UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MICROPROPAGATION SYSTEM AND REDUCTION OF HYPERHYDRICITY IN REGENERANTS OF CARNATION (DIANTHUS CARYOPHYLLUS L. CV. MALDIVES)

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BUDI WINARTO

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

October 2002

Dedicated to:

My wife Nuri Rianti M

My son Yoga Aninditya

My father Sugiyono (Alm.)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirement for the degree of Master of Science

DEVELOPMENT OF MICROPROPAGATION SYSTEM AND REDUCTION OF HYPERHYDRICITY IN REGENERANTS OF CARNATION (*DIANTHUS CARYOPHYLLUS* L. CV. MALDIVES)

By

BUDI WINARTO

October 2002

Chairperson : Dr. Maheran Abdul Aziz

Faculty : Agriculture

This study was carried out with the main objectives of developing a micropropagation system for *Dianthus caryophyllus* cv. Maldives and reducing hyperhydricity for healthy shoot production. The development of a micropropagation system included selection of explant and combination-concentration of growth regulators, optimization, multiplication of shoots, rooting and acclimatization. Hyperhydricity study included selection of types of closure and gelling agents, application of ventilated culture vessel, multiplication of recovered shoots and acclimatization of recovered plantlets. The experiment was factorial arranged in a randomized complete block design with four replications. Each treatment consisted of twelve explants per replicate.

In axillary proliferation of shoots using two types of explant and five combination-concentrations of growth regulators, node explant placed on MS medium containing 1.0 mg/L BA and 0.1 mg/L NAA was the most suitable combination in stimulating

high axillary shoot production with low rate of hyperhydricity. Lowering the concentration of NAA from 0.1 mg/L to 0.05 mg/L in combination with 1.0 mg/L BA in the optimization experiment improved axillary shoot production from 4.9 to 5.6 shoots per explant and reduced hyperhydricity to less than 30%.

In adventitious shoot formation from three explants placed on five concentrations of BA and NAA, the first young and fully developed leaves placed on MS medium supplemented with 0.1 mg/L BA and 0.01 mg/L NAA was the most suitable combination in inducing high adventitious shoot formation (43.3%) with lower hyperhydricity (60.0%) compared to other combinations tested.

MS medium containing 1.0 mg/L BA with 0.05 mg/L NAA and 0.5 mg/L BA with 0.1 mg/L NAA were the most appropriate media in inducing high shoot multiplication, whereas MS medium supplemented with 0.1 mg/L BA with 0.02 mg/L NAA and 0.1 mg/L BA with 0.01 mg/L NAA were most suitable in producing good quality shoots for rooting. High production of good quality shoots were produced only after the first subculture and reduced in the subsequent subcultures.

Half-strength MS medium was appropriate in stimulating high root formation from both axillary proliferated shoots and shoots derived adventitiously. Based on economic consideration the use of carton paper as vessel closure and 7 g/L agar were applied in the next experiment. The treatment induced 87.5% of root formation with high number of roots per explant (6.5 roots) and 2.22 cm root length with good quality roots and shoots.

All potting media for acclimatization, except jiffy-7, stimulated high survival rate (80% - 100%) for both plantlets derived from axillary shoots and those derived from adventitious shoots. In the incubation room, paddy charcoal indicated high survival rate (97.9%) with high leaf chlorophyll content in both types of plantlets, but in the screen house, a mixture of kossas peat + paddy charcoal induced the highest survival rate (100%) and leaf chlorophyll content (0.3046 mg/mg). The potted plants flowered within 4.5 to 5 months after acclimatization.

Plastic wrap in combination with agar Type 900 were the most appropriate treatment in obtaining healthy axillary shoots. The combination exhibited lower hyperhydricity (6.0%) with higher chlorophyll content (0.1288 mg/mg) and maintained a low reduction of leaf chlorophyll content at 58.5% in field derived node explants. In node explants derived from hyperhydritised shoots, the combination reduced hyperhydricity of shoots to 22.7% and increased leaf chlorophyll content to 62.0%. Whereas MS medium containing 0.1 mg/L BA and 0.01 mg/L NAA with single layer of carton paper as closure based on economic consideration was appropriate in the recovery of normal shoots from succulent condition with lower hyperhydricity (67.1%). The recovered shoots were able to multiply and produced good quality axillary shoots until the third sub-culture. They were easily rooted and successfully acclimatized in paddy charcoal and kossas peat + soil (1:1, v/v) with high survival rate (80-100%). The plants were potted and indicated a normal growth and flowered 4 to 5 months after acclimatization.

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

PERKEMBANGAN SISTEM PEMBIAKAN MIKRO DAN PENGURANGAN HIPERHIDRISITI REGENERAN BUNGA TELUKI *(DIANTHUS CARYOPHYLLUS* L. CV. MALDIVES)

Oleh:

BUDI WINARTO

Oktober 2002

Pengerusi : Dr. Maheran Abdul Aziz

Fakulti : Pertanian

Penyelidikan ini dilaksanakan dengan objektif utama untuk menghasilkan satu sistem pembiakan mikro untuk *Dianthus caryophyllus* L. cv. Maldives dan mengurangkan hiperhidrisiti bagi pengeluaran pucuk yang sihat. Kajian sistem pembiakan mikro mencakupi pemilihan eksplan dan kombinasi kepekatan pengawalatur tumbesaran, pengoptimuman, penggandaan pucuk, pengakaran, dan aklimatisasi. Kajian mengenai hiperhidrisiti mencakupi pemilihan jenis penutup kelalang dan agen pemadat, penggunaan kelalang kultur berventilasi, penggandaan pucuk yang pulih, dan aklimatisasi pokok yang pulih daripada hiperhidrisiti. Kajian berfaktor ini telah dijalankan menggunakan rekabentuk rawak berblok penuh dengan empat replikasi. Setiap rawatan mengandungi duabelas eksplan per replikasi.

Bagi pembentukan tunas aksil dengan menggunakan dua jenis eksplan dan lima kombinasi kepekatan pengawal atur tumbesaran, nod eksplan yang diletakkan pada medium MS yang mengandungi 1.0 mg/L BA dan 0.1 mg/L NAA adalah kombinasi

yang paling sesuai bagi merangsang pengeluaran pucuk aksil yang tinggi dengan kadar hiperhidrisiti yang rendah. Pengurangan kepekatan NAA dari 0.1 mg/L kepada 0.05 mg/L dalam medium dikombinasikan dengan 1.0 mg/L BA bagi eksperimen pengoptimuman meningkatkan bilangan pucuk aksil yang diperolehi daripada 4.9 kepada 5.6 pucuk per eksplan dan menurunkan kadar hiperhidrisiti kepada kurang daripada 30%.

Bagi pembentukan pucuk adventitius pula, dari tiga jenis eksplan yang diletakkan pada lima kepekatan kombinasi BA dan NAA, eksplan daun pertama yang masih muda dan berkembang yang diletakkan pada medium MS yang mengandungi 0.1 mg/L BA dan 0.01 mg/L NAA adalah kombinasi yang paling sesuai bagi merangsang pembentukan pucuk adventitius yang tinggi (43.3%) dan kadar hiperhidrisiti yang lebih rendah (60.0%) berbanding dengan kombinasi lain.

Media MS yang mengandungi 1.0 mg/L BA dengan 0.05 mg/L NAA dan 0.5 mg/L BA dengan 0.1 mg/L NAA adalah media yang paling sesuai bagi merangsang penggandaan pucuk yang tinggi. Manakala media MS yang dibekalkan dengan 0.1 mg/L BA dengan 0.02 mg/L NAA dan 0.1 mg/L BA dengan 0.01 mg/L NAA adalah yang paling sesuai bagi menghasilkan kualiti pucuk yang baik bagi pengakaran. Pengeluaran pucuk yang tinggi dan berkualiti baik telah dihasilkan sehingga subkultur pertama tetapi menurun pada subkultur seterusnya.

Medium MS berkepekatan separuh adalah sesuai bagi merangsang pembentukan akar yang tinggi pada pucuk yang diperolehi daripada tunas aksil ataupun pucuk yang diperolehi secara adventitius. Berasaskan pertimbangan ekonomi penggunaan satu

lapis kertas karton sebagai penutup kelalang dan 7 g/L agar telah digunakan bagi kajian selanjutnya. Rawatan tersebut menghasilkan 87.5% pembentukan akar dengan jumlah akar per eksplan yang tinggi (6.5) dan panjang akar (2.22) dengan kualiti akar dan pucuk yang baik.

Semua media pasuan untuk kegunaan akilmatisasi, kecuali Jiffy 7, merangsang keupayaan hidup yang tinggi (80%-100%) dan baik bagi anak pokok yang diperolehi daripada tunas aksil maupun yang diperolehi daripada tunas adventitius. Dalam bilik inkubasi, arang sekam telah memberikan keupayaan hidup pokok (97.9%) dan kandungan klorofil daun telah didapati tinggi bagi kedua-dua jenis anak pokok, tetapi rawatan dalam rumah skrin, campuran daripada kossas peat + arang sekam merangsang keupayaan hidup tertinggi (100%) dan kadar klorofil (0.3046 mg/mg) yang paling tinggi. Anak pokok yang dipasukan berbunga dalam masa 4.5 sehingga 5 bulan selepas aklimatisasi.

Kombinasi pembungkus plastik dengan agar Type 900 adalah rawatan yang sesuai bagi mendapatkan pucuk aksil yang lebih sihat. Kombinasi ini menunjukkan kadar hiperhidrisiti yang rendah (6.0%) dengan kadar klorofil daun yang lebih tinggi (0.1288 mg/mg) dan mengekalkan penurunan klorofil yang rendah sehingga 58.5% pada nod eksplan yang diperolehi daripada pokok lapangan. Pada nod yang diperolehi daripada pokok yang hiperhidrisiti, kombinasi rawatan tersebut mengurangkan hiperhidrisiti pucuk kepada 22.7% dan mempertingkatkan kadar klorofil daun kepada 62.0%. Media MS yang mengandungi 0.1 mg/L BA dan 0.01 mg/L NAA dengan satu lapis kertas karton sebagai penutup adalah sesuai bagi membaik-pulihkan pucuk daripada keadaan sukulen dengan hiperhidrisiti yang lebih rendah (67.1%). Pucuk

yang pulih didapati masih mampu mengganda dan menghasilkan pucuk aksil yang berkualiti sehingga subkultur yang ketiga. Didapati pucuk mudah diakarkan dan berjaya diaklimatisasi dalam arang sekam dan kossas peat ditambah tanah (1:1, v/v) dengan keupayaan hidup yang tinggi (80-100%). Anak pokok telah dipasukan dan menunjukkan satu pertumbuhan yang normal. Pokok berbunga dalam masa 4 sehingga 5 bulan setelah aklimatisasi.

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I certify that an Examination Committee met on 17th October 2002 to conduct the final examination of Budi Winarto on his Master of Science thesis entitled "Development of Micropropagation System and Reduction of Hyperhydricity in Regenerants of Carnation (*Dianthus caryophyllus* L. cv. Maldives)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 23 OCT 2002

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

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DECLARATION

I hereby declare that this thesis is based on my original work except 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.

BUDI WINARTO

Date: 23-10-2002

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVATIONS/NOTATIONS

A_{647}	- absorbance at 647 nanometers
A_{664}	- absorbance at 664 nanometers
AARD	- Agency for Agriculture Research and Development
ABA	- abscisic acid
ACI	- agar concentration increase
ANOVA	- analysis of variance
BA/BAP	- 6-benzyladenine/benzylaminopurine
Br	- boron
°C	- centigrade
Ca	- calcium
$CaCl_2$	- calcium chloride
CaMV	- Carnation Mottle Virus
Cl	- chloride
cm	- centimeter
CO_2	- carbon dioxide
C/N	- carbon-nitrogen ratio
CPA	- *p*-chlorophenoxy acetic acid
CRSV	- Carnation Ring Spot Virus
c.v.	- cultivar
CVMV	- Carnation Vein Mottle Virus
2,4-D	- 2,4-dichlorophenoxy acetic acid
DNMRT	- Duncan's New Multiple Range Test
DW	- dry weight
e.g.	- exampli gracia (for example)
ER	- evaporation rate
et al	- et alia
etc	- et cetere
FAA	- formaldehyde-glacial acetic acid-alcohol
f.sp	- forma specialist
FW	- fresh weight