UNIVERSITI PUTRA MALAYSIA

CROSSBREEDING BETWEEN CLEARFIELD® RICE VARIETIES WITH LOCAL WEEDY RICE IN MALAYSIA

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FP 2017 47

CROSSBREEDING BETWEEN CLEARFIELD® RICE VARIETIES WITH LOCAL WEEDY RICE IN MALAYSIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

April 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

CROSSBREEDING BETWEEN CLEARFIELD® RICE VARIETIES WITH LOCAL WEEDY RICE IN MALAYSIA

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April 2017

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The introduction of imidazolinone-resistant Clearfield® varieties has successfully control weedy rice infestations. However, there is concern about the possibility of gene introgression from Clearfield® rice to weedy *Oryza* species which is likely to take place as the incidence of natural hybridization. This could produce herbicide resistant weedy rice and thus render the controlling of weedy rice with imidazolinone herbicide ineffective. The main objective of this study was to examine whether the gene introgression from Clearfield® varieties to local weedy biotypes can occur. The study was conducted in three stages. In the first study, Clearfield® rice varieties (CL1, CL2) and four local weedy rice biotypes (WR1, WR2, WR3, WR4) were grown in a pot inside the glasshouse to evaluate the differences in the vegetative and reproductive development of local weedy rice biotypes in comparison with Clearfield® varieties. In the second study, manual pollination between two Clearfield® rice variants and four local weedy rice biotypes were performed to investigate the response of crossed F1 progenies on imidazolinone herbicide. Crossed seeds were collected and germinated in trays before the progenies were sprayed with imidazolinone herbicide (OnDuty®) at day fourteen with a rate of 214 g/ha. The third study was done to confirm whether the gene introgression has occurred in the F1 progenies using ten different Simple Sequence Repeats (SSR) primer. From the first study, both CL varieties were proved to have a high percentage of germination rate at 80% and 65% for CL1 and CL2 respectively. Three out of four of weedy biotypes have a moderate percentage of germination rate ranged from 30 to 60% while WR4 has the lowest percentage of germination rate which was only 5%. All weedy biotypes were significantly taller than Clearfield® varieties. However, no significant differences in number of tillers were observed between them except for WR4 at sixty days after seeding (DAS). All weedy biotypes flowered ten to twenty days later than the Clearfield® varieties. In the second study, WR4 crossed with CL1 produced the highest number of fertile seeds, while the lowest number is from the crossing of WR4 with CL2. Clearfield® rice parent has high resistant towards Onduty® while the WR parent is highly susceptible to Onduty® with 70% of the seedlings were shown having severe injury after 1 week of application with herbicide. Progenies of WR crossed with CL2 showed less herbicide injury compared to the progenies of WR crossed with CL1. The results also showed that between CL1 and CL2 variants, CL2 has higher compatibility to cross with all WR biotypes, with 100% were successfully survived. In the third study, it showed that SSR primer RM251 is the suitable primer to confirm the hybridization between Malaysian Clearfield® rice and weedy biotypes. As a conclusion, gene introgression from Clearfield® varieties to weedy biotypes can occur. Flowering synchronization and genetic compatibility between Clearfield® varieties and weedy biotypes can influence the rate of gene introgression.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KACUKAN ANTARA VARIETI PADI CLEARFIELD® DENGAN PADI ANGIN TEMPATAN DI MALAYSIA

Oleh

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Pengenalan varieti padi rintang racun imidazolinone, Clearfield®, telah berjaya mengawal serangan padi angin. Walau bagaimanapun, terdapat kebimbangan tentang kemungkinan berlaku introgresi gen daripada padi Clearfield® kepada padi angin spesis *Oryza* yang mungkin berlaku disebabkan insiden penghibridan semulajadi. Ini boleh menghasilkan padi angin yang rintang terhadap racun rumpai dan menyebabkan pengawalan padi angin menggunakan racun rumpai imidazolinone menjadi tidak berkesan. Objektif utama kajian ini adalah untuk mengkaji sama ada gen introgresi daripada varieti padi Clearfield® kepada padi angin boleh berlaku atau tidak. Kajian ini telah dijalankan dalam tiga peringkat. Dalam kajian pertama, varieti padi Clearfield® (CL1, CL2) dan empat jenis padi angin tempatan (WR1, WR2, WR3, WR4) telah ditanam di dalam pasu di rumah kaca untuk untuk menilai perbezaan pertumbuhan vegetatif dan pembiakan di antara padi angin dengan pertumbuhan vegetatif dan pembiakan padi Clearfield®. Dalam kajian kedua, pendebungaan secara manual antara padi Clearfield® dan padi angin tempatan telah dijalankan untuk menyiasat kerintangan F1 terhadap racun rumpai imidazolinone. Benih yang berjaya disilangkan, dikumpulkan dan dibiarkan bercambah dalam dulang sebelum progeni F1 disembur dengan racun rumpai imidazolinone (OnDuty®) pada hari ke empat belas dengan kadar semburan 214 g/ha. Kajian ketiga telah dilakukan untuk mengesahkan sama ada berlaku introgresi gen dalam progeni dengan menggunakan sepuluh jenis primer Ulang Urutan Mudah (SSR) yang berbeza. Daripada kajian pertama, kedua-dua jenis padi CL telah terbukti mempunyai peratusan kadar percambahan yang tinggi iaitu masing – masing pada 80% dan 65% untuk CL1 dan CL2. Tiga daripada empat jenis padi angin mempunyai peratusan kadar percambahan yang sederhana iaitu di antara 30 hingga 60% manakala padi angin WR4 mempunyai peratusan kadar percambahan yang paling rendah iaitu hanya 5%. Semua jenis padi angin adalah jauh lebih tinggi daripada padi Clearfield®. Walau bagaimanapun, tidak ada perbezaan ketara dalam bilangan anak padi yang dapat diperhatikan di antara mereka kecuali padi angin jenis WR4 semasa

enam puluh hari selepas pembenihan (DAS). Semua jenis padi angin berbunga lewat sepuluh hingga dua puluh hari daripada padi jenis Clearfield®. Dalam kajian kedua, WR4 yang disilangkan dengan CL1 menghasilkan bilangan biji benih yang subur paling tinggi, manakala bilangan yang paling rendah adalah dari penyilangan di antara WR4 dengan CL2. Padi induk Clearfield® mempunyai kerintangan yang tinggi terhadap Onduty® manakala padi induk WR adalah sangat mudah terdedah kepada Onduty® dengan 70% daripada anak pokok telah mengalami kecederaan teruk selepas 1 minggu disembur dengan Onduty®. Progeni WR yang disilang dengan CL2 menunjukkan kecederaan yang sedikit terhadap racun Onduty® berbanding dengan progeni daripada penyilangan WR dengan CL1.

Keputusan juga menunjukkan bahawa antara varian CL1 dan CL2, CL2 mempunyai keserasian yang lebih tinggi untuk berlaku persilangan dengan semua jenis WR, dengan 100% telah berjaya untuk terus hidup. Kajian yang ketiga menunjukkan bahawa SSR primer RM251 adalah primer yang sesuai untuk mengesahkan berlakunya penghibridan di antara varieti padi Clearfield® Malaysia dengan padi angin. Secara ringkas, gen introgresi dari varieti padi Clearfield® kepada padi angin boleh berlaku. Masa berbunga yang serentak dan keserasian genetik di antara varieti padi Clearfield® dan padi angin boleh mempengaruhi kadar introgresi gen.

ACKNOWLEDGEMENTS

In the name of Almighty Allah, Who provided me with the strength, wisdom, and will to complete my Master's journey. May His name be glorified and praised.

First and foremost, I would like to express my appreciation and sincere gratitude to my supervisor Dr. Norida Mazlan for her guidance and motivation throughout my Master's journey. Her continuous support and inspiring suggestions have been precious for the development of this thesis content. Besides my supervisor, I would like to thank the rest of my committee members: Prof. Dr. Mohd Rafii Yusop and Prof. Datin Dr. Siti Nor Akmar Abdullah for their encouragement, invaluable advice, and insightful comments.

Special thanks go to all the staff of Department of Agriculture Technology for giving me tremendous help during the completion of my experiment. Not forgotten, my greatest gratitude goes to my friends and labmates in Universiti Putra Malaysia. They were very strong in supporting me during these stressful and difficult moments.

Finally, I want to express my very profound gratitude to my family members for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

I certify that a Thesis Examination Committee has met on 28 April 2017 to conduct the final examination of Nur Hidayatul Shuhada binti Anuar on her thesis entitled "Crossbreeding between Clearfield® Rice Varieties with Local Weedy Rice in Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This is to confirm that:

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LIST OF ABBREVIATIONS

®	Registered trademark
ai	Active ingredients
AFLP	Amplified Fragment Length Polymorphism
AHAS	Acetohydroxy acid synthase
ALS	Acetolactate synthase
ANOVA	Analysis of variance
CPS	Clearfield® Production System
DAS	Days after seeding
DNA	Deoxyribonucleic acid
DOSM	Department of Statistics Malaysia
EMS	Ethyl methanesulfonate
FAO	Food and Agriculture Organization
FELCRA	Federal Land Consolidation and Rehabilitation Authority
GM	Genetically modified
IADA	Integrated Agriculture Development Area
IMI	Imidazolinone
IRRI	International Rice Research Institute
LSU	Louisiana State University
MADA	Muda Agriculture Development Area
MARDI	Malaysian Agriculture Research and Development Institute
NRE	Natural Resources and Environment
PCR	Polymerase chain reaction
RAPD	Random Amplified Polymorphic DNA

RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSL	Self-sufficiency level
SSR	Simple Sequence Primer
SSLP	Simple Sequence Length Polymorphism
t	Ton
TAE	Tris/acetate/ Ethylenediaminetetraacetic acid
TBE	Tris/Borate/Ethylenediaminetetraacetic acid
USA	United State of America
USD	United State Dollar

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Rice (*Oryza sativa*) is one of the main food sources for human consumption (Gealy et al., 2003) with Asia being the largest consumer and producer (Rajamoorthy et al., 2015). In Malaysia, rice is the third most important crop after the oil palm plant and the rubber plant, covering an area of no more than 0.7 million ha (Akinbile et al., 2011). In 2014, Malaysia self – sufficient level (SSL) for rice was 71.6% compared to 71.7% in 2013 (DOSM, 2016). About 10 - 35% reduction in rice yield is caused by the problem of weed competition with rice for sunlight, water, and nutrients (Karim et al., 2004). Weedy rice (*Oryza sativa* complex) or locally known as *padi angin* is difficult to control since it belongs to the same biological taxon as cultivated rice (Shivrain et al., 2007). Infestation of weedy rice at 35% of the rice plants caused about 60% yield loss and it can be up to 74% under serious infestation of weedy rice (Karim et al., 2004).

The introduction of Clearfield® rice is the latest technology used to manage weedy rice problem. Clearfield® rice cultivar offers an opportunity to selectively manage weedy rice with imidazolinone (IMI) herbicide and it is considered a safe product because it is not genetically modified (GM) (Sudianto et al., 2013). The cultivars will help to reduce the cost of weed management in rice cultivation and yield a higher quality of rice. In addition, the uses of the Clearfield® rice cultivars can reduce the amount of herbicides released into the environment and the ecosystem since IMI herbicide are applied in much lesser volumes (Azmi, 2013). In Malaysia, two varieties were released, known as MR220 CL1 and MR220 CL2 (BASF, 2010).

1.2 Problem Statement

There are concerns about the impact of releasing Clearfield® rice in a rice field for a long period of time. One of the concern is the possibility of transferring the resistance trait from Clearfield® rice to compatible weedy rice (Olofsdotter et al., 2000) which is likely to take place as the incidence of natural hybridization. This could produce herbicide resistant weedy rice and leads to the problem of controlling the weedy rice with IMI herbicide. The occurrence of IMI-resistant weedy rice outcrosses had been observed in some countries after a few years of Clearfield® rice commercialization. Countries like Brazil (Villa et al., 2006), United State (Burgos et al., 2007 and Zhang et al., 2006), Colombia (Gressel and Valverde, 2009) and Italy (Busconi et al., 2012) have reported occurrence of gene flow between Clearfield® rice and weedy rice in some commercial fields. The gene flow occurs when cultivated rice and weedy rice co-exist and they flower synchronously (Sudianto et al., 2013).

A study conducted by Villa et al., in 2006 showed that natural outcrossing rate of 0.065% has been reported in Brazil. In the United State of America, the natural outcrossing rate of 0.17% up to 0.763% has been reported in Louisiana and Arkansas (Shivrain et al., 2009 and Zhang et al., 2006). The resistant gene transfer from Clearfield® rice to weedy rice is evident. Therefore, without proper mitigation, the established IMI- resistant weedy rice populations will increase.

1.3 Study Objectives

The objectives of this study are:

(i) To evaluate the differences in vegetative and reproductive development of local weedy rice biotypes in comparison with Clearfield® varieties
(ii) To investigate the resistant potential of crossed Clearfield® rice with local weedy rice progenies on imidazolinone herbicide
(iii) To determine gene introgression from Clearfield® rice varieties to local weedy rice biotypes.

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