EFFECT OF PLANT GROWTH REGULATORS IN PLANT REGENERATION OF MALAYSIAN UPLAND RICE

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requirements for the award of the degree of
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To my respectable supervisor, my beloved family,

&

To all my friends, seniors, lecturers and staffs of Faculty of Biosciences and Medical Engineering (FBME)

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ABSTRACT

This study was carried out to regenerate Malaysian upland rice cultivars; Bario and Hitam by manipulating plant growth regulators. The present study showed that callus induction varied depends on cultivar used. The highest percentage of callus induction for Bario was 76.67% and 75% for Hitam cultivar on MS medium supplemented with 3 mg/L of 2,4-D and 2 mg/L of NAA respectively after 4 weeks culture. Both cultivars produced an embryogenic callus type I from scutellum with yellowish color, dry and compact structure. The embryogenic characteristics were later confirmed using histology. At week seven, highest proliferative mass of callus of Hitam cultivar callus was recorded with 1.73 g average fresh weight with diameter of 9.62 mm^2. However, lower average of fresh weight was recorded from Bario cultivar showed 1.6 g with 10.87 mm^2 diameter. Then, potential embryogenic callus was selected and transferred to MS medium supplemented with different concentrations of plant growth regulators (BAP, kinetin and NAA) for regeneration studies. The present study showed no shoot formation was recorded from Bario cultivar. The Hitam cultivar callus exhibited first green spot at week 3 and then shoot were formed. The present study showed highest shoot formation (78%) was achieved for Hitam cultivar when placed on MS contained with 1.0 mg/L BAP, 0.5 mg/L kinetin and 0.5 mg/L NAA (optimal shoot regeneration media) after 4 weeks culture. The other treatments showed potential shoot formation was MS media containing 2.0 mg/L BAP, 0.5 mg/L kinetin and 0.5 mg/L NAA and MS supplemented with 0.5 mg/L kinetin and 0.5 mg/L NAA. Similar percentage of shoot formation (33%) showed MS media containing 0.5 mg/L BAP, 1.5 mg/L kinetin and 0.5 mg/L NAA and MS supplemented with 0.5 mg/L BAP, 2.0 mg/L kinetin and 0.5 mg/L NAA. The Hitam cultivar showed 2 average numbers of shoots per callus on optimal shoot regeneration media was recorded after 6 weeks. The present study showed different plant growth regulators for Malaysian upland regeneration system was affected differently with the cultivar tested.

ABSTRAK

Kajian ini dijalankan untuk regenerasi bagi dua kultivar padi bukit Malaysia iaitu Bario dan Hitam dengan pengoptimuman parameter hormon. Kajian ini menunjukkan pembentukan kalus itu berubah bergantung kultivar yang digunakan. Peratusan tertinggi pembentukan kalus untuk kultivar Bario ialah 76.67% dan 75% untuk kultivar Hitam yang mengandungi MS media ditambahkan dengan 3 mg/L 2,4-D dan 2 mg/L NAA selepas 4 minggu kultur. Kedua-dua kultivar menghasilkan kalus embriogenik jenis 1 dari skutelum dengan warna kekuningan, struktur yang padat dan kering. Pada minggu ke-7, pertumbuhan tertinggi kalus untuk kultivar Hitam telah dicatatkan dengan purata berat segar 1.73 g dengan garis pusat 9.62 mm^2. Bagaimana pun, purata berat segar lebih rendah telah dicatatkan pada kultivar Bario Bario 1.6 g dengan 10.87 mm^2 garis pusat. Ciri-ciri embriogenik seterusnya dikukuhkan dengan histology. Kemudian, kalus embriogenik yang berpotensi telah dipilih dan dipindahkan ke MS media yang ditambah dengan kombinasi hormon yang berbeza (BAP, kinetin dan NAA) untuk regenerasi. Kajian menunjukkan tiada pertumbuhan pucuk telah dicatatkan dari kultivar Bario manakala, kalus kultivar Hitam telah menunjukkan bintik-bintik hijau pertama pada minggu ke-3 dan seterusnya penghasilan pucuk. Peratusan penghasilan pucuk yang tertinggi (78%) apabila diletakkan di atas media MS yang mengandungi 1.0 mg/L BAP, 0.5 mg/L kinetin dan 0.5 mg/L NAA selepas 4 minggu. Kombinasi hormon lain yang menunjukkan penghasilan pucuk yang berpotensi ialah media MS mengandungi 2.0 mg/L BAP, 0.5 mg /L kinetin dan 0.5 mg/L NAA and media MS yang mengandungi 0.5 mg/L kinetin dan 0.5 mg/L NAA. Peratusan penghasilan pucuk yang sama (33%) pada media MS yang mengandungi 0.5 mg/L BAP, 1.5 mg/L kinetin dan 0.5 mg/L NAA dan MS media yang mengandungi 0.5 mg/L BAP, 2.0 mg/L kinetin dan 0.5 mg/L NAA. Kultivar Hitam menunjukkan 2 bilangan purata pucuk per kalus dicatatkan setelah 6 minggu. Kajian menunjukkan kombinasi kepekatan hormon yang berbeza terhadap penjanaan semula padi bukit Malaysia telah dipengaruhi oleh perbezaan kultivar padi yang diuji.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
°C	Degree celcius
%	Percent
cm	Centimeter
mg	Miligram
mL	Mililiter
mm	Milimeter
mm^2	Milimeter square
µm	Micrometer
g	Gram
h	hour
L	Liter
2,4-D	2, 4-Dichlorophenoxyacetic Acid
BAP	6-Benzylaminopurine
NAA	1-Naphthaleneacetic acid
PGRs	Plant Growth Regulators
SE	Standard Error
v/v	Percent volume in volume

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Rice is a vital harvest crop as staple nourishment and a model plant for genomic field (Tyagi and Mohanty 2000, Bajaj and Mohanty 2005). It provides half of total dietary carbohydrate, especially in Asian countries and it is suitable diet for more than three billion people that supplied 50-80% of their daily calorie intake (Khush, 2005). Thus a considerable improvement has been done through traditional rice breeding. Rice breeding has made significant progress towards higher yield, improved quality, greater disease resistance and other important characters of agricultural importance in the past and even in future, it will still play an important role. Due to its increasing importance in nutrition and economy, it is believed that new varieties of rice that have good agronomic characters should be evolved.

Rice is a very sensitive plant to water deficiency and for this reason, many methods were made to enhance rice cultivars that are able to produce comparatively high yield in both stressed and non-stressed environments (Shahsavari *et al*., 2010). Meanwhile, upland rice was taken into consideration for more research over the last few years because it has the potential to survive in drought conditions with high production (Bernier *et al*., 2008, Geng *et al*,. 2008). Tissue culture without meddling

excessively with the genetic make-up of the individual can possibly be achieved by genetic manipulation (Shazia *et al*., 2005).

Water deficiency in agricultural production including rice has become a serious problem. Scientists believe that using new cultivars which have potential to survive with high yield may help solve or minimize the problem. Upland rice is a type of rice that is planted in dry lands and grown in rain-feeding or limited irrigation condition. Using these types of rice may save plenty of water and diminishes water pollution (Geng *et al.,* 2008). However, other biotic and abiotic factors that may limit their cultivation and production need to be genetically improved via genetic transformation. Routine tissue culture system including callus induction and regeneration is a fundamental requirement for successful genetic transformation (Li *et al*., 2007; Seraj *et al.,* 1997). It is known that, callus induction and regeneration ability highly rely on genotypes (Ge *et al.,* 2006), explant types (Lee *et al.,* 2002), carbohydrate sources (Rashid *et al.,* 2001), plant growth regulators (Zaidi *et al*., 2006), basal salts of culture medium (Zhu *et al.,* 1996) and culture conditions (Rueb *et al.,* 1994). In the literature, there are many references concerning on the optimization of tissue culture system of rice cultivars, especially using plant growth regulators (Zaidi *et al*., 2006). However, there is limited report so far on the tissue culture of Malaysian upland rice cultivars (Shahsavari *et al*., 2010).

Plant tissue culture is the *in vitro* cultivation of new plant cells from a small piece of plant tissue such as leaves, stem or cell being cultured in a sterile medium and sterile condition. The ability of a plant cell to perform this is called totipotency. All plants exhibit the property of cellular totipotency, whereby individual cell can regenerate into an entire organism (Ribnicky *et al.,* 2002). Plant cell culture is a very important instrument for essential studies on plant biochemistry and accessible methods include regeneration of differentiated cultures which are the whole plant and organ cultures; shoots, roots and adventitious roots or dedifferentiated cultures for examples calluses, cell suspensions and protoplasts (Mustafa *et al.,* 2011). It is known that, callus induction and regeneration ability highly relies on genotypes,

explant types, carbohydrate sources, plant growth regulators, basal salts of culture medium and culture conditions (Shahsavari *et al*., 2010).

Plant regeneration need to be developed first before any improvement in genetic transformation take place. Some studies have shown that using proper plant growth regulators, carbon source and adding adequate amounts of specific chemicals (i.e., tryptophan, proline & sorbitol) can result in extreme increases in the regeneration frequency, regardless of the genotype requirements (Chowdhry *et al*., 1993; Ge *et al*., 2006; Zaidi *et al.,* 2006; Geng *et al.,* 2008; Shahsavari *et al.,* 2010). Therefore, the present study was focused on determination of embryogenic characteristics of callus and development of regeneration system of Malaysian upland rice.

1.2 Objectives of Research

The objectives are as follows:

i. To characterize the embryogenic characteristics of callus through histology.
ii. To examine proliferation rate of callus induction based on fresh weight.
iii. To study the effect of plant growth regulators on regeneration frequency of Malaysian upland rice (Bario and Hitam cultivar).

1.3 Problem Statement

Rice (*Oryza sativa* L.) is an imperative cereal product comprehensively. It is not only a staple food for more than two third of the worldwide population but also serves as a model plant in genomic studies (Bajaj and Mohanty, 2005; Tyagi and

Mohanty, 2000). Malaysian upland rice is a sort of rice that is planted in dry terrains and become in waterspout sustaining or constrained watering system condition compare to wetland rice. Utilizing this rice may save plenty of water and reduces water pollution and have potential to survive with high yield may help solve or minimize these issues (Geng *et al.,* 2008).

Tissue culture technology such as *in vitro* rice cultivation is an interesting and effective way to improve the rice regeneration by optimization of plant growth regulators. This is because of the high potential for regeneration of fertile rice plants from various explants. The success in tissue culture and plant regeneration depends on plant genotypes, sources and culture condition (Hoque and Mansfield, 2004). It was shown that the regeneration rate of upland rice is moderately low and varies among cultivars (Geng *et al.,* 2008). Thus, this study was carried out to characterize the embryogenic callus through histology study and regeneration frequency from Malaysian upland rice cultivars by the effect of plant growth regulators on plant regeneration.

1.4 Scope of Research

An interaction of Malaysian upland rice cultivars and culture was carried out in embryo culture at callus induction and regeneration stages. The effect of the plant growth regulators on regeneration was observed. Two cultivars of Malaysian upland rice such as Bukit Hitam and Bario were employed to determine the effect of cultivar tested and culture media on callus induction and also regeneration. The embryogenic characteristics of callus produced were also determined by histology.

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