



***THE EFFECT OF DIFFERENT KINETIN CONCENTRATION ON SHOOT
MULTIPLICATION OF *Clinacanthus nutans* NODE CULTURE***

NUR SYAZWANI BINTI ZAKARIA

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NUR SYAZWANI BINTI ZAKARIA

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

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MULTIPLICATION OF *Clinacanthus nutans* NODE CULTURE**

By

NUR SYAZWANI BINTI ZAKARIA

A project report submitted to the

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For the award of the degree of

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CERTIFICATION

This project entitled “The Effect of Different Kinetin Concentration on Shoot Multiplication of *Clinacanthus nutans* Node Culture” is prepared by Nur Syazwani binti Zakaria and submitted to the Faculty of Agriculture in fulfillment of the requirements of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

Student's name:

Nur Syazwani binti Zakaria

180018

Student's signature

Certified by:

Mr. Azmi bin Abdul Rashid,

Project Supervisor

Department of Agricultural Technology

Faculty of Agriculture

Universiti Putra Malaysia Serdang, Selangor.

Date: _____

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LIST OF ABBREVIATIONS

%	percentage
°C	degree Celcius
µmol	micromole
ANOVA	Analysis of Variance
cm	centimetre
CRD	Completely Randomized Design
HCl	Hydrochloric acid
kPa	kilopascal
L	liter
M	molar
mg	milligram
mins	minutes
MS	Murashige and Skoog's (MS) (1962)
NaOH	Sodium hydroxide
s	seconds
SAS	Statistical Analysis Software
pH	acidity or alkalinity of a solution

ABSTRACT

Clinacanthus nutans also known as Sabah Snake Grass is a herb originated from Malaysia, Thailand and China belonging to the Acanthaceae family. This plant is a shrub with terete and glabrescent stems. It has a simple leaf arranged in an opposite manner with its shape lanceolate to ovate. This plant is traditionally used for disease treatment such as skin rashes, allergic and burns as well in curing snakebites. Recent research shows it is also efficiently used as remedies for herpes simplex and VZV lesions and alternative to cure cancer. The common method to propagate this plant is through stem cuttings, which requires much labour and time consuming and with limited number of planting materials produced. Therefore, tissue culture can be an alternative method to produce large number of offspring within a smaller space and short period of time. This experiment is carried out in *In Vitro* Laboratory, Department of Agriculture, UPM to determine the best concentration of kinetin that can maximize *in vitro* shoot proliferation from *Clinacanthus nutans* node culture. The experiment is carried out using Completely Randomised Design (CRD) with 7 treatments and 12 replications. Nodes (1cm) are excised from axenic cultures of *Clinacanthus nutans* and cultured at a slanting position in Murashige and Skoog (1962) medium with supplemented with different concentration of kinetin (0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0mg/L). The culture is incubated for 8 weeks in the culture room. It is observed that all kinetin treatments including the control treatment proliferated buds within the range of 83-100%. However, elongation of explant are higher in 1.0mg/L and 2.0mg/L kinetin compared with explant supplemented with 8.0mg/L and 10.0mg/L kinetin. This showed that, higher concentration of kinetin can inhibit explant growth.

ABSTRAK

Clinacanthus nutans, juga dikenali sebagai 'Sabah Snake Grass' merupakan tumbuhan herba daripada keluarga Acanthacea yang berasal daripada Malaysia, Thailand dan China. Tumbuhan ini merupakan pokok renek yang tumbuh tegak dan mempunyai batang yang kecil dan lembut. Tumbuhan ini sering digunakan dalam perubatan tradisional untuk merawat ruam, alergik, melecur dan gigitan ular. Baru-baru ini kajian membuktikan tumbuhan ini juga amat efektif untuk merawat penyakit yang dijangkiti oleh virus simplex herpes, varicella-zoster dan juga dapat merawat kanser. Tumbuhan herba ini biasanya dibiakkan dengan keratan batang, justeru memerlukan tenaga kerja dan masa yang panjang untuk menghasilkan anak pokok. Oleh hal yang demikian, langkah alternatif menggunakan pembiakan *in vitro* digunakan untuk menghasilkan anak pokok yang banyak dalam masa yang singkat tanpa perlu menggunakan ruang yang luas. Kajian ini dijalankan melalui kaedah sub-pengulturan eksplan *Clinacanthus nutans* pada media Murashige dan Skoog (MS) (1962) yang mengadungi kepekatan kinetin yang berbeza (0, 1.0, 2.0, 4.0, 6.0, 8.0 dan 10.0mg/L). Kajian ini dijalankan dengan menggunakan Rekabentuk Rawak Penuh. Kultur disimpan di dalam bilik kultur selama 8 minggu. Pemerhatian menunjukkan bilangan multiplikasi pucuk adalah lebih kurang sama untuk semua rawatan kinetin dan kawalan. Namun, pemanjangan eksplan bagi rawatan kinetin 1.0mg/L dan 2.0mg/L adalah lebih tinggi jika dibandingkan dengan rawatan kinetin 8.0mg/L dan 10.0mg/L. Kesimpulannya, kepekatan kinetin yang tinggi boleh merencatkan pertumbuhan eksplan *Clinacanthus nutans*.

1.0 INTRODUCTION

Clinacanthus burmanni, or *Clinacanthus nutans* is a member of the Acanthus family, Acanthaceae. It is a species that is distributed worldwide mainly around Indonesia, Malaysia, Africa, Brazil and Central America (Chen *et al.*, 2015). *C. nutans* is mainly natives in Malaysia, Indonesia, Vietnam, Thailand and China. It has many different names in different countries. In Malaysia it is known as 'Belalai Gajah' or Sabah Snake Grass. In England it is known as Drooping Clinacanthus while in Indonesia as 'Dandang Gendis'. In Thailand it is known as 'Phaya Yo' and the Chinese called it 'You Dun Cao' (Chen *et al.*, 2015).

C. nutans are widely used to treat injuries and diseases such as skin rashes, burns, fever, snake-bite as well as to cure allergic responses (Aslam *et al.*, 2015, Zulkipli *et al.*, 2017).

C. nutans is an important herbal plant in China, Malaysia and Thailand. It is used until today as it possesses anti-hepatitis and anti-herpes properties (Yuann *et al.*, 2012). In Thailand, this plant is used traditionally as a medicine to treat skin rashes, insect and snake-bite while clinically used to cure herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions as the leaves extract contain analgesic, anti-inflammatory agents and exhibited anti-herpes simplex activity thus potentially cause inactivation and inhibition of the virus infection (Sakdarat *et al.*, 2009, Yong *et al.*, 2013).

Currently, cancer is a major health threats to the Malaysian population and it has consistently reached 10-11% since 2006 and now still increasing statically proven by Malaysian Ministry of Health (2013). As researchers are still working in finding the cure for cancer, many of them are interested of using this traditional herb as an alternative mean for curing cancer. *C. nutans*, possess the antioxidant and anti-proliferation properties. It also contains potential nutraceuticals property against cultured cancer cell lines thus increase its potential as an alternate cure for cancer prevention and treatment (Yong *et al.*, 2013).

1.1 Problem Statements and Objective

Traditionally and conventionally, *C. nutans* is propagated by stem cuttings. However, due to the demand for this plant has increased recently, the current method to produce the plants cannot meet the consumers demand. Commercially, stem cutting can be excised from the mother plant and used for producing propagules but the number of planting materials produced from this method is very limited. It also required much labour to propagate this plant. As for the solution, *in vitro* propagation can be an alternative method for mass producing planting materials within a shorter period and less space requirement. Currently there is no *in vitro* study on *C. nutans* treated with different concentration of kinetin to proliferate axillary buds. Therefore this experiment is being carried out with the objective to determine the best concentration of kinetin that can maximise *C. nutans* shoot proliferation under *in vitro* condition.

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