai nilai perubatan yang tinggi dan mampu melawan kanser, permintaan terhadap Keladi Tikus (Typhonium flagelliforme semakin meningkat. Bagi mengatasi kekurangan dalam mendapatkan sumber bahan tanaman yang sihat berkualiti, satu kajian menggunakan teknik kultur tisu telah dibuat di Makmal Biologi Sel & Genetik, Jabatan Teknologi Pertanian, Universiti Putra Malaysia. Dua hormon cytokinin yang berbeza iaitu BAP dan TDZ dengan kepekatan yang berbeza diuji dan dikultur ke New Dogashima Medium. Tujuan eksperimen dijalankan adalah untuk mengkaji kesan BAP dan TDZ pada tahap kepekatan yang berbeza dan mengetahui cytokinin yang paling sesuai digunakan untuk pertumbuhan T. flagelliforme. Ekplan yang digunakan adalah bahagian pangkal pokok beserta sedikit tuber dan beberapa siri pensterilan ekplan dibuat sebelum proses kultur dijalankan. Sebanyak 12 rawatan dikendalikan iaitu BAP dan TDZ berserta 0, 0.25, 0.5, 1, 3, dan 5 mg/L masing-masing. Kedua-dua BAP dan TDZ dicampur 0.1 mg/L NAA bagi menggalakkan pertumbuhan akar bagi agar tumbuh dengan sempurna. Ekplan disubkultur ke dalam media baru yang sama setiap 2 minggu. Pemerhatian yang dicatatkan adalah berat basah (g), ketinggian ekplan (cm), bilangan pucuk dan akar bagi setiap ekplan. Peratusan kontaminasi dan kematian juga diambil kira. Kajian yang dijalankan ini menggunakan Rekabentuk Rawak Penuh (CRD). Data yang diambil setiap 2 minggu dianalisis menggunakan analisis varians (ANOVA) dan perbandingan purata diambil menggunakan prosedur Duncan (DMRT). Selepas beberapa minggu, keputusan menunjukkan ada perbezaan bererti di dalam pertumbuhan explan.

### **CHAPTER 1: INTRODUCTION**

### **1.1 Introduction**



Figure 1. Typhonium flagelliforme

*Typhonium flagelliforme* (Lodd.) Blume is a plant under Plantae Kingdom. It is belongs to family Araceae. *Typhonium* is the genus name and *flagelliforme* is the species. The common name for this plant is Rodent Tuber or called Keladi Tikus in Malays.

*Typhonium* species are common in Malaysia lowlands, frequently found in disturbed places (Dassanayake *et al.*, 1988). The plant *Typhonium flagelliforme*, (Figure 1) also known as the 'rodent tuber', is often included as an essential ingredient in various herbal remedies recommended for cancer therapies in Malaysia. The tuber tissue was found to be a good explant for inducing asexual propagation system

(Ding *et al.*, 2011). The plant is widely distributed in soft, damp and shady habitats in Southeast Asia, extending even to Nothern Australia and South India (Lai *et al.*, 2008).

*Typhonium flagelliforme* is a medicinal herb which is endowed with curative properties against a variety of illness including injuries, oedema, coughs, pulmonary ailments, bleeding and cancer (Nobakht *et al.*, 2010).

A high increasing interest of using herbal medicine and traditional medicine was reported by World Health organization (Tilburt and Kaptchuk, 2008). However, herbal medicines, like other natural resources, have very limited sources. Thus artificial regeneration of herbal plants becomes important (Nobakht *et al.*, 2009).

As news spread about the benefits of the herbs, much of what left in the wild are being harvested, depleting the natural resource. Consequently, this herbal plant must be widely grown instead of relying on the wild. Therefore there is a need to mass propagate this species to enable consistent and adequate supply of the planting materials for large-scale planting (Shabariah, 2011).

### 1.2 Justification and problem statement

Considering that *Typhonium flagelliforme* is an endangered species and the availability of planting material is scarce, the use of tissue culture technique provides a rapid method to mass produce the plant.

### **1.3 Objectives**

Specific objectives for this research are:

- 1.3.1 To study the effect of different concentrations of BAP on the multiplication of *Typhonium flagelliforme* on modified NDM medium.
- 1.3.2 To study the effect of different concentrations of TDZ on the multiplication of *Typhonium flagelliforme* on modified NDM medium.
- 1.3.3 To determine the best concentration of hormones that can be used for mass propagation of *Typhonium flagelliforme*.

### **1.4 Hypothesis**

High multiplication of individual plant of *Typhonium flagelliforme* can be produce through *in vitro* technique and will provide adequate disease-free planting material resource supply for future.

#### REFERENCES

- Al-Ramamneh, E. S., Sriskandarajah, S., and Serek, M. (2006). Plant regeneration via somatic embryogenesis in *Schlumbergera truncata*. *Plant Cell and Tissue Organ Culture*, 84, 333-342.
- Arteca, N. (1995). *Micropropagation of orchids*. New York : John Wiley & Sons, Inc. p199-241.
- Beyl, C. A. (2000). Getting started with tissue culture media preparation, sterile
   technique, and laboratory equipment. *Plant tissue culture concepts and laboratory exercises* (2<sup>nd</sup> ed., p21-38). Washington: CRC Press.
- Chen, Y. and Piluek, C. (1995). Effect of thidiazuron and N<sup>6</sup>-benzylaminopurine on shoot regeneration of Phalaenopsis.*Plant Growth Regulation*, 16, 99-101.
- Chugh, S., Guha, S., & Rao, I. U. (2009). Micropropagation of orchids: A review on the potential of different explants. *Scientia Horticulturae*, 122(4), 507-520.
- Dassanayake, M. D., and Fosberg, F. R. (1988). A Revised Hand Book to the Flora of Ceylon. London: Taylor and Drancis Publisher.
- Ding, W., Zhang, L. H., Pan, S. H., and Chen, J. S. (2011). Establishment of tissue culture and rapid propagation system of *Typhonium flagelliforme*. *Chinese Traditional and Herbal Drugs*, 42(3), 585-588.
- Evans, N. E., Coleman, J. O. D., and Kearns, A. (2003). *Plant Cell Culture*. New York : John Wiley & Sons. p12-20.
- Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., and Thorpe, T. A. (1996).Plant hormones and plant growth regulators in plant tissue culture. *In vitro Cell Development Biology*, 32, 272-289.

Horst, W. D. (1990). Microbial cell culture. Biotechnology, 1.

- Kemat, N., Kadir, M. A., Abdullah, N. A. P., and Ashraf, F. (2010). Rapid multiplication of Safed musli (*Chlorophytum borivilianum*) through shoot proliferation. *African Journal of Biotechnology*, 9(29), 4595-4600.
- Krikorian, A. D. (1995). Hormones in tissue culture and micropropagation. In: Davies,P. J., (ed.) Plant hormones. *Physiology, Biochemistry and Molecular Biology*.Dordrecht: Kluwer Academic Publishers. p774-796.
- Lai, C. S., Mas, R. H. M. H., Nair, N. K., Majid, M. I. A., Mansor, S. M., and Navaratnam, V. (2008). *Typhonium flagelliforme* inhibits cancer growth *in vitro* and induces apoptosis: an evaluation by the bioactivity guided approach. *Journal of Ethnopharmacology*, 118(1), 14-20.
- Liu, C. M., Xu, Z. H., and Chua, N. H. (1993). Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. In: Addicott, F. T., Sabater, M., Chandler and Thorpe, and Tamas. I. A. *The Plant Cell*, 5, 621-630.
- Lu, C. (1993). The use of thidiazuron in tissue culture. *In vitro Cellular Development Biology*, 29, 92-96
- Morel, G. (1965). Clonal multiplication of orchids. *The orchids: Scientific studies*. John Wiley- Interscience, New York. 169-222.
- Murdad, R., Hwa, K. S., Seng C. K., Abd Latip, M., Abd Aziz, Z., and Ripin, R. (2006).
  High frequency multiplication of Phalaenopsis gigantea using trimmed bases protocorms technique. *Scientia Horticultura*, 111, 73 79.

Nobakht, G. M., Kadir, M. A., and Stanslas, J. (2009). In vitro mass propagation of

*Typhonium flagelliforme* as affected by plant growth regulators. *African Journal of Biotechnology*, 8(24), 6840-6843.

- Nobakht, G. M., Kadir, M. A., and Stanslas, J. (2010). Analysis of preliminary phytochemical screening of *Typhonium flagelliforme*. *African Journal of Biotechnology*, 9(11), 1655-1657.
- Prakash, L., Lee, C. L., Loh, C. S., and Goh, C. J. (1996). In vitro propagation of commercial orchids; an assessment of current methodologies and development of a novel approach thin cross-section culture. In: Islam, A.S (ed.). New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd. p42-49.
- Ravanfar, S. A., Aziz, M. A., Kadir, M. A., Rashid, A. A., and Sirchi, M. H. T. (2009).
  Plant regeneration of *Brassica oleracea* subsp. *Italica* (Broccoli) CV Green
  Marvel as affected by plant growth regulators. *African Journal of Biotechnology*, 8(11), 2523-2528.
- Razdan, M. K. (2005). Introduction to Plant Tissue Culture. Second edition. USA: Science Publishers.
- Rout, G. R., Samantaray, S., and Das, p. (2000) *In vitro* manipulation and propagation of medicinal plants. *Biotechnology Advanced*, 18, 91-120.
- Sai, S. T., Keng, C. L., Pargini, N., & Teo, C. K. H. (2000). In vitro proapgation of Typhonium flagelliforme (Lodd.) Blume. In vitro Cellular & Developmental Biology, 36(5), 402-406.
- Shabariah, I. (2011). Effect of combination of different concentration of BAP and NAA on the growth and multiplication of rodent tuber (*Typhonium flagelliforme*).
   *Thesis of Faculty of Agriculture*. University Putra Malaysia, Serdang, Selangor.

- Sirchi, M. S. T., Kadir, M. A., Aziz, M. A., Rashid, A. A., Rafat, A., and Javadi, M. B. (2008). Ameriolation of mangosteen micro propagation through leaf and seed segments (*Garciana mangostana* L.). *African Journal of Biotechnology*, 7(12), 2025-2029.
- Tilburt, J. C. and Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. *Bull World Health Organization*, 86, 594-599.
- Wawrosch, C., Maskay, N., and Kopp, B. (1999). Micropropagation of the threatened Nepalese medicinal plant *Swertia chirata* Buch. Ham Ex. Wall. *Plant Cell Reproduction*, 18, 997-1001.
- William, A. T. (2009). Drug from damp soil. *Trubus Magazine*. Jakarta: Ilham Pintar Resources.
- Zhang, K., Diederich, L., and John, P. C. L. (2005). Implications for mechanism of cytokinin response and plant development. *Plant Physiology*, 137, 308-316.

Skop penyelidikan dalam Institut Penyelidikan dan Kemajuan Pertanian Malaysia.

Retrieved 10 March 2012 from <u>http://www.mardi.gov.my/web/guest/skop-</u> penyelidikan15