



***SURFACE STERILIZATION AND SHOOT REGENERATION FROM NODE
EXPLANT OF SABAH SNAKE GRASS (*Clinacanthus nutans*) USING
DIFFERENT CONCENTRATION OF CLOROX, BAP AND KINETIN***

NUR YUSLIZA BINTI MAT LAZIM

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NUR YUSLIZA BINTI MAT LAZIM

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

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by
NUR YUSLIZA BINTI MAT LAZIM

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

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CERTIFICATION

This project report entitled “**Surface Sterilization and Shoot Regeneration from Nodal Explant of Sabah Snake Grass (*Clinacanthus nutans*) using Different Concentration of Clorox, BAP and Kinetin**” is prepared by Nur Yusliza Binti Mat Lazim and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

Student’s name:

Nur Yusliza Binti Mat Lazim

Student’s Signature:

.....

Certified by:

.....
En. Azmi Abdul Rashid

Department of Agriculture Technology,

Faculty of Agriculture,

Universiti Putra Malaysia

Date:

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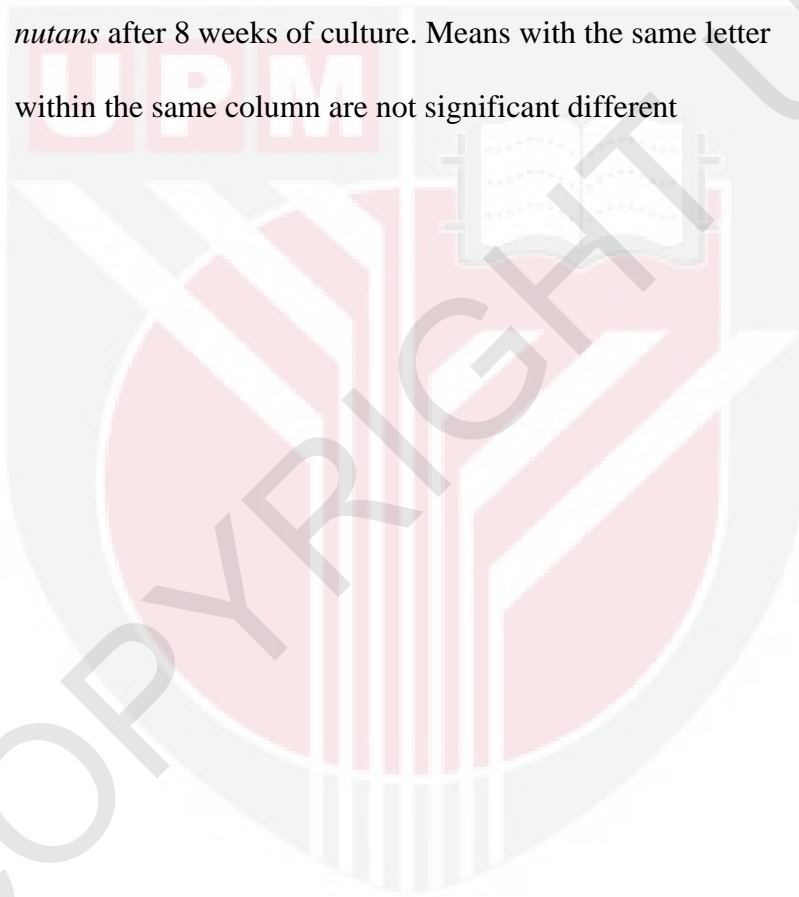
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LIST OF ABBREVIATIONS

The following abbreviations were used in the text.

BAP	6-benzylaminopurine
Kin	Kinetin
Mg/L	Milligram per liter
%	Percentage
°C	Degree Celsius
L	Liter
MS	Murashige and Skoog
MS	Mean of square
SS	Sum of square
F	Degree of freedom
pH	Hydrogen ion concentration $-\log(H)$
CRD	Completely Randomized Design
ANOVA	Analysis of variance

ABSTRACT

A study was conducted to establish a suitable surface sterilization protocol to reduce microbial contamination and increasing node explant survival of *Clinacanthus nutans*. Three different surface sterilization treatment which are the use of Clorox at 20% for 10 and 15 minutes and 30% Clorox for 10 minutes were used in this study. It was observed that immersion of node explant in 20% Clorox for 10 minutes was the best treatment in reducing microbial contamination and increasing explant survival. Microbial contamination was reduced to 20% and explant survival increased to 80% by using this sterilization protocol. Another study was carried out to determine the best concentration of 6-benzylaminopurine (BAP) or Kinetin (Kin) in triggering shoot proliferation from node explant of *Clinacanthus nutans*. The node explants of Sabah Snake Grass that are cultured on full strength MS (Murashige and Skoog 1962) medium supplemented with 0.5 mg/l BAP showed the highest shoot proliferation with 2.85 shoot formed per explant.

ABSTRAK

Satu kajian telah dijalankan bagi menentukan kesesuaian protokol pensterilan permukaan untuk mengurangkan pencemaran mikrob dan meningkatkan hidup nod eksplan *Clinacanthus nutans*. Tiga rawatan pensterilan permukaan yang berbeza yang menggunakan Clorox pada 20% untuk 10 dan 15 minit dan 30% Clorox selama 10 minit telah digunakan dalam kajian ini. Kajian ini dijalankan dengan memerhati bahawa rendaman nod eksplan dalam 20% Clorox selama 10 minit adalah rawatan yang terbaik dalam mengurangkan pencemaran mikrob dan meningkatkan eksplan hidup. Pencemaran mikrob telah dikurangkan kepada 20% dan survival eksplan meningkat kepada 80% dengan menggunakan protokol pensterilan ini. Satu lagi kajian telah dijalankan untuk menentukan kepekatan terbaik 6-benzylaminopurine (BAP) atau Kinetin (Kin) dalam mencetuskan pucuk percambahan daripada eksplan nod *Clinacanthus nutans*. Nod eksplant Daun Belalai Gajah yang dikultur pada kekuatan penuh MS (Murashige dan Skoog 1962) yang diperkaya dengan 0.5 mg / l BAP menunjukkan menembak percambahan tertinggi iaitu 2.85 pucuk membentuk setiap eksplan.

CHAPTER 1

INTRODUCTION

1.1 Introduction



Plate 1: *Clinacanthus nutans* plants

Sabah snake grass with a botanical name *Clinacanthus nutans* is widely known as a traditional medicinal plant in Southeast Asia such as Malaysia, Thailand, Indonesia and China. This plant belongs to the Acanthaceae family. The common name uses for this plant are Belalai Gajah in Malaysia, Sha Ba She Cao or E Zui Hua in mandarin, Tarum Siam in Thai and Dandang Gendis in Indonesia (Yong et al., 2013).

Sabah snake grass has several medicinal uses. This plant is widely used as traditional medicine in treating skin rashes, insect and snake bite, and snake lesion. It has a lot of advantages and have been used currently in treating kidney problem, diabetes and fracture. Apart from that, an extract from the leaves has anti-inflammatory, anti-viral, and anti-cancer properties and can be used in curing lung cancer and breast cancer. There are many clinical trials showing the successful use of a *Clinacanthus nutans* cream or lotion for the relief of minor skin inflammation (Yoke, 2013).

Clinacanthus nutans is a shrub or perennial herb that can grow up to 1m in height under a good environmental condition and agronomic practices. This plant has simple leaves with a lanceolate shape measuring 8-12mm long and 4-6mm wide. The leaves are arranged in an opposite manner. The plant has a woody stem which is jointed and grows upright. This plant has flowers that are arranged in dense cymes at the top of a branch and the crown has a tube shape (Panyakom, 2006). Propagation by stem cutting is usually practiced in *Clinacanthus nutans* planting material production. The propagation of *Clinacanthus nutans* plants by stem cutting offer limited production of planting materials. An alternative in overcoming this problem is by using *in vitro* technique to mass produce *Clinacanthus nutans* plant.

Tissue culture is a technique that are widely use currently in commercial industry and research for mass propagation of plant and also for production of disease free plants. It can also be used in crop improvement program such as induction of mutants via in vitro mutagenesis and production of transgenic plant via Agrobacterium mediated genetic transformation. Tissue culture propagation involves using a small piece of plant tissue which is cultured in a nutrient medium under sterile condition. (George and Debergh, 2008). Currently, there are a not many report on the success of *in vitro* culture of *Clinacanthus nutans*. Since this is an early study of in vitro culture of *Clinacanthus nutans* and the source of explants are from plants grown in pots outside the laboratory which is prone to microbial contamination therefore this initial study was conducted with the following objectives:

- 1) To establish a suitable surface sterilization protocol for node culture of *Clinacanthus nutan* by determining the best sterilization treatment in reducing microbial contamination of the cultures.
- 2) To determine the best concentration of 6-benzylaminopurine (BAP) and Kinetin to trigger shoot proliferation from node explants of *Clinacanthus nutans* cultured under in *vitro* condition.

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