



***SURFACE STERILIZATION AND SHOOT PROLIFERATION FROM SHOOT
TIP EXPLANT OF CAT'S WHISKERS (*Orthosiphon stamineus*) USING
DIFFERENT CONCENTRATION OF CLOROX AND BAP***

NUR HAYYU BINTI BORHAN

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CONCENTRATION OF CLOROX AND BAP

BY:

NUR HAYYU BINTI BORHAN

(170456)

This project report submitted to Faculty of Agriculture,
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DEPARTMENT OF AGRICULTURE TECHNOLOGY

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CERTIFICATION

This project entitled surface sterilization and shoot proliferation from shoot tip explant of cat's whiskers (*Orthosiphon stamineus*) using different concentration of Clorox and BAP is prepared by Nur Hayyu binti Borhan and submitted to the Faculty of Agriculture in partial fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticulture Science.

Submitted by,

NUR HAYYU BINTI BORHAN

Signature,

.....

Certified by,

(EN. AZMI BIN ABDUL RASHID)

Project supervisor,

Department of Agriculture Technology,

Faculty of Agriculture,

Universiti Putra Malaysia.

Date :

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BAP	6-Bezylaminopurine
LSD	Least Significant Difference
HSD	Honestly Significant Difference
MS	Murashige and Skoog
NaOH	Sodium Hydroxide
HCL	Hydrogen Chloride
CRD	Completely Randomized Design
SAS	Statistical Analysis System
pH	Hydrogen ion concentration/ $-\log(H^+)$
v/v	Volume per volume
<i>et al.</i>	Et alia

ABSTRACT

Orthosiphon stamineus which belongs to the Lamiaceae family is an important herb. A study was conducted to determine the best sterilization procedure for in vitro culture of *Orthosiphon stamineus* using shoot tip explant. Different concentrations of Clorox at different immersion time were used to optimize the sterilization procedure. Immersion of shoot tip explant in 25% concentration of Clorox for 15 minutes reduced microbial contamination up to 10% but increase explant survivability. Another study was conducted by culturing the shoot tip of *Orthosiphon stamineus* in half strength MS media containing different BAP concentration. The media containing 1 mg/L of BAP gave the highest shoot proliferation in shoot tip culture of *Orthosiphon stamineus* with 12.3 shoot produced per explant.

ABSTRAK

Orthosiphon stamineus tergolong dalam family Lamiaceae adalah tanaman herba yang penting. Kajian telah dijalankan untuk menentukan langkah-langkah pensterilan bagi *Orthosiphon stamineus* melalui eksplan mata tunas. Kepekatan larutan Clorox yang berlainan serta jangka masa rendaman yang berbeza digunakan. Kepekatan larutan 25% Clorox dengan masa rendaman selama 15 minit, mengurangkan kadar pencemaran sehingga 10% tetapi dapat meningkatkan kemandirian eksplan. Kajian seterusnya dijalankan dengan mengkultur mata tunas *Orthosiphon stamineus* di dalam setengah media MS mengandungi kepekatan BAP yang berlainan. Media yang mengandungi 1 mg/L BAP memberikan pengeluaran pucuk yang paling tinggi di dalam pengkulturan mata tunas *Orthosiphon stamineus* dengan kadar pengeluaran 12.3 pucuk bagi setiap eksplan.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Cat whiskers or its botanical name *Orthosiphon stamineus* belongs to the Lamiaceae family. Other common name for cat whiskers is Misai Kucing, referring to the plant's wispy appearance. This plant comes in two varieties, one with white flowers and the other with light purple flowers. *Orthosiphon stamineus* is a herbaceous shrub which can grow to a height of 1.5 m. Its leaf is simple and is arranged in an opposite manner. It is green in color and with a lanceolate leaf blade and a serrate margin. It has short petiole around 0.3 cm in length and reddish purple in colour. Its stem is quadrangle and red in color and branch profusely.

This herbal plant originates from eastern Asian countries such as Vietnam, Thailand, Indonesia and Malaysia and it is consumed as a tea known as 'Java tea'. The leaves of this plant have medicinal usage in curing atherosclerosis, nephritis, kidney stones, diabetes, inflammatory disorders and hepatic disorders such as jaundice and hepatitis (Ahamed Basheer and Abdul Majid, 2010).

In Malaysia, the production of *Orthosiphon stamineus* is still low compared to the commodity plants such as oil palm and rubber. Herbs and Spices Statistic in Malaysia by the Department of Agriculture Malaysia stated that the production of *Orthosiphon stamineus* in 2013 as 47.73 Mt with 9.7 ha planted

area. The state of Perak constitute the highest production at 4.92 metric tonne and with a planting area of 4.6 ha.

Conventionally, *Orthosiphon stamineus* can be propagated using stem cutting. The conventional method of vegetative propagation by stem cutting utilizes the ability of the axillary buds to take over the function of the main shoot in the absence of a terminal bud. However the number of cuttings that can be taken from a selected plant in a year is extremely limited because in nature the vegetative growth is periodic. A minimal size of cuttings about 24-30 cm is required in conventional methods for plant establishment and this may restrict the multiplication of this plant if the stock parent plant is limited or if the species is endangered. By using shoot tip culture where a small explant size is used (about 1-2 cm), it can favor large scale multiplication even within a limited source of material for propagation and space for incubating the propagules.

Plant tissue culture can be used as a mean for propagating *Orthosiphon stamineus* plant and this technique is becoming increasingly popular as alternative method of plant vegetative propagation. It involves asexual method of propagation and can also be used in crop improvement program of this plant. *In vitro* propagation offer many advantages over the conventional method of vegetative propagation. The rate of multiplication using this technique is extremely rapid and production of planting materials can be made all year round independent of the season. Thus, many plants can be produced in a year starting from a small piece of tissue. The success of *in vitro* propagation depends on many factors and among them includes the use of plant growth regulators. Plant

growth regulator such as cytokinin is important to enhance shoot proliferation (Zaidah and Nazri , 2005). In this study 6- Benzyl amino purine (BAP) is used to induce axillary proliferation from the shoot tip of *Orthosiphon stamineus* and there is no auxin added into the MS media. Several BAP concentrations were used in the study. Since not much research has been carried out on *in vitro* culture of this plant and the source of explant is from plants growth in pots, therefore this study is carried out with the objective:

(i) To determine the best concentration of Clorox and immersion period the explant being sterilized by Clorox in reducing microbial contamination and increasing survivability of the explant.

(ii) To determine the best concentration of BAP that can induce good shoot proliferation from the *in vitro* culture of *Orthosiphon stamineus* shoot tip explant.

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