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CALLUS INDUCTION FROM LEAF EXPLANT OF *Citrus hystrix*

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FACULTY OF AGRICULTURE
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SERDANG, SELANGOR

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CALLUS INDUCTION FROM LEAF EXPLANT OF

Citrus hystrix

BY

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A project submitted to Faculty of Agriculture, Universiti
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(Final Year Project) for the award of the degree of
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FACULTY OF AGRICULTURE
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CERTIFICATION

This project report entitled “CALLUS INDUCTION FROM LEAF EXPLANT OF *Citrus hystrix*” prepared by NOR ILYA BINTI MOHD ZAKI (156004) and submitted to the Faculty of Agriculture in partial fulfillment of the requirement course of PRT 4999 (Project) for the degree award of Bachelor of Agriculture Science.

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ABSTRACT

Citrus hystrix belongs to the Rutaceae family and is one of the most important commercial crops based on its nutritional, medicinal and economic value. An experiment was conducted for callus induction from leaf explant of *C.hystrix* and to determine the best combination of 2,4-dichlorophenoxyacetic acid (2,4-D) and Kinetin (KIN) on the callus induction with six explants per treatment. This experiment was conducted using Randomized Complete Block Design (RCBD) with 15 treatments and 3 replications. Data were analyzed using the Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used for comparison between treatment means. There was significant difference on explant survival. The highest percentage of explant survival was 68% in T5 (2.0 mg/l KIN). Besides, there was no significant difference on explant producing callus between treatments. The highest mean percentage of explant producing callus was in T5 (2.0mg/l KIN) which was 28% within 27 days of inoculation. T5 (2.0 mg/l KIN), T9 (3 mg/l 2,4-D + 1.5 mg/l KIN) and T15 (5 mg/l 2,4-D + 2.0 mg/l KIN) showed intensive callusing. It was found that T5 (2.0 mg/l KIN), T8 (3 mg/l 2,4-D + 1.0 mg/l KIN) and T10 (3 mg/l 2,4-D + 2.0 mg/l KIN) were likely to become embryogenic due to the presence of nodular structure. Thus, further studies need to be conducted to obtain the best combination of hormones on callus induction from leaf explant of *C.hystrix* employing short incubation period, producing high intensity of callusing and the ability to form embryogenic callus.

ABSTRAK

Citrus hystrix tergolong dalam keluarga Rutaceae dan merupakan salah satu tanaman komersial yang penting berdasarkan nilai pemakanan, perubatan dan ekonomi. Satu eksperimen telah dijalankan untuk mendorong pembentukan kalus pada daun eksplan *C. hystrix* dan menentukan kombinasi hormon yang terbaik antara asid 2,4-Dichlorophenoxyacetic (2,4-D) dan Kinetin (KIN). Rekabentuk RCBD telah digunakan dalam eksperimen ini dengan 15 rawatan dan 3 replikasi dan enam eksplant untuk setiap rawatan. Analisis varians (ANOVA) telah digunakan untuk menganalisa data dan Ujian Kepelbagaian Duncan (DMRT) telah digunakan untuk perbandingan purata antara rawatan yang telah digunakan. Hasil kajian menunjukkan terdapat perbezaan yang signifikan pada rawatan yang digunakan untuk peratusan eksplan yang hidup. Peratusan tertinggi eksplan yang hidup adalah 68% iaitu di dalam T5 (2.0 mg / 1 KIN). Selain itu, tiada perbezaan yang signifikan terhadap eksplan yang membentuk kalus dalam semua rawatan. T5 (2.0mg / 1 KIN) menunjukkan peratusan purata tertinggi eksplan yang membentuk kalus iaitu 28% dalam tempoh 27 hari inkubasi. T5 (2.0 mg / 1 KIN), T9 (3 mg / 1 2,4-D + 1.5 mg / 1 KIN) dan T15 (5 mg / 1 2,4-D + 2.0 mg / 1 KIN) menunjukkan kadar pembentukan kalus paling tinggi. T5 (2.0 mg / 1 KIN), T8 (3 mg / 1 2,4-D + 1.0 mg / 1 KIN) dan T10 (3 mg / 1 2,4-D + 2.0 mg / 1 KIN) berkemungkinan menjadi embriogenik dengan kehadiran kalus berstruktur nodul. Oleh itu, kajian lanjut perlu dijalankan bagi mendapatkan kombinasi hormone yang terbaik untuk membentuk kalus pada eksplan daun *C. hystrix* dalam tempoh inkubasi yang singkat, kalus berintensiti tinggi dan keupayaan untuk membentuk kalus embriogenik.

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LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetic acid
ANOVA	Analysis of variance
DMRT	Duncan Multiple Range Test
g/L	gram per liter
HCL	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KIN	Kinetin
mg/L	milligram per liter
MS	Murashige and Skoog
NAA	α -naphthalene acetic acid
NaOH	Sodium hydroxide
p.s.i	pound per square inch
pH	Hydrogen ion concentration/ $-\log H^+$
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System

CHAPTER 1

INTRODUCTION

Citrus is the most important fruit crop planted worldwide and it is recognized as queen of all fruits or golden fruits. *Citrus hystrix* is commonly known as kaffir lime in English, limau purut in Malay, jeruk purut in Indonesia and magroot in Thailand. *C. hystrix* originates from Southeast Asian country such as Malaysia, Indonesia, Thailand, India, Cambodia and Laos. This plant is usually planted as shrub in the backyard of home and not for commercialization (Jumin and Nito, 1996).

C. hystrix has many uses not only in cooking but also can be used for cleansing, herbal preparation and medicinal purposes. The rough and bumpy fruit of *C. hystrix* has little or no juice compared to other citrus species. Research had found that essential oils extracted from the peels of *C. hystrix* fruits contains antibacterial properties and induce calming effects on user (Aziz *et al.*, 2011). In Thailand, the kaffir lime's leaves are widely used for cooking their famous delicacy, the spicy *tom yam* soup. It is also mainly used in Lao curry pastes. The kaffir limes are also common in Malaysian and Burmese cuisines. The fruit and the leaves both bring out more zest into any cuisine and increase the scent as well. Many uses the kaffir lime for spicy dishes as the taste and the smell could complement perfectly with spicy foods. The juice of *C. hystrix* is used as cleanser for clothing especially in Thailand and Cambodia.

In general, *Citrus* sp. is propagated through budding, cutting or layering (Grosser, 1994). These conventional methods resulted in low yield, low resistance towards diseases, low tolerance towards environmental stress and do not perform well as a desired plant. Non-availability of propagated planting materials from elite clones for plantation is the main constraints in citrus cultivation (Singh, 2002). In recent years, tissue culture techniques have been introduced to overcome the constraint to citrus improvement and cultivation. The purposes are for rapid clonal propagation of several economic plants, restoration of vigor and yield due to infection and preservation of germplasm (Singh, 2002).

According to the Ministry of Agriculture (MOA), kaffir lime is categorized as a spice in Malaysia however, quality supply of seed and raw material are not consistent. In order to overcome the problem, tissue culture can be the basis in propagating the citrus species for the production of large number of planting materials. In tissue culture, shoot regeneration could be obtained direct from an explant or indirectly through callus formation. This study emphasized on callus formation which if successful will be followed by shoot regeneration by other researchers. Therefore, the objectives of this study were:-

- 1) To induce callus from leaf explant of *C. hystrix*.
- 2) To determine the best combination of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Kinetin (KIN) on the callus induction.

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