



UNIVERSITI PUTRA MALAYSIA

MUTATION INDUCTION OF CHRYSANTHEMUM (Dendranthema grandiflora Tzvelev) USING GAMMA AND ION BEAM IRRADIATION

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By

SHAKINAH BINTI SALLEH

© Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

February 2013

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This thesis is dedicated to

My husband;

Abdul Halim Lim Abdullah

My son;

Muhammad Hazim Lim

My daughter;

Nurul Hasya Lim

Thank you for your sacrifices, laugh and love

May Allah bless all of us

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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Chrysanthemum is one of the major temperate cut flowers in Malaysia. In chrysanthemum production, there is always a demand for new cultivars with varied flower colours and shapes coupled with other preferred traits. Induced mutation using physical mutagens including gamma ray and ion beam in combination with *in vitro* propagation can be used to generate variations in ornamental plants. The present study was conducted to generate variations in chrysanthemum to produce new cultivars through nuclear technology in combination with plant biotechnology techniques.

In establishing the protocol for *in vitro* propagation, ray florets were cultured on Murashige and Skoog (MS) medium containing five levels of α -naphthaleneacetic acid (NAA) (0, 0.2, 0.5, 1.0 and 2.0 mg/L) and four levels of 6-benzylaminopurine (BAP) (0, 0.5, 1.0 and 2.0 mg/L). Optimum concentration for callus initiation occurred in medium with 0.5 mg/L NAA + 0.5 mg/L BAP while the concentration for shoot regeneration was optimum in MS medium with 0.5 mg/L NAA + 2.0 mg/L BAP. The highest number of normal shoots was also obtained in medium with 0.5 mg/L NAA and 2.0 mg/L BAP. Medium NAA at 0.5 mg/L + BAP at 0.5 mg/L was established as optimum for callus induction from ray florets explants. Subsequent subcultures were done in medium with 0.5 mg/L NAA and 2.0 mg/L BAP for shoot development.

A radiosensitivity (radiation sensitivity) test was carried out to determine the optimum dose for mutation using acute gamma (0 to 120.0 Gy) and ion beam (0 to 30.0 Gy) for two types of explants (ray florets and nodal explants) based on their *in vitro* survival and shoot regeneration. The results showed that the optimum dose for mutation induction of ray florets explants using acute gamma was less than 41.36 Gy and the corresponding dose for ion beam was less than 10.48 Gy. The shoulder dose for irradiated ray florets explants using acute gamma and ion beam were approximately 10.0 and 2.0 Gy, respectively. The optimum dose for mutation induction of nodal explants using acute gamma and ion beam was less than 43.33 and 10.32 Gy, respectively. The shoulder dose for irradiated nodal explants using acute gamma and ion beam were approximately 10.0 and 3.0 Gy, respectively. The

result suggests that the relative biological effectiveness (RBE) for ray florets and nodal explants was 3.95 and 4.20, respectively.

The *in vitro* propagated plantlets were grown in Cameron Highlands, Malaysia (elevation: 1500 m) to evaluate the changes on morphological and flowering characteristics of plants. 16 categories of plants were established in the field: Those derived from ray florets – acute gamma treatment (10.0 and 20.0 Gy) and ion beam treatment (0.5, 1.0 and 2.0 Gy); plants derived nodal explants - acute gamma treatment (10.0, 20.0, 30.0, 40.0 and 60.0 Gy) and ion beam treatment (0.5, 1.0, 2.0, 3.0, 5.0 and 8.0 Gy). Results showed that the ion beam totally altered the plant morphology and flowering characteristics (except the response time) of plants derived from ray florets explants. Acute gamma only altered the flowering characteristics (except the response time). Leaf characteristics were altered by both mutagens. Both acute gamma and ion beam did not alter the morphology of plants derived from nodal explants but showed marked differences in the flowering characteristics especially on the number of buds and flower colours. Both mutagens altered the leaf characteristics such as leaf colour, blade length and shape. In conclusion, ray florets explants are more sensitive to both types of physical mutagens as compared to nodal explants. Ion beam was found to be more effective than acute gamma in inducing mutations for *Dendranthema grandiflora* cv. 'Reagan Red'.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**MUTASI ARUHAN KE ATAS KEKWA (*Dendranthema grandiflora*
Tzvelev) MENGGUNAKAN SINARAN GAMMA DAN ALUR ION**

Oleh

SHAKINAH SALLEH

Februari 2013

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Fakulti: Pertanian

Kekwa merupakan satu daripada bunga keratan iklim sederhana yang utama di Malaysia. Dalam industri pengeluaran bunga kekwa, permintaan yang tinggi tertumpu kepada kultivar baru yang mempunyai warna bunga dan bentuk yang lebih menarik disamping ciri-ciri baik yang lain. Untuk tujuan ini, penggunaan kaedah pembiakan *in vitro* dan mutasi aruhan menggunakan mutagen fizikal seperti sinar gamma dan alur ion didapati amat sesuai. Kajian ini dilakukan untuk menghasilkan kultivar baru bunga kekwa melalui penggunaan teknologi nuklear dan bioteknologi.

Dalam membangunkan protokol pembiakan *in vitro*, eksplan ranggi bunga telah dikulturkan di atas media Murashige and Skoog (MS) yang mengandungi 5 aras α -naphthaleneacetic acid (NAA) (0, 0.2, 0.5, 1.0 and 2.0 mg/L) dan empat aras 6-benzylaminopurine (BAP) (0, 0.5, 1.0 and 2.0 mg/L). Kepekatan optima bagi NAA dan BAP untuk membentuk kalus berlaku pada media yang mengandungi 0.5 mg/L NAA dan 0.5 mg/L BAP dan untuk penghasilan pucuk adalah optima pada media MS yang mengandungi 0.5 mg/L NAA + 2.0 mg/L BAP. Jumlah pucuk normal tertinggi berlaku pada media yang mengandungi 0.5 mg/L NAA dan 2.0 mg/L BAP. Oleh itu, media yang mengandungi 0.5 mg/L NAA dan 0.5 mg/L BAP telah digunakan bagi mengaruh pembentukan kalus daripada eksplan ranggi bunga. Kalus kemudiannya telah disubkulturkan beberapa kali pada media 0.5 mg/L NAA and 2.0 mg/L BAP bagi penggandaan pucuk.

Ujian radiosensitiviti (sensitiviti terhadap radiasi) telah dilakukan untuk menentukan dos optimum mutasi dengan menggunakan akut gama (0 hingga 120.0 Gy) dan alur ion (0 hingga 30.0 Gy) ke atas dua jenis eksplan (ranggi bunga dan tunas aksilari) berdasarkan kepada keupayaan kedua-dua jenis eksplan untuk hidup dan membentuk pucuk secara *in vitro*. Hasil kajian menunjukkan bahawa dos optimum untuk mengaruh mutasi ke atas eksplan ranggi bunga melalui akut gama adalah kurang daripada 41.36 Gy dan dengan alur ion beam adalah kurang daripada 10.48 Gy. Anggaran takat akhir penurunan (*shoulder end*) keluk regenerasi bagi eksplan ranggi bunga yang disinarkan dengan akut gama dan alur ion adalah pada dos 10.0 dan 2.0 Gy, secara berturutan. Dos optimum bagi mengaruh mutasi ke atas tunas

aksilari melalui gama akut adalah kurang 43.33 Gy dan dengan alur ion adalah kurang daripada 10.32 Gy. Anggaran takat akhir penurunan (*shoulder end*) keluk regenerasi bagi eksplan tunas aksilari yang disinarkan dengan akut gama dan alur ion adalah pada dos 10.0 dan 3.0 Gy. Kajian ini mendapati RBE (kecekapan biologi relatif) bagi eksplan ranggi bunga adalah 3.95 dan bagi eksplan tunas aksilari adalah 4.20.

Anak benih tersinar daripada eksplan ranggi bunga dan tunas aksilari telah ditanam di Cameron Highlands, Malaysia (ketinggian: 1500 m) bagi menilai perubahan morfologi dan tabiat pembungaan. 16 kategori pokok telah digunakan: pokok daripada eksplan ranggi bunga – rawatan gama akut (10.0 dan 20.0 Gy) dan rawatan alur ion (0.5, 1.0 and 2.0 Gy); pokok daripada eksplan tunas aksilari – rawatan gama akut (10.0, 20.0, 30.0, 40.0 and 60.0 Gy) dan rawatan alur ion (0.5, 1.0, 2.0, 3.0, 5.0 and 8.0 Gy). Keputusan menunjukkan bahawa alur ion telah mengubah keseluruhan morfologi pokok serta sifat pembungaan (kecuali masa untuk mula berputik) pokok daripada eksplan ranggi bunga sementara gama akut hanya mengubah sifat pembungaannya (kecuali masa untuk mula berputik). Kedua-dua mutagen telah berjaya mengubah ciri-ciri daun. Gama akut dan alur ion tidak berjaya mengubah morfologi pokok daripada eksplan tunas aksilari tetapi kedua-duanya mengubah ciri-ciri pembungaan terutamanya pada bilangan kudup dan warna bunga. Kedua-dua mutagen juga mengubah ciri-ciri daun seperti warna, panjang dan bentuk daun. Kesimpulannya, eksplan ranggi bunga adalah lebih sensitif kepada kedua-dua jenis mutagen fizikal berbanding eksplan tunas aksilari. Secara keseluruhan, alur ion adalah lebih efektif

berbanding gama akut dalam mengaruh mutasi pada *Dendranthema grandiflora* cv. 'Reagan Red'.



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I certify that a Thesis Examination Committee has met on 21 February 2013 to conduct the final examination of Shakinah binti Salleh on her thesis entitled "Mutation Induction of Chrysanthemum (*Dendranthema grandiflora Tzvelev*) using Gamma and Ion Beam Irradiation" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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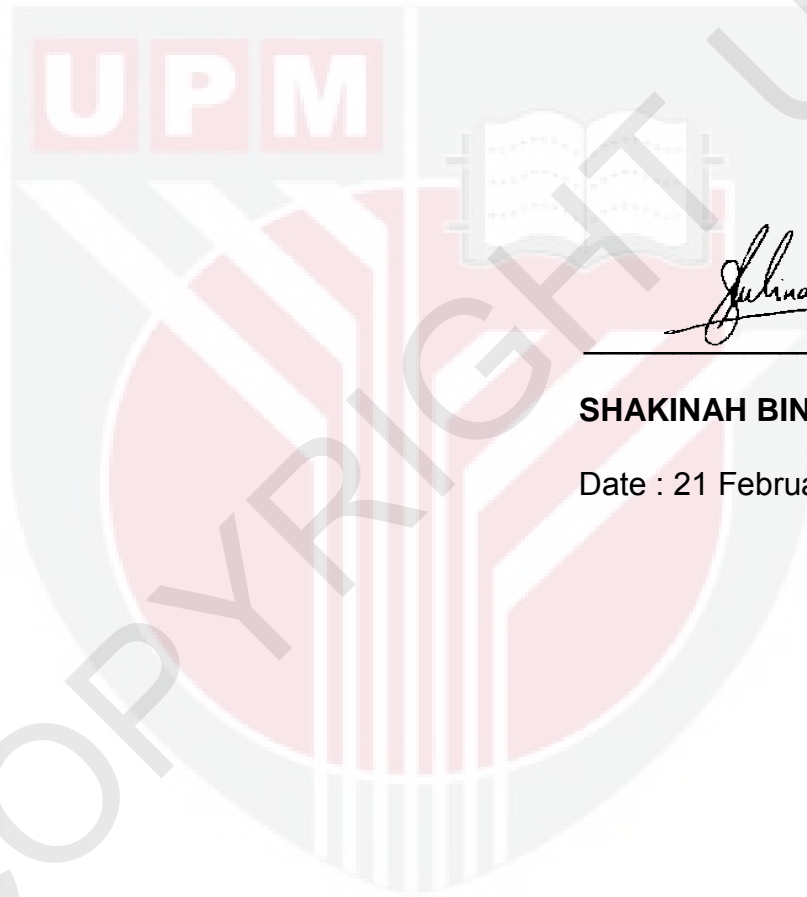
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DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



Shakinah

SHAKINAH BINTI SALLEH

Date : 21 February 2013



TABLE OF CONTENTS

	Page
DEDICATIONS	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xviii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxiii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 Overview of the chrysanthemum industry in Malaysia	3
2.2 Enforcement of Malaysian Protection of New Plant Varieties Regulation	5
2.3 <i>Dendranthema grandiflora</i> Tzvelev	6
2.3.1 Short day flowering response	6
2.3.2 Floral morphology	8
2.3.3 Leaf morphology	10
2.3.4 Plant growth habit	10
2.3.5 Flower colour	12
2.4 Propagation of chrysanthemum	14
2.5 <i>In vitro</i> propagation of chrysanthemum	14
2.5.1 Genotype	15
2.5.2 Types of explants	16
2.5.3 Culture media	19
2.5.4 Plant growth regulators (PGRs)	22
2.6 Mutation	26
2.6.1 Mutation induction	27
2.6.2 Mutagenic agent	28
2.7 Physical mutagen	28
2.7.1 Gamma rays	30
2.7.2 Ion beams	32
2.8 Radiosensitivity test	34
2.9 Screening and selection	36
2.10 Mutation breeding of chrysanthemum	39
2.10.1 Mutations in morphology of inflorescence and flower colour	40

2.10.2 Mutations in leaf characteristics	43
2.10.3 Mutations in growth habit	44
2.10.4 Mutations in physiological traits	45
3. EFFECTS OF NAA AND BAP ON CALLUS FORMATION AND SHOOT DEVELOPMENT USING RAY FLORETS EXPLANTS	47
3.1 Introduction	47
3.2 Materials and Methods	48
3.2.1 Study location	48
3.2.2 Plant materials	48
3.2.3 Surface sterilization	49
3.2.4 Medium preparation	50
3.2.5 Initiation of culture	50
3.2.6 Subculture and data collection	51
3.2.7 Experimental design and statistical analysis	53
3.3 Results and Discussion	53
3.3.1 Effects of NAA and BAP concentrations on callus growths	53
3.3.2 Effects of NAA and BAP concentrations on shoot regeneration	58
3.3.3 Effects of NAA and BAP concentrations on the number of normal shoots	63
3.4 Conclusion	64
4. RADIOSENSITIVITY TEST OF CHRYSANTHEMUM USING ACUTE GAMMA AND ION BEAM	66
4.1 Introduction	66
4.2 Materials and Methods	67
4.2.1 Irradiation	67
4.2.2 Preparation of plant materials	68
4.2.3 Phytosanitary certification	68
4.2.4 Subculture and data collection	69
4.2.5 Experimental design and data analysis	70
4.3 Results and Discussion	71
4.3.1 Lethal dose, regeneration dose and shoulder dose of ray florets explants	71
4.3.2 Lethal dose, regeneration dose and shoulder dose of nodal explants	79
4.3.3 Determination of relative biological effectiveness (RBE)	85
4.4 Conclusion	86

5. EFFECT OF ACUTE GAMMA AND ION BEAM IRRADIATION ON MORPHOLOGICAL AND FLOWERING CHARACTERISTICS OF CHRYSANTHEMUM	88
5.1 Introduction	88
5.2 Materials and Methods	89
5.2.1 Study location	89
5.2.2 Rooting, hardening and transplanting	91
5.2.3 Lighting and cultural practices	91
5.2.4 Data collection	92
5.2.5 Experimental design and data analysis	96
5.3 Results and Discussion	96
5.3.1 Morphological characteristics of plants derived from <i>in vitro</i> irradiated ray florets	96
5.3.2 Morphological characteristics of plants derived from <i>in vitro</i> irradiated nodal explants	100
5.3.3 Flowering characteristics of plants derived from <i>in vitro</i> irradiated ray florets	102
5.3.4 Flowering characteristics of plants derived from <i>in vitro</i> irradiated nodal explants	111
5.3.5 Leaf characteristics of plants derived from <i>in vitro</i> irradiated ray florets	121
5.3.6 Leaf characteristics of plants derived from <i>in vitro</i> irradiated nodal explants	130
5.3.7 Summarized of mutation effect for plants treated with acute gamma and ion beam	137
5.4 Conclusion	141
6. SUMMARY, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	143
REFERENCES	148
APPENDICES	166
BIODATA OF STUDENT	177