



UNIVERSITI PUTRA MALAYSIA

***EFFECT OF DIFFERENT CONCENTRATIONS OF BAP AND NAA ON
SHOOT REGENERATION OF TRANSGENIC BROCCOLI
(*Brassica oleracea var. italica*)***

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SHOOT REGENERATION OF TRANSGENIC BROCCOLI**

(Brassica oleracea var. italica)

**BY
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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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CERTIFICATION

This project report entitled Effect of Different Concentrations of BAP and NAA on Shoot Regeneration of Transgenic Broccoli (*Brassica oleracea* var. *italica*) is prepared by Nurfaizara binti Hamzah and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

%	Percent
°C	Celsius Degree
$\mu\text{mol m}^{-2} \text{s}^{-1}$	Micromole per meter square per second
2, 4-D	2, 4-Dichlorophenoxyacetic acid
ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
cm	Centimetre
DMRT	Duncan Multiple Range Test
et al.	et alia
g	Gram
HCl	Hydrochloric acid
IAA	Indole-3-butyric acid
IBA	Indole-3-acetic acid
KIN	Kinetin
L	Litre
M	Molar
mg/L	Milligram per litre
ml	Millilitre
NAA	1-Naphthalene acetic acid
NaOH	Sodium hydroxide
pH	Measure of acidity
RCBD	Randomized Complete Block Design
var.	Variety

ABSTRACT

Brassica oleracea L. var. *italica* Plenck (Brassicaceae) is one of the many valuable *Brassica* species, which is still less cultured under *in-vitro* condition. The study was conducted to regenerate shoots using petiole explants and to determine the best combination of 6-Benzylaminopurine (BAP) and 1-Naphthalene acetic acid (NAA) for shoot induction in *B. oleracea*. The explants were obtained from transgenic broccoli plants and placed on shoot induction medium containing basal salts of Murashige and Skoog (MS) supplemented with various concentrations of BAP (0, 1, 3, 5 and 7 mg/L) in combination with various concentrations of NAA (0, 0.05 and 0.1 mg/L). Parameters measured were the percentage of explants regenerating shoots and mean number of shoots per explant. Shoot regeneration was observed every week and data collected after 8 weeks of culture. The study was conducted using Randomized Complete Block Design (RCBD) with 10 replications per treatment. Data were analyzed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used for comparison of treatment means. The highest percentage of shoot formation (60%) and the highest mean number of shoots produced per explant (21.04) were obtained on medium containing 5.0 mg/L BAP and 0.05 mg/L NAA.

ABSTRAK

Brassica oleracea L. var. *italica* Plenck (Brassicaceae) merupakan salah satu spesies Brassica yang bernilai, tetapi masih kurang dibiakkan secara *in-vitro*. Eksperimen telah dijalankan untuk menjana pucuk menggunakan eksplan petiol serta menentukan kombinasi yang terbaik di antara BAP dan NAA untuk induksi pucuk pada *B. oleracea*. Eksplan diperoleh daripada brokoli transgenik dan diinokulasi pada medium asas Murashige dan Skoog (MS) yang mengandungi kepekatan BAP (0, 1, 3, 5 dan 7 mg/L) dan NAA (0, 0.05 dan 0.1 mg/L) yang berbeza-beza. Parameter yang diukur adalah peratus eksplan yang menghasilkan pucuk dan min bilangan pucuk per eksplan. Pemerhatian dilakukan pada setiap minggu dan data direkod selepas 8 minggu pengkulturan. Eksperimen dikendalikan menggunakan Rekabentuk Rawak Blok Lengkap (RCBD) dengan 10 replikasi bagi setiap rawatan. Data dianalisis menggunakan Analysis Varians (ANOVA) dan Duncan Multiple Range Test (DMRT) digunakan untuk perbandingan antara min rawatan. Peratusan pertumbuhan pucuk tertinggi (60%) dan min bilangan pucuk per eksplan tertinggi (21.04) diperoleh pada medium yang mengandungi 5.0 mg/L BAP dan 0.05 mg/L NAA.

CHAPTER 1

INTRODUCTION

1.1. Origin and uses of *Brassica oleracea* L. var. *italica*

Brassica oleracea var. *italica* or commonly known as broccoli is an important vegetable. Broccoli belongs to the family Brassicaceae together with cauliflower, brussel sprout, kale, cabbage, kailan and mustard. *B. oleracea* var. *italica* is a form of cabbage grown for its edible flower buds and stalk. It is native to the Eastern Mediterranean and Asia Minor. Broccoli was cultivated in Italy in ancient Roman times and was introduced to England and America in the 1700s (Prakash *et al.*, 2011).

Broccoli is a nutritious vegetable high in dietary fiber and a number of vitamins and minerals, including potassium, folic acid, and vitamin A, C and K. It is also low in calories. Other than that, broccoli also contains an anti-cancer compound, diindolymethane and small amount of selenium (The World's Healthiest Foods, 2013).

1.2. Constraints of broccoli cultivation

High temperature is the major constraint for its profitable cultivation in tropical areas. This temperate crop is normally cultivated on highland and survives successfully in low temperate area. Mid to late summer is a difficult period for producing good quality broccoli because of the adverse effects of high temperatures

coupled with low soil moisture. Fresh broccoli is highly perishable when stored in air at 20 °C (Chen *et al.*, 2001).

1.3. Significance of study

A transgenic plant is one that contains a gene or genes which have been introduced artificially into the plant's genetic makeup using a set of biotechnology techniques collectively known as recombinant DNA technology (Hussain *et al.*, 2012). Plant tissue culture carried out under aseptic conditions has important applications in plant biotechnology. Transgenic broccoli plants have been obtained by a PhD researcher in the Agrobiotechnology Laboratory, Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia and maintained in the Transgenic Greenhouse. This study was carried out to assess the ability of regeneration of the broccoli plants after being transformed with a heat tolerant gene by the researcher. The regeneration protocol could then be used for mass propagation of the transgenic lines.

1.4. Objectives of study

The objectives of this study were:

1. To regenerate shoots from petiole explants of broccoli
2. To determine the best combination of BAP and NAA for the shoot regeneration.

1.5. Hypothesis

Null hypothesis (H_0): There is no significant difference between combination of BAP and NAA on shoot regeneration of broccoli.

Alternative hypothesis (H_A): There is significant difference between combination of BAP and NAA on shoot regeneration of broccoli.



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