UNIVERSITI PUTRA MALAYSIA

IMPROVEMENT OF *IN VITRO* REGENERATION OF *BRASSICA OLERACEA* SUBSP*. ITALICA* CV. GREEN MARVEL THROUGH ORGANOGENESIS

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FP 2009 13

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By

SEYED ALI RAVANFAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

2009

In The Name of AllAH, the Most Gracious and the Most Merciful

Specially Dedicated

To

My beloved wife Shaghayegh

My parents Seyed Naser and Zarintaj

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agriculture Science

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April 2009

Chairman : Associate Professor Maheran Bt Abd. Aziz, PhD

Faculty : Agriculture

Broccoli (*Brassica oleracea* subsp. *italica*) cv. Green Marvel is an important vegetable crop grown in Cameron Highlands, Malaysia. Being an F_1 hybrid crop, selfing will result in segregation in the F_2 generation. In Malaysia, the production of hybrid seeds of cv. Green Marvel cannot be carried out due to the absence of parental stock material for hybridization. Therefore, the development of *in vitro* regeneration system is necessary to produce uniform and true-to-type plants of the cultivar. Currently, *in vitro* regeneration of other broccoli cultivars have been carried out but none has been reported on the cultivar Green Marvel.

This study was carried out with the main objective of establishing a protocol for plant regeneration of broccoli cv. Green Marvel through multiple shoot formation from hypocotyl, shoot tip and cotyledon explants, followed by rooting and

acclimatisation. All experiments were conducted and arranged in a Randomised Complete Block Design (RCBD).

In the experiment on multiple shoot formation from hypocotyl explants, the concentrations of 6-benzylaminopurine (BAP) alone or in combination with α-naphthaleneacetic acid (NAA) or indol-3-butyric acid (IBA) were assessed. In treatments containing only BAP, 3 mg/l of the plant growth regulator produced the highest percentage of explant with shoots (96.7%) and the highest mean number of shoots per explant (6.03). In treatments containing BAP and NAA, the highest percentage of explant with shoots (93.3%) occurred on 1 and 3 mg/l BAP while the highest mean number of shoots (6.0) was obtained on 5 mg/l BAP. In treatments containing BAP and IBA, BAP alone enhanced shoot multiplication. The highest percentage of hypocotyl explant with shoots (100%) and highest mean number of shoots produced per explant (6.33) occurred on 3 mg/l BAP. Therefore, 3 mg/l BAP was considered the most suitable for adventitious shoot formation from hypocotyl explants of broccoli cv. Green Marvel.

In the experiment on shoot multiplication from shoot tip explants, the concentrations of BAP either alone or in combination with NAA or IBA were assessed. The result showed that BAP at 5 mg/l produced the most number of shoots than any combinations of BAP with NAA or IBA. In treatments containing BAP and NAA, the highest percentage of explants with shoots (100%) and the highest mean number of shoots per explant (3.76) were on 5 mg/l BAP. In treatments containing BAP and

IBA, the highest percentage of shoot tip explants with shoots (96.7%) and the highest mean number of shoots produced per explant (2.83) also occurred on 5 mg/l BAP. BAP at 5 mg/l BAP was therefore the optimum hormonal treatment for shoot proliferation from shoot tip explants of broccoli cv. Green Marvel.

In the experiment on multiple shoot formation from cotyledon explant using different combinations of BAP and NAA, the highest percentage of explant producing shoots (53.3%) and the highest mean number of shoots produced per explant (0.43) occurred on treatment combination consisting of 3 mg/l BAP and 1 mg/l NAA. The treatment combination consisting of 3 mg/l BAP and 1 mg/l NAA is therefore the most suitable for high frequency of shoot regeneration from cotyledon explant of broccoli cv. Green Marvel.

In the experiment on rooting of shoots using different concentrations of auxins, the highest percentage of shoots with roots (100%) and the highest mean number of roots produced per explant (6.5) occurred on 0.2 mg/l IBA, but did not differ significantly compared to MSO medium. Meanwhile the highest mean length of root (2.46 cm) was attained on hormone-free MS medium (MSO). Thus, MSO medium was considered the most suitable medium for rooting of broccoli cv. Green Marvel shoots.

In the acclimatisation study, various types of potting mixture were assessed. The highest percentage of survival (83.3%) occurred on medium containing sand+ soil

(1:1) while the maximum root length (4.37 cm) and the highest plant height (7.87 cm) were attained in potting medium containing peatmoss+ perlite+ vermiculite (3:1:1). The mixture of peatmoss+ perlite+ vermiculite (3:1:1) was therefore considered the most suitable potting medium for broccoli cv. Green Marvel. Plantlets were well rooted and ready for field transfer after four weeks of acclimatisation.

The study on multiple shoot formation concluded that BAP alone at 3 and 5 mg/l were more effective in inducing shoot proliferation on the hypocotyl and shoot tip explants of broccoli cv. Green Marvel, respectively, while BAP in combination with NAA was required for effective shoot formation from the cotyledon explants. The study on rooting showed that MSO was most suitable for high percentage of root formation, mean number of roots produced per explant and root length attained. In the acclimatisation study, the most suitable potting mixture consisted of peatmoss+ perlite+ vermiculite.

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

PEMBAIKAN REGENERASI *IN VITRO BRASSICA OLERACEA* SUBSP. *ITALICA* CV. GREEN MARVEL MELALUI ORGANOGENESIS

Oleh

SEYED ALI RAVANFAR

April 2009

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Brokoli (*Brassica oleracea* subsp. *italica*) cv. Green Marvel adalah tanaman sayuran yang penting di Cameron Highlands, Malaysia. Oleh kerana tanaman ini jenis hibrid F_1, kacukan sendiri akan menghasilkan segregasi di peringkat generasi F_2. Di Malaysia, pengeluaran biji benih hibrid cv. Green Marvel tidak dapat dilakukan kerana ketiadaan bahan stok induk untuk penghibridan. Oleh itu, pembentukan sistem regenerasi *in vitro* diperlukan untuk pengeluaran anak pokok cv. Green Marvel yang sekata dan tulen. Pada masa ini, regenerasi *in vitro* kultivar brokoli yang lain telah dijalankan tetapi belum ada laporan mengenai brokoli cv. Green Marvel.

Kajian ini dijalankan dengan objektif utama untuk menghasilkan suatu protokol regenerasi untuk cv. Green Marvel melalui pembentukan pucuk berganda daripada hipokotil, tunas hujung, dan eksplan kotiledon, diikuti dengan pengakaran dan

aklimatisasi. Kesemua eksperimen dilakukan dan diatur menggunakan Rekabentuk Blok Penuh Berawak (RCBD).

Dalam eksperimen pembentukan pucuk berganda daripada eksplan hipokotil, kepekatan 6-benzilaminopurin (BAP) secara sendiri atau gabungan dengan asid α-naftalenasetik (NAA) atau asid indol-3-butirik (IBA) telah dikaji. Dalam rawatan yang mengandungi BAP sahaja, 3 mg/l bahan pengawalatur tersebut menghasilkan peratus eksplan membentuk pucuk yang tertinggi (96.7%) dan min bilangan pucuk per eksplan yang tertinggi (6.03). Bagi rawatan yang mengandungi BAP dan NAA, peratus eksplan membentuk pucuk yang tertinggi (93.3%) berlaku pada rawatan 1 dan 3 mg/l BAP, manakala min bilangan pucuk per eksplan yang tertinggi (6.0) adalah dari rawatan dengan 5 mg/l BAP. Bagi rawatan yang mengandungi BAP dan IBA, rawatan BAP bersendiri meningkatkan penggandaan tunas. Peratus eksplan membentuk pucuk yang tertinggi (100%) dan min bilangan pucuk per eksplan tertinggi (6.33) berlaku pada rawatan dengan 3 mg/l BAP. Oleh itu, 3 mg/l BAP dianggap yang paling sesuai untuk pembentukan pucuk adventisius daripada eksplan hipokotil brokoli cv. Green Marvel.

Dalam eksperimen pembentukan pucuk berganda daripada eksplan tunas hujung, kepekatan BAP sama ada dibekalkan secara sendiri atau gabungan dengan NAA atau IBA telah dikaji. Hasil kajian menunjukkan BAP pada kepekatan 5 mg/l menghasilkan pucuk yang paling banyak berbanding gabungan BAP dengan NAA atau IBA. Bagi rawatan yang mengandungi BAP dan NAA, peratus eksplan

membentuk pucuk tertinggi (100%) dan min bilangan pucuk per eksplan tertinggi (3.76) adalah pada rawatan dengan 5 mg/l BAP. Bagi rawatan yang mengandungi BAP dan IBA, peratus eksplan membentuk pucuk tertinggi (96.7%) dan min bilangan pucuk per eksplan tertinggi (2.88) juga terhasil pada rawatan dengan 5 mg/l BAP. BAP pada kepekatan 5 mg/l adalah rawatan hormon yang optimum untuk proliferasi pucuk daripada eksplan tunas hujung brokoli cv. Green Marvel.

Dalam eksperimen pembentukan pucuk berganda daripada eksplan kotiledon menggunakan gabungan BAP dan NAA yang berbeza, peratus eksplan membentuk pucuk tertinggi (53.3%) dan min bilangan pucuk per eksplan tertinggi (0.43) berlaku pada kombinasi rawatan yang mengandungi 3 mg/l BAP dan 1 mg/l NAA. Rawatan kombinasi 3 mg/l BAP dan 1 mg/l NAA adalah yang paling sesuai untuk regenerasi yang tinggi daripada eksplan kotiledon brokoli cv. Green Marvel.

Dalam eksperimen pengakaran pucuk pada kepekatan auksin yang berbeza, peratus pucuk berakar tertinggi (100%) dan min bilangan akar per pucuk tertinggi (6.5) berlaku pada 0.2 mg/l IBA, tetapi tidak berbeza dengan signifikan berbanding medium MSO. Manakala purata panjang akar tertinggi (2.46 cm) dihasilkan pada medium MS tanpa hormon (MSO). Maka, medium MSO dianggap yang paling sesuai untuk pengakaran pucuk brokoli cv. Green Marvel.

Dalam kajian aklimatisasi, pelbagai jenis medium pemasuan telah dikaji. Peratus kebolehan hidup tumbuhan tertinggi (83.3%) berlaku pada medium yang

mengandungi pasir+tanah (1:1), manakala panjang maksimum akar (4.37 cm) dan ketinggian tumbuhan yang tertinggi (7.87 cm) dihasilkan pada medium pemasuan yang mengandungi peatmoss+perlit+vermikulit (3:1:1). Medium yang mengandungi peatmoss+perlit+vermikulit (3:1:1) dianggap sebagai medium pemasuan yang paling sesuai. Anak pokok berakar dengan baik dan sedia untuk dialih ke lapangan setelah empat minggu aklimatisasi.

Daripada kajian ke atas pembentukan pucuk berganda dapat disimpulkan bahawa BAP pada 3 dan 5 mg/l adalah lebih efektif dalam meningkatkan proliferasi pucuk masing masing pada eksplan hipokotil dan tunas hujung brokoli, manakala kombinasi BAP dan NAA diperlukan untuk pembentukan pucuk yang berkesan daripada eksplan kotiledon. Kajian tentang pengakaran menunjukkan medium MSO adalah paling sesuai untuk menghasilkan peratus pengakaran, min bilangan akar per eksplan serta panjang akar tertinggi. Bagi kajian aklimatisasi, campuran pemasuan paling sesuai terdiri daripada campuran peatmoss+perlit+vermikulit.

ACKNOWLEDGMENTS

IN THE NAME OF ALLAH

Thanks and Praise is due to Allah who gave me strength and determination to complete my study. I would like to express my gratitude and sincere thanks to those who have helped me in preparing and conducting the research and finishing this thesis. Therefore, it pleases me to express my deep gratitude to them.

I would like to thank the chairman of my supervisory committee, Associate Professor Dr. Maheran Abdul Aziz, for her supervision, kind guidance and advice in the completion of the thesis. I appreciate her patience and sincere approach to motivate, help, advice and guide me to finish my study. Thanks are also extended to my committee member, Associate Professor Dr. Mihdzar Abdul Kadir, and I am grateful to Mr. Azmi Abdul Rashid, for his continuous help and suggestion while I was carrying out my project. The experience and knowledge, gained has resulted in a significant and positive effect on my work.

Not forgetting my dearest friends, especially Arash Raafat, Hossein Torabi, Mohammad Javadi, Fatemeh Hadadi and Hossein Kamaladini. Thanks for your support and encouragement in times of need.

Last but not least, thanks to my beloved wife Shaghayegh, my father Naser, my mother Zarin and my brothers Roham and Mohammad. I owe you every thing.

I certify that a Thesis Examination Committee has met on 13 April 2009 to conduct the final examination of Seyed Ali Ravanfar on his thesis entitled "Improvement of *In Vitro* Regeneration of *Brassica oleracea* Subs. *Italica* Cv. Green Marvel Through Organogenesis" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the student be awarded the Master of Science.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment for the degree of Master of Agricultural Science. The members of the Supervisory committee are as follows:

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Date: 8 June 2009

DECLARATION

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

_____

Seyed Ali Ravanfar

Date:

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BAP	6-benzylaminopurine
BA	N^6-benzyladenine
cm	centimeter
cv	cultivar
2, 4-D	2, 4-dichlorophenoxyacetic acid
2-ip	γ, γ-(dimethylallyl) aminopurine
α	level of significance
DNMRT	Duncan's New Multiple Range Test
e.g.	exempli gratia (for example)
et al.	et alia
etc.	et cetera
F_1	First filial generation
g	gram
g/l	gram per litre

H	hour (s)
IAA	indol-3-acetic acid
IBA	indol-3-butyric acid
KIN	kinetin
KNO_3	potassium nitrate
L	liter
M	molar
mg	milligram
mg/l	milligram per liter
ml	milliliter
mm	millimeter
μ mol m^{-2} s^{-1}	micromole per meter square per second
μM	micromolar, 10^{-3} mM
MS	Murashige and Skoog
NAA	α-naphthalene acetic acid
NO_3	nitrate
PGR	Plant Growth Regulator

pH	-log (H+)
RCBD	Randomized Complete Block Design
sp.	species
TDZ	thidiazuron
var	variety

CHAPTER 1

INTRODUCTION

Broccoli, (*Brassica oleracea* subsp. *italica*), a cultivar of wild cabbage, originated along the northern and western coasts of the Mediterranean Sea. It is a hardy vegetable which develops best during the cool seasons of the year. When broccoli plants of most varieties are properly grown and harvested, they can yield over an extended period of time, because the side heads develop after the large, central head is removed. Two crops per year (spring and fall) may be grown. New heat tolerant varieties allow broccoli to be produced in all but the hottest parts of the season (Wolford and Banks, 2000).

Other cultivar groups of *Brassica oleracea* include: cabbage (Capitata Group), cauliflower (Botrytis Group) kale and collard greens (Acephala Group), kohlrabi (Gongylodes Group), and brussels sprouts (Gemmifera Group). Chinese broccoli (Alboglabra Group) is also a cultivar group of *Brassica oleracea*. The edible part of the broccoli plant is the tender stem and unopened flower buds. They are a good source of vitamin A, D, C, calcium, and riboflavin or B_2. It is usually boiled or steamed, but may be eaten raw and has become popular as a raw vegetable (Diindolylmethane Information Resource Center, 2007).

Broccoli is high in soluble fiber with potent anti-cancer properties which include diindolylmethane and selenium. The 3' diindolylmethane found in broccoli is a potent