UNIVERSITI PUTRA MALAYSIA

GENETICS, GENOTYPE STABILITY ANALYSES AND EVALUATION OF NEWLY-DEVELOPED HIGH-YIELDING HYBRID RICE IN MULTILOCATION

ELIXON SUNIAN @ ELIXSON BIN SULAIMAN

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

Specially dedicated to my family for their unwavering support and patience during my studies. And it's from them that I've learnt and grown.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Mohd Rafii Yusop, PhD
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Hybrid rice varieties have been reported to produce 15-20% yield increment over the best conventional inbred varieties due to the heterosis or hybrid vigour. This yield advantage of hybrid rice can be a viable option to increase national rice production and farmers' income. Therefore, this study was conducted to develop locally adapted high-yielding and stable hybrid rice for the Malaysian environment. The main objective of the study was to develop superior high-yielding and stable rice hybrids across environments for local cultivation. The study was divided into two main experiments; Experiment 1 was conducted to examine the combining ability effects of 120 F_1 hybrids and 34 parents with the specific objectives were to develop and evaluate F_1 hybrids derived from line × tester based on yield and growth performance, to estimate general and specific combining abilities of hybrid for yield, yield components and grain quality and to quantify heterosis values base on check varieties, mid-parents and better parents of the newly developed F_1 hybrids. The experiment was conducted during off season 2016 (April – September) at MARDI Center of excellence for Rice Crop, Seberang Perai, Penang. Results of Experiment 1 showed that the top 20 hybrids that had significantly high mean yield which ranged from 46.62 to 54.46 g/plant as compared to the grand mean value (37.83 g/plant) were recorded in H17, H41, H35, H119, H108, H49, H105, H59, H94, H37, H107, H60, H43, H65, H27, H52, H46, H16, H3 and H2. These hybrids also showed the standard heterosis value was above 15% which is an important character for the selection of potential hybrids. Their grain characters were suitable to the Malaysian market which prefer rice with a long (> 6.21 mm length) and slender (> 3.00 mm of length to width ratio) grain, whereas the amylose content in the category of low (< 20%) to intermediate (20 – 25%). The combining ability was estimated where the rice testers T4 was a potential parent which had significantly positive GCA (general combining ability) effects mainly for yield (2.56), filled grains per panicle (4.25), total grains per panicle (4.37) and thousand grains weight (2.54). The best rice restorer line was L17 which recorded significantly positive GCA values particularly for yield (8.11), filled grains per panicle (12.57) and thousand grains weight (1.81). The SCA (specific combining ability) effect was

estimated where hybrids H108, H65, H27, H114, H41, H35, H37, H81, H3, H105, H60, H2, H44, H17, H43, H118, H6, H49, H64 and H39 recorded significantly positive SCA values ranged from 4.84 - 15.05. Meanwhile, Experiment 2 was conducted to evaluate the G×E interaction effects and genotype stability of 20 selected hybrids which were tested in eight environments with a combination of four locations and two planting seasons. The specific objectives of Experiment 2 were to quantify the effect of environments on the expression of phenotypic values of the selected hybrid rice across different environments and to identify stable and high yield hybrid rice for commercial cultivation in Malaysia. Most of the evaluated quantitative traits were highly influenced by G×E (genotype × environment) interaction except for panicle length, grain length, grain width, milled grain length, milled grain width and milling recovery. Pooled data over environments revealed that eight hybrids; G19, G3, G18, G13, G8, G7, G14, and G12 recorded the highest mean yield (8.21, 8.10, 7.80, 7.49, 7.34, 7.24, 7.22 and 7.16 t/ha, respectively) as compared to the best check variety G21 (5.90 t/ha). The univariate and multivariate hybrid stability analyses for yield indicated that high yielding hybrids namely, G19, G3, G18, G8 and G14 had highly stable performance across the environments. The univariate hybrid stability and GGE (Genotype Main Effects + Genotype × Environment Interaction) biplot analysis revealed that hybrid G7 was highly stable performance across the environments, contrary in AMMI (Additive Main Effect and Multicative Interaction Effect) stability analysis found that this hybrid was unstable. Generally, there were three patterns of hybrid's interaction across the environments. The first categories are well-adapted across the environment (G19, G3, G18, and G8), the second categories are hybrid that is adapted to specific environments (G12) while, the third categories are the poorly adapted hybrids in all environments (G2, G4, G5 and G17) hence, these hybrids can be discarded. From this study, the newly developed hybrids rice, G19, G3, G18, H8 and G12 are recommended for local verification trial and upscaling before being released for cultivation in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

GENETIK, ANALISA KESTABILAN GENOTIP DAN PENILAIAN MULTILOKASI PADI HIBRID BAHARU BERHASIL TINGGI

Oleh

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Varieti padi hibrid telah dilaporkan boleh mencapai hasil melebihi 15-20% berbanding varieti konvensional inbred yang disebabkan oleh heterosis atau kecergasan hibrid. Kelebihan hasil padi hibrid ini boleh menjadi pilihan untuk meningkatkan pengeluaran padi negara dan pendapatan petani. Oleh itu, kajian ini telah dijalankan untuk membangunkan varieti hibrid berhasil tinggi dan stabil pada persekitaran Malaysia. Objektif utama kajian ini adalah membangunkan varieti padi hibrid berhasil tinggi dan stabil terhadap persekitaran untuk penanaman lokaliti. Kajian telah dibahagikan kepada dua eksperimen utama; Experimen 1 telah dijalankan untuk menilai kesan keupayaan gabung terhadap 120 hibrid F_1 dan 34 induknya dengan objektif khusus adalah membangunkan dan menilai hibrid F_1 yang dijana daripada titisan × penguji berdasarkan kepada ciri hasil dan prestasi pertumbuhan, menentukan keupayaan bergabung am dan spesifik oleh hibrid untuk ciri hasil, hasil komponen dan kualiti biji dan mengukur nilai heterosis berdasarkan kepada varieti kawalan, *mid-parent* dan *better parent* oleh hibrid yang telah dijana. Eksperimen telah dijalankan pada musim luar 2016 (April – September) di Pusat Kecemerlangan MARDI untuk tanaman padi, Seberang Perai Pulau Pinang. Keputusan kajian Experimen 1 menunjukkan 20 hibrid terbaik yang mempunyai purata hasil yang signifikan pada julat 46.62 g hingga 54.46 g/rumpun berbanding purata hasil keseluruhan (37.83 g/rumpun) telah direkodkan pada H17, H41, H35, H119, H108, H49, H105, H59, H94, H37, H107, H60, H43, H65, H27, H52, H46, H16, H3 dan H2. Hibrid ini juga menunjukkan nilai heterosis piawai adalah melebihi 15% yang mana ia merupakan ciri penting didalam pemilihan potensi suatu hibrid. Ciri kualiti bijinya juga sesuai untuk pasaran Malaysia yang mengutamakan biji beras panjang (panjang > 6.21 mm) dan tirus (nisbah panjang dan lebar > 3.00 mm), manakala kandungan amilosanya dalam kategori rendah (< 20%) ke sederhana (20 – 25%). Keupayaan bergabung juga telah dinilai yang mana padi penguji T4 merupakan induk berpotensi yang telah menunjukkan nilai GCA (keupayaan bergabung am) yang signifikan dan positif terutama untuk hasil (2.56), bilangan biji bernas per tangkai (4.25), jumlah biji per tangkai (4.37) dan berat seribu biji (2.54). Titisan padi restorer yang terbaik adalah L17 yang telah merekodkan nilai GCA yang signifikan dan positif terutamanya untuk hasil (8.11), bilangan biji bernas per tangkai (12.57) dan berat

seribu biji (1.81). pengaruh SCA (keupayaan bergabung spesifik) telah dinilai yang mana hibrid H108, H65, H27, H114, H41, H35, H37, H81, H3, H105, H60, H2, H44, H17, H43, H118, H6, H49, H64 and H39 merekodkan nilai SCA yang signifikan dan positif pada julat 4.84 - 15.05. Manakala, Eksperimen 2 telah dijalankan untuk menguji kesan interaksi G×E (genotip × persekitaran) dan kestabilan genotip 20 hibrid terpilih telah diuji di lapan persekitaran dengan kombinasi empat lokasi dan dua musim penanaman. Objektif khusus Experimen 2 ialah untuk mengukur pengaruh persekitaran keatas ekpresi nilai fenotipik oleh hibrid terpilih terhadap persekitaran yang pelbagai dan mengenalpasti hibrid berhasil tinggi dan stabil untuk penanaman komersil di Malaysia. Kebanyakan ciri kuantitatif yang dinilai adalah amat dipengaruhi oleh interaksi G×E (genotip × persekitaran) kecuali panjang tangkai, panjang biji padi, lebar biji padi, panjang beras, lebar beras dan pengilangan. Gabungan data dari keseluruhan persekitaran menunjukkan lapan hibrid; G19, G3, G18, G13, G8, G7, G14 dan G12 telah merekodkan purata hasil yang tertinggi (masing-masing 8.21, 8.10, 7.80, 7.49, 7.34, 7.24, 7.22 dan 7.16 t/ha) berbanding dengan varieti kawalan G21 (5.90 t/ha). Analisa kestabilan hibrid secara univariat dan multivariat keatas hasil menunjukkan hibrid berhasil tinggi iaitu G19, G3, G18, G8 dan G14 mempunyai kestabilan yang amat tinggi pada pelbagai persekitaran. Analisa kestabilan hibrid secara univariat dan analisa biplot GGE (*Genotip Main Effects + Genotype × Environment Interaction*) mendapati hibrid G7 adalah amat stabil di pelbagai persekitaran, yang bertentangan dengan analisa AMMI (*Additive Main Effect and Multicative Interaction Effect*) mendapati hibrid ini adalah tidak stabil. Secara amnya, terdapat tiga corak interaksi hibrid terhadap pelbagai persekitaran. Kategori pertama ialah adaptasi yang baik pada pelbagai persekitaran (G19, G3, G18 dan G8), kategori kedua ialah hibrid yang beradaptasi pada persekitaran yang spesifik (G12), manakala, kategori ketiga ialah hibrid yang adaptasinya yang lemah pada semua persekitaran (G2, G4, G5 dan G17), oleh itu hibrid ini boleh disingkirkan. Daripada kajian ini, padi hibrid baharu yang dibangunkan iaitu G19, G3, G18, G8 dan G12 adalah disyorkan untuk penilaian penentusahan setempat dan skala perintis sebelum ianya diistiharkan untuk penanaman di Malaysia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. Members of the Supervisory Committee are as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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LIST OF ABBREVIATIONS

%	Percentage
^{o}C	Degree Celsius
AEC	Average environment coordinate
AMMI	Additive main effect and multiplicative interaction effect
ANOVA	Analysis of variance
ASV	AMMI Stability Value
cm	centimeter
CMS	Cytoplasmic male sterile
g	gram
G × E	Genotype × environmental interaction
GGE	Genotype main effects plus genotype × environment interaction
GA_3	Gibberellic acid
HRDC	Hybrid Rice Research Consortium
IRRI	International Rice Research Institute
kg	kilogram
LSD	Least significant difference
PCA	Principal Component Analysis
SAS	Statistical analysis software
SVD	Singular value decomposition
SVP	Singular value partition
t/ha	tonnes per hectare
SE	Standard error
WA	Wild abortive

CHAPTER 1

INTRODUCTION

1.1 Background

Rice is one of Malaysia's most important food crops. Malaysian rice hectarage remained stable at 0.68 million ha, with an average yield of 4.07 t/ha (USDA, 2021). Rice parcel areas decreased from 291,086 to 284,162 ha between 2015 and 2019 (DOA, 2019). Deterioration of paddy cultivation areas, particularly in rice fields on the outskirts of cities, is always aimed at converting land into industrial activities (Fikry and Norshafadila, 2017). Malaysia's population is expected to rise from 32.4 million in 2018 to 32.6 million in 2019, with a 0.62% increase (DOSM, 2019). The constant increase in population size, combined with the decline in cultivated areas, necessitates immediate intervention by increasing current production to meet future challenges.

Increasing current yield potential necessitated the use of high-yielding varieties, with hybrid rice technology playing a significant role. According to China's experiences, the adoption of hybrid rice technology has significantly increased China's self-sufficiency in rice production. To date, hybrid rice cultivation has occupied more than 60% of China's rice-growing areas, totalling 17 million ha (Yuan, 2014; Cheng *et al*., 2007). Hybrid rice has a yield advantage of more than 20% over conventional indica and japonica rice varieties due to high heterosis and diverse genetic background (Qian *et al*., 2016). Hybrid heterosis derived from the crossing of two genetically dissimilar inbred parents would lead to superiority in vigour for increased yield, panicle length, more grains per panicle, productive tiller, etc. (Haiyang and Xing, 2018; Virmani *et al*., 2003). Yonghui *et al*. (2020) reported the super hybrid rice Yliangyou 3218 which showed a higher grain yield as compared with super rice Zhendao 11 inbred with high above-ground biomass production and an increasing number of effective tillers and spikelets per panicle.

Malaysia is still lagging in terms of hybrid technology adoption. Several hybrids from other countries have previously been evaluated in Malaysia with promising results. However, many imported hybrid seeds have poor medium grain quality, lower yield than popular inbred varieties, and are susceptible to diseases such as blast, false smut, and bacterial leaf blight. As a result, local hybrid rice with agro-climate suitability and consumer-preferred grain quality should be developed. Hybrid rice technology requires the development of highly stable parental inbred. Similarly, there is a need to investigate hybrid rice technology in Malaysia in terms of genetic diversity, agronomy performance, and crop management to make hybrid rice technology adoption work efficiently. MARDI released a local hybrid rice variety, Kadaria 1, in 2019, indicating the need to introduce more local rice hybrid varieties with higher yields to diversify varietal options for farmer cultivation.

Superior parents must be bred to develop a high-yielding rice hybrid. In the development of hybrid rice, information on yield per se performance does not always provide a better

indication of combining ability. As a result, selecting potential parents using combining ability analysis could assess the value of parents in producing a superior hybrid. General combining ability provides information on the parent's choices, which can be used for future breeding or crop improvement. Meanwhile, specific combining ability provides information on the best hybrid combination for a specific phenotypic trait, such as yield performance. Furthermore, the superior hybrid must have a high superior parent and standard heterosis.

Among the limiting factor for hybrid, development is genotype by environment interaction (GEI). This affects the performance of developed hybrids in different agro-ecological conditions. Although, some hybrids may have wide adaptability across environments while, some are highly influenced by the change in the environment which performs inconsistently in other locations (Akter *et al.*, 2019). Exploration of varietal adaptability is thus critical in the development of superior hybrid rice (Peng *et al*., 2016).

1.2 Problem statements

The rice crop continues to be the primary source of income for more than 32 million Malaysians. According to FAO (Food and Agricultural Organization) reports, Malaysia rice production in 2019 was 2.67 million tonnes but is expected to fall to 2.58 million tonnes in 2020. (FAO, 2021). With the cultivated area under rice stabilising at around 0.68 million hectares, yield plateauing at 4.02 – 4.07 t/ha, declining productivity trend, and shrinking of rice parcel areas due to other activities (urban development, industrials, etc.), there is an urgent need to increase current yield output through exploration of other technologies and development of high yielding varieties in addressing the problems associated with local production. One of the innovations that have been a driving force in overcoming challenges and providing a solution that benefits the rice industry in most countries is hybrid technology.

Farmers in Malaysia commonly cultivate inbred varieties of rice, which is one of the major impediments to achieving maximum yield potential in production. Furthermore, imported hybrid rice seeds are costly and poorly adapted to local agroecology, and grain quality is not preferred by local consumers. In Malaysia, hybrid rice technology is considered new and needs to be explored in terms of genetics, agronomy, and crop management. A hybrid rice breeding programme is critical for the development of high-yielding, stable-performance rice varieties for the Malaysian environment. As a result, the development of locally adapted hybrid rice seeds is a promising option for increasing national rice productivity, which will contribute to national self-sufficiency and raise rice farmers' income. Hybrid rice varieties have been used for commercial cultivation in countries such as China, India, the Philippines, and the United States, contributing to the country's higher output.

1.3 Significance of the study

Hybrid rice technology is needed to explore in the research and development (R&D) of breeding of high yielding varieties which have the potential and impact in the effort to increase local production. This is due to the facts that; i) hybrid rice varieties have higher yield advantages about 15 - 20% higher than the inbred variety due to the heterosis or hybrid vigour, thus, farmers can earn extra income. ii) the effect of combining ability of both general and specific combining are important indicators of potential values for assessing inbred rice lines in hybridisation as a step to develop superior local hybrids. iii) the candidate hybrids and their phenotypic response to environmental fluctuation are not similar among genotypes leading to G×E interaction (iv). Yield stability performance differs across environments which lead to released varieties possibly different in adaptation and consistency in yield performance across environments, therefore, it's compulsory to identify rice hybrid with high yield and well-adapted across major rice granaries before being released for commercial.

1.4 Objectives

The main objective of this study was to develop superior high-yielding and stable rice hybrids across environments for local cultivation. The specific objectives were:

i. To develop and evaluate F_1 hybrids derived from line×tester based on yield and growth performance
ii. To estimate general and specific combining abilities of hybrid for yield, yield components and grain quality.
iii. To quantify heterosis values base on check varieties, mid-parents and better parents of the newly developed F_1 hybrids
iv. To quantify the effect of environments on the expression of phenotypic values of the selected hybrid rice across different environments.
v. To identify stable and high yield hybrid rice for commercial cultivation in Malaysia.

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