PARTICLE BOMBARDMENT-MEDIATED TRANSFORMATION OF MALAYSIAN RICE MR 232 CALLUS

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Alhamdulillah

Specially dedicated to my dear family,

My Beloved Parents,
Abdul Manap b. Abdul Kadir & Minah bt. Awang

My Brother and Sister
Razif and Farzana

Fellow Lecturers and Friends

Thank you very much for everything

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ABSTRACT

Rice is a staple food for more than two-third of the world's population. As human population arises, the production of rice must also be increased via various approaches, and one of it is plant transformation. In this study, eight weeks old embryogenic callus derived from the scutellum of mature seed embryos of indica rice variety MR232 was biolistically transformed. The mature rice seed was firstly cultured on Murashige and Skoog medium supplemented with 1mg/L of 2, 4 –D and 5mg /L NAA for 8 weeks to induce callus. The callus produced showed embryogenic calli. The percentage of embryogenic calli induction was 40.7%. A white to yellowish embryogenic calli was recorded. While, in control treatment where the medium was not supplemented with any plant growth regulators, no callus induction was recorded. This embryogenic callus was then subjected to particle bombardment under room temperature. The callus of MR232 was bombarded with gold microparticles coated with plasmid pCAMGloipt harbouring the *gusA* gene which encodes β-glucuronidase. Physical parameters were observed in the bombardment, which were target distance and helium pressure. After bombardment, half of the calli were subjected to GUS assay while the other half was used for PCR analysis. The histochemical GUS assay was conducted to analyse the transformation efficiency. Calli bombarded at 9cm target distance showed the highest percentage of GUS activity (recorded as blue spots) with 22.8%. While, for helium pressure parameter, calli bombarded using 900 psi of helium pressure showed the highest percentage of GUS activity with 71.43%. However, PCR analysis results no detection of GUS fragment from the bombarded calli showing that the transformation was not stable.

ABSTRAK

Padi adalah tanaman yang menjadi sumber makanan ruji kepada dua per tiga penduduk dunia. Seiring dengan pertambahan populasi dunia, penghasilan padi perlu dipertingkatkan melalui pelbagai kaedah dan salah satu kaedah yang boleh diambil adalah melalui pemindahan gen. Dalam kajian ini, kalus embriogeni berumur 8 minggu dari benih matang padi indika kultivar MR232 digunakan dalam pembedilan zarah. Benih matang padi dikultur di atas media Murashige dan Skoog yang ditambah dengan 1mg/L 2,4-D dan 5mg/L NAA. Peratusan induksi kalus yang direkod adalah sebanyak 40.7% dan kalus yang dihasilkan menunjukkan ciri-ciri embriogenik iaitu berwarna putih dan kekuningan. Benih matang padi yang dikultur di atas media kawalan menunjukkan tiada pertumbuhan kalus direkod. Kalus embriogenik ini kemudiannya digunakan untuk pembedilan zarah. Zarah emas digunakan dan disalut dengan plasmid pCAMGloipt yang membawa gen β-glukuronides dan dibedil ke atas kalus embriogeni padi kultivar MR232. Parameter fizikal diuji dalam pembedilan ini, iaitu jarak sasaran dan tekanan gas helium. Kalus kemudiannya dibahagikan kepada dua bahagian untuk pengujian pengasaian GUS dan analisis PCR. Keberkesanan pemindahan gen dianalisis menggunakan kaedah pengasaian GUS yang mana bintik biru yang terbentuk pada kalus embriogeni direkodkan. Peratusan keberkesanan pemindahan gen paling tinggi dihasilkan oleh kalus embriogeni yang dibedil dengan jarak sasaran 9cm iaitu sebanyak 22.8% dan dengan 900 psi tekanan gas helium iaitu sebanyak 71.43%. Walau bagaimanapun, tiada gen GUS yang dikesan dalam analisis PCR menunjukkan pemindahan gen adalah tidak stabil.

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LIST OF SYMBOLS/ABBREVIATION

°C	Celcius degree
%	Percentage
CaMV	Cauliflower Mosaic Virus
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
g	Gram
HCl	Hydrochloric Acid
LB	Luria Bertani
mg	Milligram
mL	Milliliter
M	Molar
mM	Millimolar
MS	Murashige and Skoog (1962)
He	Helium
psi	Pressure per square inch
2, 4-D	2, 4-Dichlorophenoxyacetic acid
NAA	1-Naphthaleneacetic acid
Na_2HPO_4	Disodium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
$K_4[Fe(CN)_6]$	Potassium ferrocyanide
$K_3[Fe(CN)_6]$	Potassium ferricyanide
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
GUS	β-glucuronidase

MADA	Muda Agriculture Development Authority
GFP	Green fluorescent protein
USDA	United States Department of Agriculture
KADA	Kemubu Agricultural Development Authority
PCR	Polymerase Chain Reaction
bp	Base pairs

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Rice is one of the three leading food crops in the world and the second most widely grown cereal after wheat. Rice, wheat and maize directly supply more than 50% of all calories consumed by the entire human population. However, because a considerable amount of wheat used as animal feed, rice becomes the only major cereal crop that is consumed almost exclusively by humans (Van Nguyen and Ferrero, 2006). Rice provides 21% of global human per capita energy and 15% of per capita protein. Although rice protein ranks high in nutritional quality among other cereals, its protein content is modest. Rice also provides minerals, vitamins, and fiber. But through milling process, all of these constituents were reduced. Because of that, rice is mainly eaten as carbohydrate source in people's daily food intake (Maclean *et al*., 2002)

Rice from genus *Oryza* comprises of two cultivated and twenty-one wild species. While the species *Oryza glaberrima* is grown only in a small scale in West Africa, species *Oryza sativa* is cultivated all over the world. Rice was a primary food source for more than half of the world's population. *Oryza sativa* also called as Asian

rice, paddy rice, common rice, lowland rice and upland rice were consumed as a major staple food by more than 3 billion people in Asia (Khush, 1997).

Half of the world's population consumes rice as their staple food. 90% of this consumption was in Asia (Datta, 2004). Following the success of the Green Revolution in the early 1960s, a steady rise in Asia's per capita rice consumption from 85 kilograms per year in the early '60s to nearly 103 kilograms in the early'90s was recorded. Global per capita consumption also witnessed a rise from 50 to 65 kilograms per annum during the same period. The rising per capita consumption plus the growing population more than doubled global rice consumption during this period from 150 to 350 million tons (Mohanty, 2013).

Worldwide, area harvested each year with rice accounts up to 154 million hectares. Rice is mainly cultivated in Asia where China has the largest cultivation area of rice (cultivation of rice). According to FAO (2014), global paddy production in 2013 was recorded at 744.9 million tonnes. This rough paddy was then producing 496.6 million tonnes of milled rice. Global rice utilization in 2013-2014 was recorded with 490.3 million tonnes (milled basis) and is expected to increase up to 500 million tonnes in 2014-2015. Although, the forecast showed that 83% will be used as human food consumption, a rise also was forecasted in animal feed and other uses.

1.2 Problem Statement

In recent years, declining in the availability of land for cultivation has affected the production of rice worldwide. Dhlamini *et al.* (2005) stated that cereals production including rice was facing major problems caused by biotic and abiotic stresses. When combined, biotic stresses like diseases and pests as well as abiotic stresses like drought, cold, and salinity lead to 30%-60% yield loss each year all over the world. Other than

that, several other factors have also affected the rice growth yield such as declining productivities in rice production systems, pressures from abiotic stresses like drought, cold and salinity, pressures from biotic stresses like diseases and pests, increasing in productions costs and low returns in developing countries (Wang *et al.*, 2012). As the world's population now having grown from 6 to 7 billion people, rice consumers also are projected to increase annually. This resulted in the rate of population growth exceeding the rate of food grain production.

In order to overcome this problem, many improvements have been taken including conventional breeding to produce desirable trait in the plant. However, this attempt still faces a limited success. Recently, advancement in agricultural biotechnology has achieved a major breakthrough by the application of gene transfer technique that has been showed to have advantages compare to the conventional methods. Gene transfer technique allowed the introduction of genes from unrelated sources to be inserted into the rice genome (Khush, 1997).

1.3 Objectives

The objectives of this study are:

1. To record callus induction of MR2 232 on MS media contained 1mg /L of 2, 4-D and 5mg/L of NAA

2. To investigate physical parameters affecting the transformation efficiency using GUS assay and PCR analysis

3. To amplify the GUS gene from transformed callus

1.4 Scope of Research

The scope of this study was focused on manipulating the physical parameters of particle bombardment that were Helium pressure and target distance on transformation efficiency percentage. Prior to particle bombardment, the rice seed of MR232 was cultured onto Murashige and Skoog (MS) media in order to obtain the callus (explant). Analysis of the transformation efficiency was calculated and followed with histochemical analysis for GUS activity according to each parameter in order to investigate the optimized parameters for bombardment.

1.5 Significance of Study

Malaysian *indica* rice, MR 232 is a new rice variety produced by the Malaysian Agricultural Research and Development Institute (MARDI) and was introduced in 2006. MR 232 poses many advantages including resistant to blast, red tungro disease, sheath brown rot disease and moderately resistant to fungus attacks. However, the characteristics of the MR232 including broad and longer flag leaf, tall plant and poor root system lead it to be very susceptible to lodging. The significance of this study is to improve the transformation efficiency of particle bombardment process that can be used to introduce new gene into MR232 genome to improve its physical disadvantages.

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