



**UNIVERSITI PUTRA MALAYSIA**

***GENETIC VARIABILITY AND RELATIONSHIP OF PHENOLIC  
CONTENTS, ANTIOXIDANT CAPACITY AND GRAIN YIELD  
COMPONENTS OF SELECTED COLOURED RICE ACCESSIONS***

**HAMIDAH MOHD SARIF**

**FP 2020 46**



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By

**HAMIDAH MOHD SARIF**

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

**March 2019**

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

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March 2019

**Chairman : Professor Mohd Rafii bin Yusop, PhD**  
**Faculty : Agriculture**

Rice is one of the most important food crops and a primary source of calories for more than half of the world's population. Nowadays, there is increasing demand for the coloured rice due to growing knowledge of health benefits. Coloured rice is one of the alternative healthy foods since it contains high antioxidant, phenolic compounds, and other nutrients that have significant benefit on human health. In this study, genetic diversity was evaluated among 32 coloured rice accessions. Rice accessions were obtained from Rice Genebank, Malaysian Agricultural Research and Development Institute (MARDI) and were planted at the field for multiplications, morphological and yield components assessment. The evaluation was carried out in a randomized complete block design (RCBD) with three replications. The analysis of variance results showed that all the agro-morphological characteristics, yield and yield components, grain quality, and antioxidant properties were significant differences among the accessions. Correlation analysis revealed that phenolic content positively high correlated with plant height, leaf area, heading days, number of tillers, thousand grain weight, dehulled grain width, amylose content and gel consistency. Most of the characteristics studied had high heritability values (>70%) except for numbers of tillers (40.72%), alkaline spreading value (40.68%), broken rice percentage (22.98%) and head rice recovery (7.74%). These indicated that the characters with high heritability values (>70%) were highly influenced by genetic factor than environmental factors in their phenotypic expression. The coloured rice accessions had broad genetic divergence and high heritability values will be given high priority in the selection for successful improvement of rice grain quality. Correlation analysis showed that phenolic content was positively significant correlated with all the morphological traits and yield components. The total antioxidant capacities (TAC) for all the coloured rice accessions were ranged from 12.10 to 34.90  $\mu\text{M}/100\text{g}$  total equivalent antioxidant capacity (TEAC). The highest of TAC content was identified in accession DNJ 128 (34.90  $\mu\text{M}/100\text{g}$  TEAC). For molecular diversity study, out of 59 simple sequence repeats (SSR) markers used, 34 markers showed polymorphism among the coloured rice accessions. The polymorphic information content (PIC) ranged from 0.86 (RM137) to

0.97 (RM400) with an average 0.92 which indicated that SSR markers used in this study were effective to reveal the polymorphic among the rice accessions. Cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) showed that all the coloured rice accessions were grouped into four clusters. From this study, six coloured rice accessions namely, YTM 15, Pulut Merah 3, Padi Randau, Ringan Bawang, DNJ 128 and DV 107 produced high yield with good grain quality and high antioxidant capacity were selected for future rice breeding program.



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

**KEPELBAGAIAN GENETIK DAN HUBUNGKAIT ANTARA KANDUNGAN FENOLIK, KAPASITI ANTIOKSIDAN DAN KOMPONEN HASIL BIJIAN AKSESI TERPILIH PADI BERWARNA**

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Padi merupakan salah satu tanaman makanan yang terpenting dan merupakan sumber utama kalori kepada lebih daripada separuh penduduk dunia. Pada masa kini, terdapat peningkatan permintaan terhadap beras berwarna kerana peningkatan pengetahuan terhadap khasiat kesihatannya. Beras berwarna merupakan salah satu alternatif makanan untuk kesihatan kerana ianya mengandungi antioksidan yang tinggi, sebatian fenolik, dan nutrien lain yang mempunyai manfaat yang signifikan terhadap kesihatan manusia. Dalam kajian ini, kepelbagaian genetik telah dinilai di kalangan 32 aksesi terpilih padi berwarna. Aksesi-aksesi padi berwarna tersebut telah diperolehi dari Genbank Padi, Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI) dan telah ditanam untuk pembiakan, penilaian morfologi dan komponen hasil. Penilaian ini dijalankan menggunakan rekabentuk blok penuh terawak (RCBD) dengan tiga replikasi. Keputusan analisa varians menunjukkan semua ciri agro-morfologi, hasil dan komponen hasil, kualiti bijian, dan kandungan antioksidan memberikan perbezaan yang ketara di kalangan aksesi. Analisa korelasi menunjukkan bahawa kandungan fenolik adalah berkorelasi tinggi secara positif dengan tinggi pokok, luas permukaan daun, hari terbit bunga, tempoh matang, berat seribu bijian, lebar beras, kandungan amilosa dan konsistensi gel. Kebanyakan ciri-ciri yang dikaji memberikan nilai keterwarisan yang tinggi (>70%) kecuali bilangan anak pokok (40.72%), nilai penyebaran alkali (40.68%), peratus beras hancur (22.98%) dan peratus kepala beras (7.74%). Ini menunjukkan bahawa ciri-ciri yang mempunyai nilai keterwarisan yang tinggi (>70%) adalah sangat dipengaruhi oleh faktor genetik berbanding faktor-faktor sekitaran dalam ekspresi fenotipiknya. Aksesi yang mempunyai percapahan genetik yang luas dan nilai keterwarisan yang tinggi di kalangan aksesi padi berwarna tersebut akan diberi keutamaan yang tinggi dalam pemilihan untuk kejayaan dalam pembaikan kualiti bijian beras. Analisa korelasi menunjukkan bahawa kandungan fenolik berkorelasi signifikan secara positif dengan semua ciri-ciri morfologi dan komponen hasil. Jumlah kapasiti antioksidan (TAC) untuk semua aksesi padi berwarna adalah di antara 12.10 hingga 34.90  $\mu\text{M}/100\text{g}$  trolox setara kapasiti antioksidan (TEAC). Kandungan TAC tertinggi didapati di dalam aksesi DNJ 128 (34.90  $\mu\text{M} / 100\text{g}$  TEAC). Bagi kajian diversiti

molekular, dari 59 penanda jujukan mudah berulang (SSR) yang telah digunakan, 34 penanda menunjukkan polimorfisme di kalangan aksesori padi berwarna tersebut. Kandungan maklumat polimorfik (PIC) adalah berjangka dari 0.86 (RM137) hingga 0.97 (RM400) dengan purata 0.92 di mana ianya menunjukkan bahawa penanda SSR yang digunakan dalam kajian ini adalah efektif untuk menunjukkan polimorfik di kalangan aksesori padi tersebut. Analisa kluster menggunakan *unweighted pair group method with arithmetic mean* (UPGMA) menunjukkan bahawa semua aksesori padi berwarna telah dikelaskan kepada empat kluster. Dari kajian ini, enam aksesori padi berwarna iaitu, YTM 15, Pulut Merah 3, Padi Randau, Ringan Bawang, DNJ 128 dan DV 107 yang mengeluarkan hasil yang tinggi serta kualiti bijian yang baik dan kapasiti antioksidan tinggi telah dipilih untuk program pembiakan padi masa hadapan.



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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 Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

%	Percentage
SSR	Simple sequence repeat
PCR	Polymerase chain reaction
PIC	Polymorphic information content
ha	Hectare
USDA	United State Department of Agriculture
TAC	Total antioxidant capacity
DMPD	N, N-dimethyl-p-phenylenediamine
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
LDL	Low-density lipoprotein
ORAC	Oxygen radical absorbance capacity
PCL	Photo chemiluminescence
TEAC	Trolox equivalent antioxidant capacity
TRAP	Total radical trapping antioxidant parameter
ABTS	2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid
IRRI	International Rice Research Institute
SES	Standard Evaluation System
UPOV	Union of the Protection of New Varieties
2-AP	2-acetyl-1-pyrroline
GT	Gelatinization temperature
<i>SSIIa</i>	<i>Starch synthase IIa</i>
DNA	Deoxyribonucleic acid
QTL	Quantitative trait locus

hB	Heritability
Kg/ha	Kilogram per hectare
Syn.	Synonyms
MARDI	Malaysian Agricultural Research and Development Institute
NPK	Nitrogen, Phosphorus, Kalium
GPS	Global Positioning System
°	Degree
MS	Main season
cm	Centimeter
mm	Millimeter
NaOH	Natrium hydroxide
M	Molar
ml	Milliliter
Conc.	Concentration
g	Gram
µl	Microliter
g/L	Gram per liter
Mg/ml	Milligram per milliliter
nm	Nanometer
Mg GAE	Milligram Gallic Acid Equivalents
ph	Potential of hydrogen
ANOVA	Analysis of variance
SE	Standard Error
SAS	Statistical Analysis System
LSD	Least significant difference
d.f	Degree of freedom

EMS	Error mean square
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
NTSYS	Numerical Taxonomy System
$\sigma^2_p$	Phenotypic variance
$\sigma^2_g$	Genotypic variance
$\sigma^2_e$	Mean square of error
MSG	Mean square of accessions
PCV	Phenotypic coefficient of variance
GCV	Genotypic coefficient of variance
X	Mean of trait
$h^2_B$	Broad sense heritability
PCA	Principle Component Analysis

## CHAPTER 1

### INTRODUCTION

Rice is one of the most consumed cereals in the world. Rice is also the main food and source of nutrition for most Malaysian people. The world population is increasing and will reach around 8 billion by 2025 (Rosenberg, 2012). About 90% of the world's rice is grown and consumed in Asia (Tyagi et al., 2004). It is consumed as white and coloured rice types but white type is the most consumed by people. However, currently there is increasing demand for the coloured rice such as black, brown and red rice, due to its growing knowledge of health benefits (Descalsota et al., 2019).

Coloured rice is one of the alternative healthy foods since it contains high antioxidant and other nutrient that have significant benefit on human health. The assessment of the nutrient contents and antioxidant properties are important for selection of superior coloured rice accessions for commercial cultivation. Nutrition content and antioxidant properties such as protein, vitamins and minerals, amino acid, zinc, iron, phenolic and glycemix index are important traits in determining the quality of rice grain. Antioxidant plays an important role in repairing DNA and reduces risk of cancer and other diseases. Abdullah et al. (2018) reported that coloured rice contains higher antioxidant properties as compared to white rice. Selection of rice cultivars with a high antioxidant level and good agronomic trait may facilitate the breeding and commercial production of pigmented rice in Malaysia.

Agro-morphological characteristics yield, and yield component attributes are commonly used to estimate diversity among the coloured rice accessions. Morphological characters are easy to study and cost effective but diversity analysis, based on quantitative traits alone may not be completely reliable because the traits are influenced by the environmental factors (Fufa et al., 2005).

Therefore, molecular marker is an important tool for assessment of genetic diversity among coloured rice accessions. Plant breeders now have more interest in molecular characterization because of minimal environmental effects (Tatikonda et al., 2009; Glaszmann et al., 2010). Molecular characterization of germplasm materials provides information on genetic distance among the accessions and it is useful for crossing programmes from diverse genetic backgrounds to increase heterosis value (Henry, 1997).

Even though studies on the genetics and breeding of rice are very advanced but there are scarce information and research about coloured rice accessions in Malaysia. Documentation of the coloured rice germplasm should be carried out for effective breeding programme. As a primary step to achieve this study, it is important to evaluate the genetic diversity of Malaysia and exotic coloured rice for local utilization in coloured rice varietal development.

Therefore, the objectives of this study were:

- i) To determine the agro-morphological characteristics, yield and yield components and antioxidant properties performance of 32 coloured rice accessions.
- ii) To estimate genetic variance components and heritability values of the rice population
- iii) To determine molecular diversity of the 32 coloured rice accessions using simple sequence repeats (SSR) markers
- iv) To select potential coloured rice accessions for development of new variety for local cultivation.



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