AMPLIFICATION OF PARTIAL RICE FLORIGEN FROM MALAYSIAN UPLAND RICE CULTIVAR HITAM AND WAI

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This research work has been dedicated to my late mother Hajiya Ramatu. May ALLAH reward her with Aljannah Firdausi and her soul rest in peace.Ameen.

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ABSTRACT

Rice is one of the global sources of food where it is grown in almost 148 million hectares annually with about 11% of the world planted land. Wetland rice is commonly grown and required extra land for good irrigation system. To compensate for land scarcity, upland rice which requires less water consumption is one alternative. Regardless of wetland or upland, rice is still being planted once a year all over the world although it could be harvested faster during short day condition. To meet global rice demand, there is a need to understand the effect of photoperiod on rice florigen and flowering to produce better yield. The aim of the study was to amplify rice florigen from Malaysian upland rice to improve its potential by amplifying *Hd3a* and *RFT1* genes. Following amplification and verification via sequencing, cloning of the purified PCR product and construction of phylogenetic tree was carried out. Malaysian upland rice is grown in long day condition hence only partial *RFT1* gene was successfully isolated. The partial gene sequence was aligned with 10 other *RFT1* gene belonging to Indica and Japonica varieties and showed that Malaysian upland rice cultivars Hitam and Wai evolved from Japponica cultivars. Findings from this study suggested high similarity of *RFT1* gene between various cultivars and further research on this gene is hoped to provide better understanding of flowering time of Malaysian upland rice for crop improvement.

ABSTRAK

Beras adalah salah satu sumber makanan global di mana setiap tahun ia ditanam di hampir 148 juta hektar iaitu kira-kira 11% daripada tanah dunia. Padi sawah adalah jenis yang biasa ditanam dan memerlukan tanah tambahan untuk sistem pengairan yang baik. Untuk mengimbangi kekurangan tanah, padi tanah tinggi yang tidak memerlukan penggunaan air yang banyak adalah satu alternatif. Tidak kira kawasan paya atau tanah tinggi, padi masih ditanam sekali setahun di seluruh dunia walaupun ia boleh dituai lebih cepat dalam keadaan hari siang yang pendek. Bagi memenuhi permintaan beras global, terdapat keperluan untuk memahami kesan fotokala pada florigen padi untuk menghasilkan hasil yang lebih baik. Tujuan kajian ini adalah untuk mengamplifikasi florigen padi dari padi tanah tinggi Malaysia untuk meningkatkan potensinya dengan amplifikasi gen *Hd3a* dan *RFT1*. Setelah proses amplifikasi and pengesahan melalui penjujukan, pengklonan PCR produk tulen dan pembinaan pokok filogenetik telah dijalankan. Padi tanah tinggi Malaysia ditanam dalam keadaan hari siang yang panjang maka hanya sebahagian gen *RFT1* berjaya dipencilkan. Urutan jujukan sejajar dengan 10 gen *RFT1* lain daripada variasi Indica dan Japonica menunjukkan bahawa kultivar Hitam dan Wai berasal daripada padi tanah tinggi Malaysia mempunyai evolusi sejajar dengan variasi Japponica. Hasil daripada kajian ini mennunjukkan persamaan yang tinggi antara gen *RFT1* daripada pelbagai kultivar dan penyelidikan lanjut mengenai gen ini diharapkan dapat memberikan pemahaman yang lebih baik daripada masa berbunga padi tanah tinggi Malaysia untuk penambahbaikan tanaman.

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LIST OF ABBREVATIONS

RFT1	-	*Rice Flowering Time 1*
Hd3a	-	*Heading date 3a*
Eh1	-	*Early Heading Date 1*
Hd1	-	*Heading Date 1*
OsGI	-	*Oryza sativa gigantae*
PCR	-	Polymerase Chain Reaction
gDNA	-	Genomic Deoxy Ribonucleic Acid
DAS	-	Days After Sowing
FT	-	Flowering Time
LB	-	Luria Broth
LBA	-	Luria Broth Agar
µL	-	Microliter
mL	-	Mililiter
bp	-	Base Pairs
QTL	-	Quantitative Trait Loci
SAM	-	Shot Apical Meristem
UN	-	United Nation
FAO	-	Food Agricultural Organization
NCB1	-	National Centre for Biotechnology Information
M	-	Marker
Mg	-	Miligram

Kb	-	Kilobase
nm	-	Nanometer
et al	-	And other persons

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CHAPTER 1

INTRODUCTION

1.1 Study background

Plants are generally found in different environment. They have adapted to many external factors with an impact on growth, development and most importantly the ability of the plant to produce food and seed (Brambilla and Fornara, 2013).

Rice as a cereal plant is one of the source of food in which more than one third of the globe population depend on it. It is grown in almost 148 million hectares land annually which is about 11% of the land used in the world. About 90% rice produced in the world is consumed by Asian that has about 60% of the world population (Khush, 1997). More than 3 billion individuals of the world use rice as their essential food which provide about 80% of the calories needed (Sohrabi *et al*., 2012).

Rice alone provide more than one fourth of the total calories consumed in the world. It was reported by Food Agricultural Organisation (FAO) (1999) that rice remains the most important staple food in more than 15 countries across the Asia together with the pacific region. It also predominate 10 countries found between the

Caribbean, Latin America, seven countries in North America and Sub Saharan Africa respectively. Rice is a member of family *poaceae* which is made of 2 cultivars. These two species are mainly *Oryza sativa* also known as Asia cultivated rice and is found worldwide while the *Oryza glaberrima* which is found within the west African countries only, which is either planted as wetland or upland.

Wetland rice is a type of rice that is planted in waterlogged area which is difficult to handle. Upland rice is found in most part of the world except Antarctica but is found mostly in Asia, Latin America and Africa. This is the type of rice that is cultivated in dry soil with inadequate water and the yield is very low due to environmental stress (Sohrabi *et al*., 2012).

Out of 150 million hectares of land utilised for rice cultivation in Asia, only a small proportion of it is upland rice which is around 11% (Sohrabi *et al*., 2012). Most of the upland rice in Sabah and Sarawak is mainly for home consumption. Due to the enviromental stresses, the upland rice does not produce high yield. Therefore improving its yield production is necessary so as to meet the food security in the world. With the recent technology in the area of plant molecular studies, upland rice flowering associated closely with the yield can be increased. Flowering time can be described as heading date in cereal plants, which is the agronomical determinant for acclimatization to precise plantation area and growing seasons for existing species of planted rice.

Photoperiod is the most important factor that helps to provide the plants with suitable signals for flowering. Regulation of flowering is among important processes of plants that relate to its fruit and seed formation. Flowering time is regulated by many factors in environment such like light during the day, water and temperature supply. Flowering is classified into 3 classes according their response to photoperiod. These include long-day plant that produce flowering within the long-day (LD) condition, short day plants that promote flower under short day (SD) condition lastly neutral day plant which operates independently of photoperiod as shown in Table 2.1. Subsequent progress and recent advacement in the understanding of

photoperiodic flowering in *Oryza sativa* helps to reveal some conserved pathways which promotes flowering under short day condition and at the same time suppressed flowering under long day condition.

Genetic factors in addition to enviromental factors regulate the time of flowering in plant. Genetic mechanism about photoperiodic response for the long LDP has been studied through the *Arabidopsis* while the short day plants (SDPs) like rice show a remarkable similarity (Yano *et al*., 2001). Today, the world has depended on rice as a major source of food. Many studies have been carried out to improve its production using conventional approach. Through molecular studies, improvement of rice yield would be achieved. Studies to give background information on flowering genes of Malaysian upland rice could help improve its low yield.

1.2 Problem Statements

The consumption of rice is increasing at the speed of 1.5% per annum while its production presently increase at the speed of 1% per annum only (Jeon *et al*., 2011). According to the United Nation (UN) estimation, the world population will be 8 billion by 2025 while the present population is 6.7 billion, therefore there is need for the production to increase from 445 million tonnes at present to 486 million tones by 2025 (Jeon *et al*., 2011). Food Agricultural Organisation estimate by 2050 the world rice requirement will be 524 million tonnes which need yearly increase of 2 million tonnes from the present level of production (Jeon *et al*., 2011). Upland rice need to be improved so as to utilise the large abundance of land in Asia. To meet with the challenge of producing more rice from the existing land resources, therefore upland rice cultivar with a high yield are required. To achieve this in Malaysian upland rice there is a need to further understand the rice florigen hence this study could serve as flatform for increasing rice yield and seed production.

1.3 Objectives

1 To optimize PCR Amplification of *Hd3a* and *RFT1* genes using specific primers from Malaysian Upland Rice cultivars Wai and Hitam

2 To clone the ampilified PCR product into intermediate vector

3 To compare the amplified *RFT1* sequence with other known *RFT1* sequences from various *O. sativa* cultivars for creation of phylogenetic tree.

1.4 Scope of Study

Upland rice from the local cultivars were grown up until 14 weeks of age and the leaves harvested at different stages for genomic DNA extraction followed by PCR with specific primers. Amplified PCR product of correct band size was purified and sent for sequencing which was verified and later cloned into *E.coli.* Sequence obtained was aligned with selected *RFT1* gene sequence from NCBI to create phylogenetic tree using the amplified partial sequence.

1.5 Significance of Study

Due to the climate of Malaysia categorized under neutral day or long day condition, rice can only be planted once in a year as it took up until 7 months to obtain the yield. The success in optimizing *Hd3a* and *RFT1* genes from genomic DNA serve as a very important platform on how these genes regulate flowering in

upland rice of Malaysia. To date no such information has been reported on Malaysian upland rice. This serve as a very important platform to see how these genes can be manipulated to adapt to upland environment which in turn will help increase upland rice production in Malaysia. In the long run, manipulation of the gene will aid upland rice to be planted twice in a year hence, increasing its production.

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