ESTIMATION OF MAJOR GENETIC DETERMINANTS OF GRAIN PROPERTIES OF SELECTED MALAYSIAN AND SRI LANKAN RICE VARIETIES

By

BANNEKA MUDIYANSELAGE KUMUDUNI SENARATHNE MENIKE

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

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DEDICATION

This thesis is dedicated
To my dear husband Priyantha,
Kids Oveen and Dahamli

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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The world rice breeding programs aim at the improvement of the yield while enhancing grain quality to meet health aspects. Physical, physicochemical composition, antioxidant activities and molecular analysis of selected Malaysian and Sri Lankan improved rice varieties were conducted. Five Malaysian *indica* rice varieties namely, MARDI Siraj 297 (MS297), MR219, MR220, MR263, MR284 and twelve Sri Lankan rice varieties namely, Bg 252, Bg 300, At 311, Bg 94-1, Bg 352, Bg 358, Bg 359, At 362, Bw 367, Ld 368, At 405 and Bg 406 were employed in this study. The results found a significant difference in grain length and width where Bw 367 was the shortest (3.71 mm), and the longest was MS297 (7.36 mm). In terms of grain width, At 405 variety is the lowest (1.63 mm), and Bg 352 is the widest (2.31 mm). Significant differences in milling characteristics where the percentage of brown rice (%Br) is the highest in At 311 (80.8 %) and the lowest in Bg 252 (77.8 %), Bg 352 shows the highest %TMR value (75.5%), MR284 shows lowest (69.6%), and then %HG, Bg 358 shows the highest (73%), and the lowest is At 311 (58%). There were significant differences of apparent amylose contents (AAC) and gelatinization temperatures (GT) (ANOVA, $p<0.05$). All Malaysian rice varieties and At 311 had intermediate AAC meanwhile, other Sri Lankan rice varieties possessed high AAC. In addition, all Malaysian varieties, and Bg 300, Bg 359, Bw 367 and Ld 368 had intermediate GT levels, however, Bg 352, At 362, At 405, and Bg 406 showed low GT values. The antioxidant activities of total phenolic content (TPC), total flavonoid content (TFC), ferrous reduction potential (FRAP), and DPPH scavenging activity for both Malaysian and Sri Lankan rice varieties were conducted. Data analysis using ANOVA at $p<0.05$ with $n=4$ for antioxidant data while, multivariate analytical methods of Hierarchical cluster analysis (HCA), Principal components analysis (PCA), and Pearson correlations were performed using MINITAB window ver. 17. Results showed that there is significance difference observed (ANOVA, $p<0.05$) which is TPC range between 275 to 844 mgTAE100^{-1}g, TFC range is 318 to 691 QE100^{-1}g, and FRAP range is 0.23 to 1.34 mM100^{-1}g, and DPPH range is 54.45 to 39.79 mg/mL. Red-pigmented rice which is Ld 368, At 362, Bg 252, At 311, and Bg 406 has high antioxidant activity, this is due to its higher phenolic and flavonoid content than white rice. HCA and PCA were used to classify the varieties into groups. There were four clusters in HCA at 55 similarity levels.-In PCA analysis, the length, shape, TFC, and AAC are related to PC1 (37.6%) and the first four components explained 82.3% of the total variability. Pearson's analysis showed strong correlations of pericarp-colour with antioxidant activities (TPC (r=0.86), TFC (r=0.84), FRAP (r=0.88), DPPH (r=0.76)). Furthermore, TFC with TPC (r=0.71), FRAP (r=0.76), and thousand-grain weight with length (r=0.63), while significant negative correlation showed length with TMR (r= (-0.61)) and HG% (r= (-0.80)). Genetically distant parents can be used as parental materials to have different genetic makeup. Identification of the presence of 'waxy' genes that encode amylose content, phylogenetic relationships based on 'waxy' gene sequences and genetic variation through SSR marker analysis for Malaysian and Sri Lankan rice varieties has been done. Fifteen micro satellite markers were evaluated. Molecular analysis was conducted by Bio Edit 7.2, NCBI Blast, ClustalW and MEGA-11.0 software's. The availability of a waxy gene encoding GBSS1 that determines amylose contents in selected rice varieties was confirmed. Two single nucleotide polymorphisms at A/C in Exon 6 at position 3911, and T/C in the splicing site of Intron 6 at position 3922 of the waxy gene were observed. All the selected varieties with high AAC were shown the sequence of 'AGTTATA' at the putative leader intron 5' splice site. These indices can be used as molecular marker for differentiate amylose classes. Phylogenetic relationships were identified according to amplified sequences of Malaysian and Sri Lankan improved rice varieties. Six polymorphic micro satellite markers were identified and the most polymorphic ones were RM312, RM259, and RM202. Identified variations and relationships of selected Malaysian and Sri Lankan rice varieties can be utilized as important genetic sources for incorporating future rice breeding programs.

Keywords: Rice, Physical composition; Physicochemical properties; Antioxidant activity; Molecular analysis; Health aspects; Genetic relationships

SDG: GOAL 2: Zero Hunger, GOAL 3: Good Health and Well-Being, GOA 4: Quality Education

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

PENGANGGARAN PENENTU GENETIK UTAMA BAGI CIRI BIJIRIN VARIETI PADI MALAYSIA DAN SRI LANKA TERPILIH

Oleh

BANNEKA MUDIYANSELAGE KUMUDUNI SENARATHNE MENIKE

Jun 2022

Pengerusi : Profesor Madya Rosimah binti Nulit, PhD
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Program pembiakan padi dunia adalah bertujuan meningkatkan hasil dan juga meningkatkan kualiti bijirin yang dapat memenuhi aspek kesihatan. Komposisi fizikal, fisikokimia, aktiviti antioksida dan analisis molekul pada varieti padi Malaysia dan Sri Lanka telah dijalankan. Lima varieti padi indica Malaysia iaitu MARDI Siraj 297 (MS297), MR219, MR220, MR263, MR284 dan dua belas varieti beras Sri Lanka iaitu Bg 252, Bg 300, At 311, Bg 94-1, Bg 352, Bg 352. Bg 359, At 362, Bw 367, Ld 368, At 405 dan Bg 406 dikaji. Objektif pertama adalah mengkaji ciri fizikal dan komposisi fisikokimia. Hasil kajian mendapati perbezaan bererti pada panjang dan lebar bijirin di mana Bw 367 adalah terpendek (3.71 mm), dan terpanjang adalah MS297 (7.36 mm). Pada kelebaran bijirin, varieti At 405 adalah terendah (1.63 mm), dan Bg 352 adalah paling lebar (2.31mm). Perbezaan berertipada ciri pengilangan di mana peratus beras perang (%Br) adalah paling tinggi pada At 311 (80.8 %) dan terendah pada Bg 252 (77.8 %,), Bg 352 menunjukkan nilai %TMR yang tertinggi (75.5%), MR284 menunjukkan paling rendah (69.6%), dan seterusnya %HG, Bg 358 menunjukkan tertinggi (73. %), dan paling rendah adalah At 311 (58. %). Kandungan amilosa ketara (AAC) dan suhu gelatinisasi (GT) adalah sangat berbeza antara variety (ANOVA, $p<0.05$). Kesemua varieti beras Malaysia dan hanya satu varieti Sri Lanka, At 311 menunjukkan AAC sederhana manakala, varieti Sri Lanka yang lain menunjukkan AAC yang tinggi. Kesemua varieti Malaysia dan Bg 300, Bg 359, Bw 367 dan Ld 368 mempunyai tahap GT sederhana, walaubagaimanapun, Bg 352, At 362, At 405, dan At 406 menunjukkan tahap GT yang rendah. Aktiviti antioksidan iaitu jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), potensi pengurangan ferus (FRAP), dan aktiviti penghapusan DPPH bagi kedua-dua varieti beras Malaysia dan Sri Lanka dilakukan. Analisis data menggunakan ANOVA pada $p<0.05$ manakala analisis multivariat, kelompok Hierarki (HCA), analisis komponen Utama (PCA), dan korelasi Pearson menggunakan MINITAB ver.17. Kajian mendapati terdapat perbezaan bererti (ANOVA, $p<0.05$) pada kesemua aktiviti antioksidan di mana julat TPC antara 275 -844 mgTAE/100^{-1}g, julat TFC antara 318.0-691.0 QE100^{-1}g, julat FRAP antara 0.0-1.34mM100^{-1}g, dan julat DPPH ialah 54.45 -39.79 mg/mL. Beras merah iaitu Ld 368, At 362, Bg 252, At 311 dan Bg 406 mempunyai tinggi aktiviti antioksidan, ini disebabkan oleh kandungan fenolik dan flavonoidnya lebih tinggi berbanding beras putih. Analisis HCA dan PCA digunakan untuk mengelaskan varieti kepada kluster. Analisis HCA mendapati empat kluster pada tahap persamaan 55. Ini menunjukkan terdapat hubungan genetik antara varieti padi. Analisis PCA menunjukkan panjang, bentuk, TFC dan AAC berkaitan dengan PC1 (37.6%) dan keempat-empat komponen pertama ini menandakan 82.3% daripada jumlah kebolehubahan. Analisis Pearson menunjukkan korelasi tinggi antara warna perikap dengan aktiviti antioksidan (TPC (r= 0.86), TFC (r= 0.84), FRAP (r= 0.88), DPPH (r=0.76)). Korelasi antara TFC dengan TPC (r=0.71), FRAP (r=0.76), dan berat bijiran dengan panjang (r=0.63), manakala korelasi negatif antara panjang dengan TMR (r= -0.61) dan %HG (r=-0.80). Pengenalpastian ke atas kehadiran 'waxy' gen yang mengkodkan kandungan amilosa, hubungan filogenetik berdasarkan jujukan 'waxy' gen dan variasi genetik melalui analisis penanda SSR bagi varieti beras Malaysia dan Sri Lanka telah dilakukan. Pengekstrakan DNA, PCR, elektroforesis gel, penjujukan gen dan penilaian lima belas penanda satelit mikro telah dijalankan. Analisis molekular menggunakan Bio Edit 7.2, NCBI Blast, perisian ClustalW dan MEGA11.0. Pengkodan 'waxy' gen GBSS1 yang menentukan kandungan amilosa dalam varieti beras telah berjaya dikenalpasti. Dua polimorfisme nukleotida tunggal iaitu A/C pada Exon 6 pada kedudukan 3911, dan T/C dalam tapak intron 6 'waxy' gen telah berjaya ditemui. Selanjutnya, semua varieti padi dengan AAC tinggi telah ditunjukkan pada urutan 'AGTTATA' di tapak intron 5'. Oleh itu, indeks ini boleh digunakan sebagai penanda molekul untuk membezakan kelas amilosa yang berbeza. Hubungan filogenetik telah dianalisis ke atas varieti beras Malaysia dan Sri Lanka dan empat belas jujukan gen yang diperolehi daripada NCBI. Keputusan mendapati enam penanda mikro satelit polimorfik telah jumpai dan tiga dari penanda iaitu RM312, RM259, dan RM202 adalah penanda utama. Identifikasi variasi dan hubungkait antara beras Malaysia dan Sri Lanka boleh digunakan sebagai sumber genetik, manakala data kualiti bijirin boleh digunakan untuk program pembiakbakaan padi masa masa hadapan.

Kata Kunci: Padi, Komposisi fizikal; Sifat fizikokimia; Aktiviti antioksidan; Analisis molekul; Aspek kesihatan; Hubungan genetik.

SDG: MATLAMAT 2: Kelaparan Sifar, MATLAMAT 3: Kesihatan yang Baik dan Sejahtera, MATLAMAT 4: Kualiti Pendidikan

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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. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AFLPs	Amplified Fragment Length Polymorphism
APS	Ammonium persulfate
ATP	Adenosine triphosphate
A_{600nm}	Optical density at wavelength 600 nanometre
bp	Base pairs
$CaCl_2$	Calcium chloride
cDNA	Complementary deoxyribonucleic acid
°C	Degree Celsius
cm	centimetre
CTAB	Cetyltrimethyl-ammoniumbromide
dNTPs	Deoxynucleotidetriphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetra acetic acid
FAO	Food and Agriculture Organization of the United Estate
g	gram
KI	Potassium Iodide
L	Litre
M	Molar(s)
MAS	Marker-Assisted Selection
min	minute(s)
mm	Millimetres
M	Molar
µL	Microliter

PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fraction Length Polymorphism
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeat or Microsatellites
STR	Short Tandem Repeats
TAE	Tris-Acetate EDTA buffer
TBE	Tris-Borate-EDTA buffer
U	Unit
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice (*Oryza sativa* L.) serves as the primary source of staple food for more than half of the global population (Pang et al., 2018; Fitzgerald et al., 2009). The main drivers of the rising global demand for rice are the expanding economy, population growth, and other sociodemographic factors (Rahman et al., 2021). The current challenge of global rice breeding programs is not only to improve the yield but also to ensure grain quality to meet consumer acceptance (Al-daej, 2021; Butardo et al., 2019; Claire et al., 2016). According to Rita et al. (2017); Champagne et al. (1997) quality has been modified to fit the eating and cooking habits and food preferences of different nations.

Rice quality is generally controlled by genetic and environmental factors (Han et al., 2004). According to Terdoo & Feola (2016) quality characteristics can be categorized as intrinsic or extrinsic attributes. Rice's intrinsic characteristics include cleanliness, purity, colour, aroma, taste, grain size and shape, grain homogeneity, and volume expansion while extrinsic attributes include packaging, labelling, credibility, branding, and details. The main compound in rice kernel is starch and its physicochemical properties such as apparent amylose content, gel consistency, and gelatinization temperature are considered key components of grain quality (Kong et al., 2015; Fitzgerald et al., 2009). According to Chen et al. (2017); Kong et al. (2015); Kim et al. (2006), high-amylose varieties aid in the development of cultivars with greater resistant starch and a lower glycemic index.

Antioxidants are substances capable of playing an important role in defending the cells against free radicals which are toxic by-products of natural cell metabolism (Ibria et al., 2017; Masisi et al., 2016). Rice comprises a variety of antioxidant abilities that play beneficial roles in health advancements. Dietary antioxidants include vitamins like tocopherols, tocotrienols, ascorbate, and phytic acid as well as other direct antioxidants like phenolic acids, flavonoids, anthocyanins, proanthocyanidins. It also contains antioxidant-supporting elements such as Se, Fe, Cu, Zn, riboflavin, and other B vitamins (Dennis et al., 2019; Goufo & Trindade, 2014). Rice is a potential fount of different phytochemicals with antioxidant activities based on the cultivar, other physicochemical properties, and processing methods (Ziegler et al., 2018; Masisi et al., 2016).

Waxy gene (*Wx*) encoding Granule-Bound Starch Synthase I (*GBSS I*) determines amylose content in rice endosperm, while starch branching enzymes encoded by *Sbe* genes are involved in the formation of amylopectin (Zhang et al., 2019; Wang et al., 1995). Potential molecular markers are available to detect alleles associated with five classes of amylose (Tripathy et al., 2016). According to Chen et al. (2017), molecular profiling of these germplasms is important for genetics and breeding studies. Markers are a valuable resource in tagging genes for tracking down specific chromosomal introgressions in breeding populations (Escano et al., 2020). Microsatellite markers consist of 1-6 base pairs (Mohanty et al., 2021). DNA markers are referred to as a segment of DNA revealing differences in sequences because of base deletion, insertion, and substitution between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. Polymorphism can be detected by Southern blotting and polymerase chain reaction (Nogoy et al., 2016).

Evaluation of genetic diversity is necessary for crop improvement, conservation, utilization, and genetic resource management (Wang et al., 2021; Razak et al., 2020; Rani et al., 2020; Srivastava et al., 2019; Chakravarthi & Naravaneni 2006).

1.2 Problem Statement and Justification of Study

Future efforts to improve rice yields, quality, and food security are expected to face greater challenges due to climate change (Gunaratne et al., 2021; Firdaus et al., 2020). The United Nations Sustainable Development Goals also address issues like climate change, and environmental degradation on a worldwide scale (Georgeson & Maslin, 2018). The number of people who are malnourished has increased and Africa and South Asia are the most severely impacted regions. Global rice consumption is predicted to rise from 676 million tons in 2010 to 763 million tons in 2020 and then to 852 million tons in 2035. This reflects an increase of 176 million tons overall over the subsequent 25 years (Bull et al., 2020). Therefore, the world rice breeding programs aim the improvement of yield, and grain quality to meet health aspects and achieve food security. Breeders are challenged to improve varieties with better quality traits. There is an important relationship between diet and health (Upadhyay & Karn, 2018). Lack of proper knowledge of nutritional and health aspects affects the quality of the products. Genetic resources are the most valuable basic raw material for the improvement of any crop. According to Xiao-yu et al. (2013), different varieties possess variations in morphological, physical, physicochemical, and cooking qualities. Rice varieties having slowly digestible starch are potentially characterized to have a low glycaemic index with high amylose content that is correlated with resistant starch and helps in the management of diet-related diseases (Al-Mssallem, 2014; Kim et al., 2006). Furthermore, rice contains a variety of bioactive compounds and photochemical, known as antioxidants (Goufo & Trindade, 2014; Walter & Marchesan, 2011; Iqbal et al., 2005). Concentration and types of compounds vary among different varieties due to genetic factors and environmental factors (Han et al., 2004). Knowledge of starch biosynthesis, antioxidant properties, and genetic resources with a diversity of nutritional and health features are necessary to produce outstanding varieties. Exploration and evaluation genetic diversity of rice is important for the sake of increasing yield or quality. A combination of morphological and molecular markers is more effective for detecting genetic diversity and identifying relationships of germplasm

1.3 Objectives of Study

This study's main objective was to evaluate the physical, and physicochemical composition, antioxidant activities, and molecular analysis of selected Malaysian and Sri Lankan rice for incorporation into the future breeding program. The specific objectives are:

1. To estimate the physical and physicochemical properties of selected Malaysian and Sri Lankan improved rice varieties.
2. To determine the antioxidant activities of selected Malaysian and Sri Lankan improved rice varieties.
3. To identify the presence of the waxy gene responsible for amylose contents, the phylogenetic relationships based on the PCR amplified waxy gene sequences and genetic variations through SSR marker analysis of selected Malaysian and Sri Lankan improved rice varieties.

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