



UNIVERSITI PUTRA MALAYSIA

***SHOOT MULTIPLICATION AS AFFECTED BY DIFFERENT EXPLANT
POSITION ON MOTHER STOCK PLANT OF CLINACANTHUS NUTANS***

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**FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR DARUL EHSAN**

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By

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CERTIFICATION

This project entitled “Shoot multiplication as affected by different explant on mother stock plant of *Clinacanthus Nutans*” is prepared by Mohamad Zamir Hadi bin Ismail and submitted to the Faculty of Agriculture in fulfilment of the requirements of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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List of Abbreviations

%	percentage
mg	milligram
L	Litre
mm	millimetre
cm	centimetre
m	metre
M	molar
μmol	micromole
w/v	weight to volume ratio
°C	degree celcius
kPa	kilopascal
s	second
mins	minutes
MS	Murashige and Skoog's medium (1962)
BA	benzyladenine
BAP	6-benzylaminopurine
IAA	indole-3-acetic acid
NAA	1-naphthaleneacetic acid
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
SAS	Statistical Analysis Software
ANOVA	Analysis of variance

ABSTRACT

Clinacanthus nutans of the family Acanthaceae or commonly known as Sabah Snake Grass is a famous tropical herb used for medicinal purpose. This plant is widely used in Southeast Asia especially in Malaysia as an anti-inflammatory drug for the treatment of wound and disinfection. Recently, it is used in treating cancer. This plant is usually propagated by stem cutting which has low reproductive capacity. The development of rapid propagation methods for *C. nutans* is required to satisfy human demand for its medicinal products. The purpose of this study is to establish an effective protocol for *in vitro* multiplication of *C. nutans* shoots. The study is conducted by sub-culturing different explants of *C. nutans* on Murashige & Skoog 1962 (MS) medium added with 2 mg/L 6-Benzylaminopurine (BAP). Shoot tip and different node positions on stock plant of *C. nutans* are used as the explants. The study is conducted using a Completely Randomized Design. The cultures are maintained for 8 weeks in the culture room with a light intensity of $30\mu\text{mol}/\text{m}^2/\text{s}$. It is observed that the number of shoot proliferated was significantly higher ($p < 0.05$) when using shoot tip explant compared to node explants excised at different position on the mother stock plant. The shoot tip explant is the best explant for inducing shoot proliferation. Sub-culture of the explant needs to be carried out at sixth till seventh week.

ABSTRAK

Clinacanthus nutans daripada keluarga *Acanthaceae* dikenali sebagai Sabah Snake Grass merupakan herba tropika terkenal yang digunakan untuk tujuan perubatan. Tumbuhan ini telah digunakan secara meluas di Asia Tenggara terutamanya di Malaysia sebagai ubat anti-radang untuk merawat luka dan pembasmian kuman. Baru-baru ini, *C. Nutans* digunakan dalam merawat kanser. Tumbuhan ini biasanya dibiakkan dengan keratan batang namun mempunyai keupayaan pembiakan yang rendah. Penghasilan kaedah pembiakan *C. Nutans* yang lebih cepat diperlukan untuk memenuhi permintaan orang ramai terhadap produk perubatan. Tujuan kajian ini adalah untuk mewujudkan satu protokol pembiakan *in vitro* yang berkesan bagi memperbanyakkan penghasilan pucuk *C. Nutans*. Kajian ini dijalankan melalui sub-pengkulturan eksplan *C. Nutans* yang berbeza pada media Murashige & Skoog 1962 (MS) yang ditambah dengan 2 mg/L 6-Benzylaminopurine (BAP). Tip pucuk dan nod yang berbeza kedudukan pada pokok stok *C. Nutans* digunakan sebagai eksplan. Kajian ini dijalankan dengan menggunakan Rekabentuk Rawak Penuh. Kultur disimpan selama 8 minggu di bilik kultur dengan intensiti cahaya $30\mu\text{mol}/\text{m}^2/\text{s}$. Pemerhatian menunjukkan bahawa bilangan multiplikasi pucuk adalah jauh lebih tinggi ($p < 0.05$) apabila menggunakan eksplan tip pucuk berbanding explan nod yang berbeza kedudukan pada pokok induk. Eksplan tip pucuk adalah eksplan yang terbaik untuk mendorong multiplikasi pucuk. Sub-kultur explan perlu dilakukan pada minggu ke-enam hingga minggu ke-tujuh.

CHAPTER 1

INTRODUCTION

Clinacanthus nutans (Burm f.) Lindau is a well-known medicinal herb belonging to Acanthaceae family that have long been traditionally used in the treatment of insect or snake bites and herpes infection. It is commonly known as ‘Sabah Snake Grass’ or ‘Belalai Gajah’ in Malaysia, Drooping Clinacanthus in English, ‘Pyaya yo’ in Thailand and ‘You Dun Cao’ in Mandarin (Chen *et al.*, 2015). *C. nutans* is often cultivated as small shrubs and is native to tropical Asia, primarily indigenous in Malaysia, Indonesia, and Thailand (Nesheim *et al.*, 2006). It is widely grown in tropical Asia and has been used as important medicinal herb in Malaysia, Thailand and China (Chen *et al.*, 2015). In South East Asia, *C. nutans* has been traditionally used for treatment of skin infection, diarrhea, fever, diabetes mellitus, diuretics, dysuria, dysentery, and heals burns (Kunsorn *et al.*, 2013). It has also been discovered to cure problem of gall bladder stone, regulating normal menstrual cycle, and liver cleansing (Shim *et al.*, 2013). Previously, *C. nutans* was reported to possess painkiller, anti-diabetic, antioxidant, anti-inflammatory activity, and as the remedy for chickenpox and herpes zoster which is caused by Varicella zoster virus (VZV) (Yarnell and Abascal, 2005; Arullappan *et al.*, 2014; Aslam *et al.*, 2014). Its extract has been decoded in clinical use in the form of a medicinal cream to treat herpes and hepatitis infection (Kongkaew & Chaiyakunapruk, 2011). Besides, *C. nutans* is widely used commercially in Malaysia and other Asian countries to cure liver problem, kidney syndrome, nasal cavity cancer, uterine fibroid, urinates neuropathies, gout, and uric acid (Arullappan *et al.*, 2014). Recently, *C. nutans* or Sabah Snake Grass has been utilized by locals in Malaysia as a cure for

cancer. However, its effectiveness has not yet been proven scientifically and it could be taken as an alternative treatment for cancer patients. The method of consuming *C. nutans* or Sabah Snake Grass was introduced by blending the leaves of the grass and drink as normal juice. It has created a fast growing market for herbal based products such as Sabah Snake Grass tonic nowadays.

Currently, the demand for medicinal plant is increasing and it may reduce the sustainable supply of the medicinal herbs in the future. Unthreatening endangered species, controlling over-collecting, avoiding unsustainable planting practices, and preventing pollution are important in the sustainable management of valuable medicinal plants. The traditional medicinal plant *C. nutans* is conventionally propagated by stem cutting which has low reproductive capacity. The development of rapid propagation methods for *C. nutans* is needed to increase productivity that has to satisfy human demand for its medicinal products. Hence, plant tissue culture techniques or *in vitro* culture method could be an alternative technique for the production of *C. nutans* plants that have medicinal properties.

The use of tissue culture techniques for herbaceous plant propagation has increased considerably over the past few decades. Reliable and efficient protocols for plant regeneration *in vitro* through stimulation of axillary shoot proliferation from protoplast, cell, organ, embryo or through organogenesis directly from callus have been developed for many important herbaceous plants such as *Andrographis paniculata* (Hempedu Bumi), *Orthosiphon stamineus* (Misai Kucing), *Phyllanthus niruri* (Dukung Anak) and *Gynura procumbens* (Sambung Nyawa) (McCown, 2000; Leng and Lai-Keng, 2004; Cimanga *et al.*, 2004; Purkayastha *et al.*, 2008; Keng *et*

al., 2009). *C. nutans* is one of the important perennial herbs with medicinal values that can be regenerated through *in vitro* propagation. Therefore, it is important to develop *in vitro* propagation protocol of *C. nutans* from the best explant part in order to increase plant productivity so as to achieve better plant product quality.

1.1 Research problems

C. nutans become important due to its effect in treating cancer and the demand for this plant has increased recently in Malaysia. The current method of propagation through stem cutting was unable to match the population demand. Therefore, *in vitro* plant propagation can be an alternative means for mass production of *C. nutans* planting materials in short period of time. Currently, no report has been made on successfulness of *in vitro* culture using different *C. nutans* explant parts except a study by Ng (2013) that showed success in callus induction of *C. nutans* but no plant regeneration was observed.

1.2 Objective

The specific objective of this study is:

1. To determine the best explant from mother stock plant of *C. nutans* in enhancing shoot multiplication.

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