UNIVERSITI PUTRA MALAYSIA

EVALUATION AND SELECTION OF RICE MUTANTS FOR HIGH YIELD AND QUALITY FODDER PRODUCTION

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By

OLADOSU YUSUFF ABISOLA

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

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DEDICATION

My incomparable parents

and invaluable teachers in all realms of my studies

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

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October 2014

Chairman: Mohd Rafii Yusop, PhD.
Institute: Tropical Agriculture

Increasing genetic variability with mutagenic agents has been broadly employed in plant breeding because this method alters one or more desirable traits, creating an effective breeding programme. Based on this background, this study was performed to evaluate the genetic diversity of derived mutant lines and to establish the relation between yield and its components in them using multivariate analysis to select the best genotype in grain yield and high nutritional quality. To achieve this objective, two field trials were performed on 45 rice genotypes and four Malaysian commercial varieties to evaluate their growth and yield traits, and to perform a comparative nutrient analysis. From data collected on quantitative traits, analysis of variance, variance components, heritability, genotypic and phenotypic coefficient variation, and expected genetic advance were estimated. All the vegetative and yield traits showed significant variations among the genotypes. Additionally, all of the traits correlated positively with the final yield, except for number of tillers.

To complement this study, molecular methods were used to study to further confirm the outcome of this research, establishing the relation between the two methods. The genetic diversity of the 45 genotypes was analysed using 25 inter-simple sequence repeats markers (ISSRs). The data were analysed using NT-SYS software to estimate the genetic relations among the rice accession from different sources, i.e., Malaysia, Bangladesh and Vietnam, to detect level of polymorphism within and between populations. The analysis of the molecular variance showed that 90% of total genetic variations were due to differences within populations, whereas 10% of total genetic variations were due to differences among populations. A positive correlation was observed between similarity distance between the morphological traits and the ISSR molecular data. Both methods, morphological traits and molecular markers are useful tools for assessing the genetic variability and diversity of the mutant rice genotypes.

Data on fodder quality, namely, proximate composition of cell wall content analyses, primary and trace mineral components varied significantly among the genotypes for all of the traits except acid detergent fibre (ADF), acid detergent lignin (ADL), organic matter digestibility (OMD), metabolisable energy (ME), cellulose and hemicellulose. The results from this study indicated that ion beam radiation caused significant changes in the agronomic performance of majority of the mutant lines compared with the parental variety (MR219), based on genetic diversity results and agronomic traits performance. Evaluation over two locations, mutant lines ML21,

ML10, ML4, ML6 and ML9 performed better in terms of yield production compared to other mutants. The result from the nutritional characteristic assessment revealed that ML24 and ML22 rice straw was better in CP, N, P, Mg content compared with other genotypes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PENILAIAN DAN PEMILIHAN PADI MUTAN BAGI PENGELUARAN HASIL TINGGI DAN FODER BERKUALITI

Oleh

OLADOSU YUSUFF ABISOLA

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Peningkatan dalam kepelbagaian genetik melalui ejen mutagenesis telah meluas digunakan dalam pembiakbakaan tumbuhan kerana kaedah ini dapat mengubah satu atau lebih ciri yang diingini dalam program pembiakbakaan yang berkesan. Berdasarkan maklumat asas ini, kajian ini telah dijalankan bagi menilai kepelbagaian genetik mutan yang diaruh dan untuk menentukan hubungan antara ciri hasil dan komponennya melalui analisis multivariat bagi memilih genotip yang terbaik dalam penghasilan bijian dan mempunyai kualiti pemakanan ternakan yang tinggi. Untuk mencapai objektif tersebut, dua ujian lapangan telah dijalankan ke atas 41 genotip padi mutan dan empat varieti komersil Malaysia bagi ciri pertumbuhan dan hasil serta membuat analisis perbandingan nutrient pemakanan. Dari data kuantitatif yang telah diambil, analisa varians, varians komponen, keterwarisan, pekali variasi genotip dan fenotip, dan kemajuan genetik dijangka telah dinilai. Semua genotip menunjukkan variasi untuk semua ciri vegetatif dan hasil. Selain itu, semua ciri berkorelasi secara positif terhadap hasil akhir, kecuali bilangan anak padi.

Untuk mengukuhkan lagi kajian ini, kaedah molekul telah digunakan untuk mengesahkan lagi hasil kajian ini, serta bagi mengkaji hubungan antara kedua-dua kaedah tersebut. Kepelbagaian genetik pada 45 genotip telah dianalisis dengan menggunakan 25 penanda mudah jujukan berulang pertengahan (ISSRs). Data dianalisis dengan menggunakan perisian NT-SYS bagi menganggarkan hubungan genetik antara aksesi padi yang berlainan sumber, iaitu, Malaysia, Bangladesh dan Vietnam, untuk mengesan tahap polimorfisma di dalam dan di antara populasi. Analisis varians molekul menunjukkan bahawa 90% daripada jumlah variasi genetik adalah disebabkan oleh perbezaan dalam populasi, manakala 10% daripada jumlah variasi genetik disebabkan oleh perbezaan di antara populasi. Didapati korelasi positif bagi jarak kesamaan di antara ciri morfologi dan data molekul ISSR. Kedua-dua kaedah iaitu ciri morfologi dan penanda molekul adalah kaedah yang sangat berguna untuk menilai kepelbagaian genetik dan genotip padi mutan ini.

Data kualiti makanan ternakan, iaitu komposisi proksimat dan analisis kandungan dinding sel, komponen mineral utama dan surih adalah berbeza di kalangan genotip untuk semua ciri kecuali gentian detergen asid (ADF), lignin detergen asid (ADL), bahan organik penghadaman (OMD), tenaga metabolisma (ME), selulosa dan hemiselulosa. Keputusan kajian ini menunjukkan bahawa radiasi sinaran ion menyebabkan perubahan ketara dalam prestasi agronomi kebanyakan waris mutan

berbanding dengan varieti induk (MR219) iaitu berdasarkan keputusan kepelbagaian genetik dan ciri agronomi. Berdasarkan penilaian di dua lokasi, mutan ML21, ML10, ML4, ML6 dan ML9 menunjukkan prestasi yang lebih baik dari segi pengeluaran hasil berbanding mutan lain. Hasil daripada penilaian ciri nutrisi mendapati bahawa jerami mutan ML24 dan ML22 memberikan kandungan CP, N, P, Mg yang lebih baik berbanding dengan genotip yang lain.

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- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

%	-	Percentage
ANOVA	-	Analysis of variance
AMOVA	-	Analysis of molecular variance
bp	-	Base pair
cm	-	Centimetre
CTAB	-	Cetylmethylammonium bromide
CV	-	Coefficient Variation
DNA	-	Deoxyrobose nucleic acid
DNase	-	Deoxribonuclease I
dNTPs	-	deoxribonucleotide triphosphate
EDTA	-	Ethylene diamine tetracetate
G	-	Gram
HCL	-	Hydrochloric acid
H_2O_2	-	Hydrogen perioxide
IRRI	-	International Rice Research Institute
L	-	Liter
M	-	Molar
Mg	-	Milligram
Min	-	Minute
Ml	-	Millilitter
NaCl	-	Sodium Chloride
Ng	-	Nanogram
Nm	-	nanometer
^0C	-	Degree celcius
PCR	-	Polymerase chain reaction
PVP	-	polyvinylpolypyrrolidone
Rpm	-	Rotation per minute
TBE	-	Tris-borate-EDTA

Tm	-	Melting temperature
UV	-	Ultraviolet
µg	-	Microgram
µl	-	Microliter
V	-	Vol

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rice is considered one of the largest cereal crops. Playing a significant role in the diets of more than half of the world's population (Khush, 2005), rice contributes 43% of total food grain production and 46% of all cereal production globally. Rice occupies the third position after wheat and maize in terms of cultivation area and production. This crop not only provides carbohydrate but also supplies several essential nutrients, such as protein, iron, calcium, thiamine, riboflavin, niacin and vitamin E, to the human body (Akinbile et al., 2011). In Southeast Asia, where agriculture is the predominant source of livelihood, over 115 million ha of land were estimated to be devoted to the production of rice, oil palm, maize, coconut and natural rubber (ADB, 2009). Rice has been widely consumed by this region's population for over 4000 years and is the staple food of approximately 557 million people (Manzanilla et al., 2011). In 2007, the average annual consumption per capita was approximately 197 kg (FAOSTAT, 2012) and provided 49% of calories and 39% of the protein in the diet (FAOSTAT, 2012).

Rice production in Malaysia began long ago, and now, the country has a reasonable potential to produce different types of rice varieties, which are primarily grown in eight granary areas in Peninsular Malaysia (Azmi and Mashhor, 1995). In Malaysia, four primary rice environments exist, which are classified as irrigated, partially irrigated, rainfed lowland and upland regions. The irrigated lowland predominates in Peninsular Malaysia; rainfed lowland rice is more common in Sabah, and upland rice prevails in Sarawak.

Food security and socioeconomic stability become points of great concern and importance. However, the wide gap between rice production and its demand increase on a daily basis because of increases in the human population. Thus, the grain yield per unit area must be increased to eliminate these present and future problems. Various research outcomes have shown that production should be increased by 40% by 2030 to meet the increasing demand of the inhabitants of the world (Khush, 2005). The current reasons for the low yield in rice production are that the farmers grow several traditional varieties that are tall, late maturing, prone to lodging and susceptible to biotic and abiotic stresses.

Moreover, the limited nature of agricultural water resources and arable lands in most parts of the world in both developed and underdeveloped countries (Rosegrant, 2010) that occurs because of competition between agricultural cultivation and urbanisation (FAO 2004) and climatic changes has a significant effect on arable land, rendering this land unsuitable for crop production (FAO, 2010). These reasons are the primary causes of food insecurity.

To address the above constraints, rice breeders have employed both conventional and non-conventional breeding methods to develop high yielding varieties (Shu, 2009). Consequently, different countries have developed different plant varieties through induced mutations in the areas of breeding purpose, plant technology and physiological assessment (Domingo et al., 2007; Fu et al., 2008). With the help of these mutants, identifying the elements of desired traits, such as high yield potential, semi-dwarfism, high level of disease and pest tolerance, salt tolerance and early maturity, has become easy. Induced mutations have played a significant role in the development of improved rice varieties across the globe (Qayyoum et al., 2000; Baloch, 2002; Baloch et al., 2003). Thousands of useful mutant varieties of fruits and crops, such as cotton, cassava, wheat, peanut, pears, rice, and barley, have been developed from physical-chemical radiation breeding (William, 2007).

In Malaysia, the use of mutations has a limited approach, which could be more widely adopted. However, these techniques have caused the release of rice mutants, such as MA03 (Nazir et al., 1998); SPM 129, SPM 130 and SPM 142 (Azlan et al., 2004); Mahsuri (Hadzim et al., 1988); Muda 2 (Alias et al., 1988); PS 1297 and Manik 817 (Mohamad et al., 1989); SPM 29 and SPM 39 (Mahmud et al., 1995); Q 34 (Hadzim et al., 1994); and MRQ 50 (Phang et al., 2004). These mutants are considered to have shorter plant stature, earlier maturity, photoperiod insensitivity, higher quality grain and traits, and resistance biotic and abiotic factors and can be considered successful because they have made significant contributions in one way or another to the varietal development in Malaysia.

In contrast, rice, which is one of the world's largest cereal crops, generates a relatively large amount of crop residues. Only approximately 20% of rice straw was used for industrial purposes, such as ethanol, paper, and fertilisers, and for domestic purposes, such as fodders. The remains of rice straw are removed from the field, left over, or burnt, which causes environmental pollution by increasing the amount of greenhouse gas in the air (El-Gammal and Shakour, 2001). However, the potential use of these crop residues in animal feeds will be of greater benefit than wasting these residues because ruminant animals are capable of converting food with high fibre content into cellulose. Although, rice straw is known for its low-quality roughage for animal consumption, rice straw feed values have a wide range in different varieties (Nori et al., 2009). Over the past three decades, plant breeders have become aware of the need to utilise the entire plant, thereby improving the utility of crop residues (Rexen and Munck, 1984). Enhancing the nutritive value of rice straw with chemical, biological or physical treatments has been the primary focus of recent studies (Liu and Ørskov, 2000; Selim et al., 2004). Physical treatments, such as crushing, are related to breaking the silicified encrusting layer of straws. The chemical treatment of straw with alkalis, such as ammonia and sodium hydroxide, and a biological approach using bacterial colonisation on cellulose and voluntary straw intake have commonly been used for improving digestibility (Sarnklong et al., 2010). However, the selection of varieties with higher nutritive value straw would be a less expensive and more logical approach.

Genetic diversity plays a vital role in plant breeding. Genetic divergence is an efficacious tool for an effective choice of parents for hybridisation and breeding programmes (Vivekananda and Subramaniam, 1993). Using genetic divergence, scientists can detect genome structures and discover novel methods for modifying and developing crops (Ahmadikhah et al., 2008). Collected information from genetic diversity studies can assist in the logical utilisation of genetic resources within and among closely related crop varieties. Genetic diversity analyses assist breeders in monitoring germplasms and in forecasting potential genetic achievements (Chakravarthi and Naravaneni, 2009). Therefore, rice genetic diversity studies are essential for varietal identification, classification, proper purity maintenance, conservation and breeding (Saini et al., 2004).

1.2 Problem statement

The Malaysian government's decision to achieve 100% self-sufficiency in rice might be a result of the height of the world food crisis in June 2008, when Malaysia suddenly found itself unable to guarantee sufficient rice for the nation in three consecutive months in mid-2008. Rice-exporting countries, including Thailand, Vietnam, and India, either banned or limited their rice exports during the food crisis; thus, Malaysia unexpectedly found no one from whom to buy rice. The food crisis exposed Malaysia's persistent and increasing food insecurity problem. Malaysia has vast agricultural land, and as a tropical country, Malaysia has many opportunities for rice cultivation, with the possibility of attaining self-sufficiency in rice by developing high yield varieties and utilising crop residues as a source of fodder for animals instead of allowing these residues to rot in the paddy field. Malaysia produces only 2.54 million MT of rice, and the rice demand shortfall is met by importation. Under this context, the productivity of paddy fields must be increased from the current 4.5 to 10 t/ha. Therefore, information regarding the genetic diversity and heritability pattern of both indigenous and introduced genotypes of rice cultivars must be performed to enhance the breeding of high yield varieties with good-quality fodder that are also suitable for Malaysia's climate.

1.3 Research objectives

The primary objectives of this research were to select the best varieties with increased grain yield and high quality fodder among the 45 rice accessions obtain from different sources. However, the specific objectives of study were as follows:

1. To determine the genetic diversity, heritability and variation in morphological traits among the rice genotypes using quantitative traits.
2. To characterize the genetic diversity among the rice genotypes, as revealed by ISSR markers.
3. To select the best genotype in term of yield and higher nutritive value for fodder production.

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