UNIVERSITI PUTRA MALAYSIA

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By

TENGKU LAILA KAMALIAH

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In the name of Allah, The Most Beneficent, The Most Merciful

Say: "Truly, my prayer and my service of sacrifice and my life and my death,

are (all) for Allah, the Cherisher of the Worlds:

(Q.S. 6:162)

to :

My beloved husband,

Irdam Halim

My parents (H. Tengku Mustafa Kamal and Hj. Tengku Cici Hazariah)

My parents-in-law (Abdul Halim and Fatimah Kala)

My brother (Tengku Julian Mustafizar)

mg/L BA + 30 g/L sucrose can be used for subculturing multiple shoots derived from shoot tip explants. Subculturing should be limited to three subcultures.

There were two treatment options for induction of multiple shoots from nodal explants: 5 mg/L $AgNO_3$ or 0.1 mg/L NAA (naphthalene acetic acid), combined with 3 mg/L BA and 45 g/L sucrose. For subculturing of multiple shoots derived from nodal explants, medium containing 2 mg/L BA + 30 g/L sucrose can be used. Subculturing should be limited to three subcultures.

The specific objectives of the study on shoot elongation, rooting and acclimatisation were: to find out suitable medium for shoot elongation, to establish suitable media type and concentration of auxin for rooting, to determine an appropriate medium for acclimatisation of micropropagated plantlets and to determine stomata characteristic and number of stomata per mm^2 of leaves of plantlets in test tube, under acclimatised and *ex vitro* condition. All experiments were conducted and arranged in a Completely Randomised Design (CRD), except for experiment on "Effect of Growth Media on *Ex Vitro* Performance", which was conducted in a Randomised Complete Block Design (RCBD).

The results showed that full strength MS medium without growth regulators was suitable for shoot elongation and rooting. Soil and oil palm frond compost mixture (1:1, v/v) was a suitable medium for acclimatisation. Plantlets were well rooted and ready to be transferred to field condition after

Experiments were also conducted with the specific objectives to induce callus from cotyledon explants and to study the effect of 2,4-dichlorophenoxy acetic acid (2,4-D), kinetin and sucrose on callus formation. The best treatment for callus formation was 1 mg/L 2,4-D with 30 g/L sucrose. Higher level of 2,4-D decreased percentage of callus formation. The presence of kinetin at all concentrations tested and the addition of sucrose above 30 g/L decreased the production of callus.

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

REGENERASI PLANTLET DAN INDUKSI KALUS KOBIS HIBRID (*BRASSICA OLERACEA* L. VAR. *CAPITATA* CV. TROPICANA)

Oleh

TENGKU LAILA KAMALIAH

Oktober 2002

Pengerusi : Azmi Abdul Rashid, M.Phil.

Fakulti : Pertanian

Penyelidikan ini dilaksanakan dengan objektif utama untuk mewujudkan sebuah protokol untuk meregenerasi anak pokok *Brassica oleracea* L. subsp. *capitata* cv. Tropicana melalui penggandaan tunas dan untuk menginduksi kalus dengan menggunakan eksplan kotiledon. Kajian ini terdiri dari beberapa bahagian iaitu penggandaan tunas; pemanjangan pucuk, pengakaran dan aklimatisasi: dan induksi kalus.

Objektif khas daripada kajian penggandaan tunas adalah untuk menentukan pengawalatur tumbesaran dan kepekatan sukrosa dan argentum nitrate ($AgNO_3$) yang sesuai untuk menginduksikan tunas berganda dan untuk mengkaji kesan daripada 6-benzylaminopurina (BA) terhadap pengggandaan tunas. Kesemua eksperimen dilaksanakan dan disusun mengikut Rekabentuk Rawak Lengkap.

Hasil kajian menunjukkan bahawa BA lebih baik dari kinetin untuk menggalakkan pembentukan tunas berganda daripada eksplan mercu pucuk. Argentum nitrat ($AgNO_3$) pada kepekatan 0.5 mg/L dikombinasikan bersama 3 mg/L BA dan 45 g/L

sukrosa meningkatkan penggandaan tunas. Medium yang mengandungi 0.5 mg/L BA + 30 g/L sukrosa boleh digunakan untuk pengsubkulturan. Pengsubkulturan sebaiknya dihadkan sehingga tiga kali sahaja.

Ada dua pilihan rawatan untuk menggalakkan penggandaan tunas daripada eksplan buku iaitu: 5 mg/L $AgNO_3$ atau 0.1 mg/L NAA (asid naptalena asetik), dikombinasikan bersama 3 mg/L BA dan 45 g/L sukrosa. Bagi pengsubkulturan tunas daripada eksplan buku, medium yang mengandungi 2 mg/L BA + 30 g/L sukrosa boleh digunakan. Subkultur sebaiknya dihadkan sehingga tiga kali sahaja.

Objektif khas daripada kajian pemanjangan pucuk, pengakaran dan aklimatisasi adalah untuk mengetahui medium yang sesuai untuk menggalakkan pemanjangan pucuk, untuk mewujudkan jenis medium dan kepekatan auksin yang sesuai untuk pengakaran, untuk menentukan medium yang sesuai untuk mengaklimatisasi anak pokok yang dibiak secara pembiakan mikro dan untuk menentukan ciri-ciri stomata dan bilangan stomata per mm^2 bagi daun anak pokok tabung uji, yang telah diaklimatisasi dan ditanam dipersekitaran *ex vitro*. Kesemua eksperimen telah dilaksanakan dan disusun mengikut Rekabentuk Rawak Lengkap, kecuali eksperimen "Kesan Medium Pertumbuhan terhadap Prestasi Anak Pokok di Persekitaran *Ex Vitro*", yang disusun mengikut Rekabentuk Rawak Berblok Penuh.

Hasil kajian menunjukkan bahawa medium MS berkepekatan penuh tanpa pengawalatur tumbesaran sesuai digunakan untuk menggalakkan pemanjangan pucuk dan pengakaran. Campuran tanah dan kompos pelepah kelapa sawit (1:1, v/v) adalah medium yang sesuai untuk aklimatisasi. Anak pokok berakar dengan baik dan

tersedia untuk dipindahkan ke kondisi ladang sesudah 4 minggu dalam kondisi aklimatisasi.

Kajian telah juga dilaksanakan dengan objektif khas untuk menginduksi kalus dari eksplan kotiledon dan untuk mengkaji kesan 2,4-asid diklorofenoksi asetik (2,4-D), kinetin dan sukrosa terhadap pembentukan kalus. Rawatan yang terbaik untuk pembentukan kalus adalah 1 mg/L 2,4-D bersama 30 g/L sukrosa. Peningkatan kepekatan 2,4-D menurunkan peratus pembentukan kalus. Kehadiran kinetin pada seluruh kepekatan yang diuji dan penambahan sukrosa melebihi 30 g/L menurunkan kadar pembentukan kalus.

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LIST OF ABBREVIATIONS/NOTATIONS

A_{647}	absorbance at 647 nanometers
A_{664}	absorbance at 664 nanometers
ACCA	1-amino-cyclopropane-1-carboxylic-acid
$AgNO_3$	silver nitrate
$Ag_2S_2O_3$	silver thiosulfate
2,4-D	2,4-dichlorophenoxyacetic acid
2-iP	γ,γ-(dimethylallyl) aminopurine
α	level of significance
ANOVA	analysis of variance
Arc sin	$\sin^{-1}(X)$
AVG	aminoethoxyvinylglycine
BA	6-benzylaminopurine
cm	centimeter
CRD	completely randomised design
cv.	cultivar
DNMRT	duncan new multiple range test
e.g.	exempli gratia (for example)
et al.	et alia
etc.	et cetera
FAA	formaldehyde-glacial acetic acid alcohol
F_1	first filial generation
Fe-EDTA	iron ethylene diamine tetra acetic acid
g	gram

g/L	gram per litre
IAA	indole acetic acid
IBA	indole butyric acid
mg	milligram
mg/L	milligram per litre
mg/mg	milligram per milligram
mL	millilitre
mm	millimetre
µmol m^{-2} s^{-1}	micromole per metre square per second
MS	Murashige and Skoog (1962) basal medium
N	nitrogen
NAA	naphthalene acetic acid
NCSS	Number Cruncher Statistical System
%	percent
pH	$-\log (H^+)$
RCBD	randomised complete block design
SAM	S-adenosylmethionin
sp.	species
√	square root
var	variety
v/v	volume to volume

CHAPTER 1

GENERAL INTRODUCTION

Background

Cabbage is among the important vegetable crops in the world. It is cultivated in many countries in the northern and southern hemisphere. Asia clearly leads producing 58% of the world cabbage production (Rubatzky and Yamaguchi, 1997). In tropical Asia the consumption of early maturing cabbage, with firm and round heads, is rapidly increasing.

In Malaysia, the annual consumption of cabbage per capita is the second highest in 1991 after Chinese mustard (Kamil *et al.*, 2001). Cabbage is regarded as a good source of calcium (22.8 mg/100 g fresh weight) and dietary fibre (2.5 g/100 g fresh weight) (Lucarini *et al.*, 1999). In addition, it possesses anticarcinogenic properties. Two bioactive sulfur-containing phytochemicals, glucosinolates and S-methyl cysteine sulfoxide, which is formed in cabbage appear to have potential value as cancer chemopreventive agents (Verhoeven *et al.*, 1997).

In Malaysia, seeds of *Brassica*, including cabbage, are the second most commonly imported vegetable seeds. The total amount of *Brassica* seeds imported was about 391,246 kg and exceeding RM 300,000 in 1998 (Puteh and Abidin, 1999). Most of the *Brassica* seeds imported were F1 hybrids.

Malaysia imports not only seeds of cabbage but also fresh cabbage from Indonesia and Australia. Quantity import of fresh cabbage increases from year to year. Malaysia imported 16,770.76 ton cabbage in 1980, 28,835.12 ton in 1990 and 30,596.06 ton in 1995 (Lin, 1997).

The production of hybrid seeds of cabbage is almost impossible in Malaysia because of climatic hindrance. Vernalisation is needed for the induction of flowering. Moreover, the parent material for hybridisation and production of hybrid seed in Malaysia is not available. Therefore an alternative method of producing and maintaining plants having F_1 hybrid characteristic is through *in vitro* culture. On the other hand, high regeneration capability through tissue culture system is crucial to the success of *Brassicas* genetic engineering through genetic transformation (Takasaki *et al.*, 1997).

In vitro plantlet regeneration can be achieved through axillary proliferation, adventitious shoot formation and somatic embryogenesis. Axillary proliferation can be obtained by using shoot tip culture or axillary bud culture. Adventitious shoot formation and somatic embryogenesis can be achieved directly from explants or indirectly via callus formation. Indirect somatic embryogenesis is the most promising regeneration system for future genetic improvement of cabbage. Genetic improvement of cabbage through conventional breeding is hindered by sexual incompatibility. Therefore, the development of protocol to induce embryogenic callus and somatic embryogenesis are needed to obtain pest and disease resistant cabbage in the future.

Plate I shows the possible steps of clonal propagation of white cabbage, beginning from germination of seed, multiple shoot induction, rooting, acclimatisation and field planting. In future, this protocol can also be used for cabbage genetic improvement.

Objectives

The protocol for *in vitro* plantlet regeneration of white cabbage cv. Tropicana has not been developed. In addition, regeneration of whole plants from cultured tissues or cells is a prerequisite for successful applications of *in vitro* techniques for gene transfer and mass propagation. Therefore, the objectives of this research were: (a) to establish a protocol for plantlet regeneration of *Brassica oleracea* L. var. *capitata* cv. Tropicana through multiple shoot formation and (b) to induce callus using cotyledonary explants, which if embryogenic, can be used for inducing somatic embryos.