

**GENETIC DIVERISTY STUDY BASED ON SEED MORPHOLOGY OF
RICE LANDRACES FROM KOTA BELUD, SABAH**

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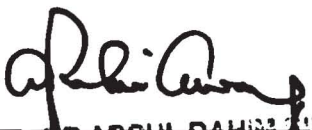
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ABSTRACT

The measurement of morphological variation is the easiest indicator of genetic diversity. Rice landraces often exhibit tremendous morphological diversity. Exploring diversity in a collection of rice landraces is very important for identifying new, undiscovered genes and further improvement of germplasm. Hence, this study was carried out to determine the seed morphological variation of 45 rice landraces which were obtained from Kota Belud, Sabah. A total of 13 seed morphological characteristics, namely awning, awn colour, apiculus colour, lemma and palea colour, lemma and palea pubescence, sterile lemma colour, sterile lemma length, seed length, seed shape (length-width ratio), 100-grain weight, seed coat colour, endosperm type, and scent were evaluated based on the descriptor set by IBPGR-IRRI. Observations on the morphological characters of seed were recorded and analyzed by using Microsoft Excel. Shannon-Weaver diversity index, H' was used to estimate each characteristic's diversity index. The H' value of awning, awn colour, apiculus colour, lemma and palea colour, lemma and palea pubescence, sterile lemma colour, sterile lemma length, seed coat colour, endosperm type and scent was 0.3460, 0.4292, 0.5409, 0.2444, 0.2944, 0.3656, 0.2257, 0.1479, 0.6296 and 0.6126, respectively. The diversity index, H' was found to range from 0.1479 to 0.6296 in this study. The overall H' value was 0.4913. As for the quantitative parameters, 100-grain weight ranged from 1.76 g to 3.29 g. Seed length ranged from 5.4 mm to 7.9 mm whereas width ranged from 1.9 mm to 2.9 mm. The varying degree of the mixed characteristics is due to many genes that are responsible in controlling one characteristic. As a conclusion, Sabah rice landraces possess a range of diversity in their morphology. Such diversity is an important reservoir of useful genes which can be used to enrich the existing varieties with essential favourable agronomic traits.

KEPELBAGAIAN GENETIK BERDASARKAN MORFOLOGI BIJI BENIH PADI TRADISIONAL DARI KOTA BELUD, SABAH

ABSTRAK

Pengukuran variasi morfologi merupakan penunjuk kepelbagaian genetik yang paling mudah. Padi tradisional sering menunjukkan kepelbagaian morfologi yang luar biasa. Penerokaan kepelbagaian genetik dalam koleksi padi tradisional sangat penting dalam mengenalpasti gen yang baru dan seterusnya pembaikan germplasm yang lebih lanjut. Oleh itu, kajian ini dijalankan bagi menentukan tahap variasi morfologi biji benih untuk 45 jenis padi tradisional yang diperolehi dari Kota Belud, Sabah. Sebanyak 13 ciri-ciri morfologi biji benih, iaitu sengkuap, warna sengkuap, warna apiculus, warna lema dan palea, pubertas lema dan palea, warna lema steril, panjang lema steril, panjang benih, bentuk biji benih (nisbah panjang-lebar), 100-berat biji benih, warna kot benih, jenis endosperm, dan aroma dinilai berdasarkan deskriptor yang telah ditetapkan oleh IBPGR-IRRI. Pemerhatian ke atas ciri-ciri morfologi biji benih direkodkan dan dianalisis dengan menggunakan Microsoft Excel. Indeks kepelbagaian Shannon-Weaver, H' digunakan untuk membuat anggaran tahap indeks kepelbagaian bagi setiap ciri. Nilai H' untuk sengkuap, warna sengkuap, warna apiculus, warna lema dan palea, pubertas lema dan palea, warna lema steril, panjang lema steril, warna kot biji benih, jenis endosperm dan aroma masing-masing ialah 0.3460, 0.4292, 0.5409, 0.2444, 0.2944, 0.3656, 0.2257, 0.1479, 0.6296 dan 0.6126. Indeks kepelbagaian, H' berada dalam lingkungan 0.1479 hingga 0.6296 untuk kajian ini. Nilai keseluruhan H' adalah 0.4913. Bagi parameter kuantitatif, 100-berat biji benih berjulat dari 1.76 g hingga 3.29 g. Panjang biji benih berjulat dari 5.4 mm hingga 7.9 mm manakala lebar biji benih berjulat dari 1.9 mm hingga 2.9 mm. Kepelbagaian genetik mengikut varieti, H' berjulat dari 0 hingga 0.1780. Perbezaan dalam ciri-ciri yang bercampur adalah disebabkan wujudnya banyak gen yang bertanggungjawab dalam mengawal satu ciri. Kesimpulannya, beras tradisional Sabah mempunyai kepelbagaian dari segi morfologi. Kepelbagaian itu merupakan gen yang boleh digunakan untuk memperkaya jenis padi yang telah wujud sebelum ini dengan sifat agronomik yang baik dan penting.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

2-AP	2-acetyl-1-pyrroline
Σ	Sum
DLL	Dwarfl
FAO	Food and Agriculture Organization
GPA	Global Plan of Action
H'	Shannon-Weaver Index
HYV	High Yielding Variety
IRRI	International Rice Research Institute
Kb	Kilo base pairs
QTLs	Quantitative Trait Loci

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rice (*Oryza sativa* L.) is one of the significant cereal commodities (Lopez, 2008) and is planted on about one tenth of the earth's arable land (Muhammad Rashid *et al.*, 2009). It is the single largest source of the food energy to half of humanity (Eckardt, 2000; Kurata and Yamazaki, 2006). Globally, rice is distributed with a high concentration in Asia (Vaughan *et al.*, 2003). However, there is an urgent need to take all the necessary steps to enhance the productivity of this crop in order to meet the future demand for food which is anticipated from the increased world population (Sundaram *et al.*, 2007). Many breeders are seeking for noble genes found in the rice landraces, which had been reported to post useful gene(s) in order to overcome this shortage (Reeves and Cassaday, 2002).

Rice landraces are defined by Brown in 1978 as "geographically or ecologically distinctive populations which are conspicuously diverse in their genetic composition both between landraces and within them". They are the traditional varieties of rice which are grown by farmers and passed down from one generation to another generation (Pusadee *et al.*, 2009). According to Harlan (1992), rice landraces are identifiable by their unique morphologies and well-established local names.

Rice landraces have been largely replaced by the genetically uniform modern varieties in many parts of Asia (Pingali and Rajaram, 1998). The adoption of new modern varieties means that the land area planted with landraces are gradually disappearing (Guei, 2000). The modern, high yielding rice varieties which are often invariant for many genetic markers lead to the most extreme lost of diversity. Such



loss of diversity have serious consequences for the crop from the epidemic disease susceptibility to the lack of evolutionary potential for adaptation to the changing environmental conditions (Pusadee *et al.*, 2009).

The diversity in crop varieties is important for the development of agriculture in order to increase food production, poverty alleviation and promote growth of economic. Shahidullah *et al.* (2009) stated that the diversity which is available in the germplasm serves as an insurance against the unknown needs and conditions too. Thereby, it contributes to the stability of farming systems at local, national and global levels (Singh *et al.*, 2000).

Landraces constitute a good source of unique genes for stress tolerance, high yield stability, adaptability to the environments and genetically dynamics (Frankel *et al.*, 1995; Guei and Traore, 2001). Thus, evaluation and characterization of rice landraces should form an important constituent of the collection efforts. This is because Ogunbayo *et al.* (2007) stated that rice landraces have an enormous built-in genetic diversity due to several generations of growing and selection by farmers and breeders.

The most common characterization method is by observing the morphology of the seed grains. The measurement of the morphological variation which can be obtained easily is the indicator of the genetic diversity. Morphological characters may be ecologically adaptative (Schaal *et al.*, 1991) which are good indicators of genetic variation, local differentiation, or ecotypes.

Grain morphology is among the first visible character for the selection and marking of quality (Sadar *et al.*, 2007). Most farmers prefer medium- to long-grained rice in the rainfed lowlands of Asia. Grain appearance is an important consideration for most of the rice consumers (Ikehashi and Khush, 1979; Jennings *et al.*, 1979; Juliano, 1985). Although all grains are equally translucent after cooking and clarity of endosperm does not affect the taste or texture of rice, people will still prefer rice that has clear endosperm in most of the regions. Aromatic rice is being preferred in some parts of Asia and draws a premium price in certain specialty markets. Webb *et al.* (1979, 1985) noticed that there is a particular trend of cooking quality characters which is related to the grain shape.

Genetic variability for agronomic traits as well as the quality traits in almost all crops is essential in the crop improvement program. This is because this component is transmitted to the next generation (Singh, 1996). Thus, the diversity in grain morphology needs to be examined.

1.2 Justification

Landraces are precious genetic resources. This is because they contain huge genetic variability that can be used to complement and broaden the gene pool of the advanced genotypes (Kobayashi *et al.*, 2006). Besides, landraces rice often exhibit tremendous morphological diversity. Exploring diversity in a collection of landraces rice is very important for identifying new genes and further improvement of the germplasm (Aggarwal *et al.*, 2002; Brondani *et al.*, 2006; Jayamani *et al.*, 2007; Thomson *et al.*, 2007). It is belief that there are many undiscovered useful genes which can be found in the landraces rice. These genes aid in improving the resistance of rice towards biotic and abiotic stresses, grain shape, high yield stability, and aroma of rice (Frankel *et al.*, 1995; Guei and Traore, 2001).

Diversity based on the phenological and morphological characters vary with the environmental conditions (Kaylan and Rambabu, 2006). The exact potential of the local landraces genetic, differences from the commercial varieties and the magnitude of heterogeneity especially in the Sabah landraces are not well catalogued. Therefore, the need to characterize available landraces has become essential in the modern crop improvement as suggested by Frey *et al.* (1984), Dale *et al.* (1985) and Rezai and Frey (1990). This study was conducted to identify the seed morphology or phenotypic characteristics of Sabah rice landraces from the Kota Belud District which will become the source of valuable information for the future breeding program.

1.3 Research Objective

The objective of this study was to determine the diversity level of rice landraces from Kota Belud, Sabah based on the seed morphology characteristics.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice (*Oryza sativa* L.) belongs to the tribe of Oryzeae which is under the Poaceae sub-family in the grass family of Gramineae (Poaceae). The genus of *Oryza* has been divided into few sections by biosystematists (Chang *et al.*, 1965).

O. sativa, a diploid species has AA as its genomic formula. There are 24 chromosomes which can be found in it (Chang *et al.*, 1965). The genetic structure of the *Oryza* genus has been provided by extensive studies using a variety of means: morphological studies, cytogenetics, interspecific hybridization, biochemical and molecular markers (Chang *et al.*, 1965).

The characteristics of a rice plant is illustrated as an annual grass with round, hollow, jointed culms, rather flat and sessile leaf blades, and a terminal panicle. The lifespan of a rice plant can be more than a year under favourable conditions (Chang *et al.*, 1965).

2.2 Rice Landraces

Landraces are widespread and popular among farmers. Besides, they are an important part of agriculture as their diverse array in a crop creates genetic diversity in agriculture (Modi, 2004). Fukai *et al.* (1991), Gomez and Kalami (2003), Gomez *et al.* (2003), Irie *et al.* (2003) and Kohli *et al.* (2004) stated that landraces are known to be heterogeneous mixtures of genotypes that carry a range of stress-tolerance genes and others. Besides that, landraces also possess preferred traits which could be used to



produce new cultivars or to incorporate desirable traits into varieties (Lynch *et al.*, 1992). These include stress tolerance, high yield stability, adaptability to environments and genetic dynamics (Frankel *et al.*, 1995; Guei and Traore, 2001). Besides, landraces are also important genetic resources for resistance to pests and fungal diseases. For instance, Indian landraces Velluthachira, Bengale and Bhumansam are resistant to rice gall midge; Chemban is resistant to brown plant hopper; Tadukan is resistant to blast; whereas Buhjan and Laka are resistant to sheath blight (Siddiq *et al.*, 2005).

Landraces have been the mainstay of agricultural systems in many developing countries (Hill *et al.*, 1998). However, introduction of modern high yielding rice varieties has caused the landraces unpopular among some farmers (Li *et al.*, 2004) to the extent that some of the landraces have been replaced (Mohapatra *et al.*, 2004). For example, observation in India by Kohli *et al.* (2004) reported that, the primary centre of rice origin had an unspecified large number of landraces. However, most of them were out of cultivation.

Rao *et al.* (2002) warned that the danger of genetic erosion in many varieties of rice landrace in Laos was due to the widespread adoption of modern varieties by farmers. Joshi and Witcombe (2003) argued that introducing modern varieties into areas which are predominated by landraces results in an increase of allelic diversity. Yet, the increase in allelic diversity would still result in the loss of some rice landraces over time.

Although landraces may have lower yields compared to the modern varieties under some conditions, they are still grown largely in approximately 50% of the rainfed rice areas in Asia (Li *et al.*, 2004). This is because they can adapt to specific local conditions better and they are developed for regional uses of rice (Parzies *et al.*, 2004). The genetic variability which can be found within landraces affords the possibility of genetic flexibility. In other words, landraces have the potential to adapt to the conditions of local field and they can adapt to changing environments, farming practices, and specific uses, namely animal versus human consumption (McCouch, 2004).

Genetic divergence among the genotypes plays an important role in the selection of parents which have wider variability for different characters (Nayak *et al.*,

2004). This fact was proved by Chaudhary and Sarawgi (2002) who reported that 50 rice landraces which had been studied by them differed for 19 morphological and quality traits. Chaudhary and Motiramant (2003) evaluated another 54 aromatic rice landrace accessions for the same 19 descriptors in order to obtain genetic variability information and character association of grain quality and yield attributes. A wide range of variation was recorded for most of the characters in the study carried out by them. Heritability in broad sense was very high for all the characters which exhibited high heritability coupled with high genetic advance except the harvest index. Significant variation for morphological and economic traits was also recorded among 11 landraces which were grown in the Tamil Nadu region of India as reported by Gomez and Kalami (2003).

Furthermore, Wood and Lenne (1997) stated that the genetic diversity of traditional landrace varieties is the most immediately useful and economically valuable component of rice biodiversity. Hence, an understanding of the structure, apportionment, and dynamics of local landrace variation is required in order to conserve, manage, and use such germplasm resources efficiently (Pusadee *et al.*, 2009).

Fukuoka *et al.* (2000) reported that morphologically similar landraces revealed great diversity upon random amplified polymorphic DNA analysis in a study of genetic variation for aroma among landraces rice in the Red River Delta, Vietnam. Chaudhary and Sarawgi (2002) reported that other studies of genetic variation in rice landraces have been based on morphological and quality traits only.

2.3 Rice Morphology

The study of rice morphology and development is interdisciplinary (Smith and Robert, 2003). Morphological characters are being used as discrete markers in order to provide indicators for the management of crop, identify the growth stage of plant, and provide selection criteria in the programs of crop improvement (Moldenhauer and Gibbons, 2003). Morphological characters are being used to monitor the development of plant. Monitoring is done by visual identification of the plant critical growth stages. For example, emergence, tillering, the first visible sign of panicle formation, booting, heading, and maturation (Moldenhauer *et al.*, 1994). Besides, number of emerged

leaves on the main culm is related to number of leaf of the cultivar (Miller *et al.*, 1993; Nemoto *et al.*, 1995; Counce *et al.*, 2000).

2.3.1 Root

There are two major types in the root system of *O. sativa*, namely crown roots (including mat roots) and nodal roots. Both of them develop from nodes. However, the crown roots develop from underground nodes whereas nodal roots develop from nodes above the ground.

Rice has a fibrous root system: the temporary seminal roots which also known as the embryonic roots and secondary adventitious roots. The fibrous roots last for only a short time after the germination of seed. Embryonic roots will first form in the seedlings follow by the adventitious roots which are produced from the underground nodes of young culms.

2.3.2 Culms

The culm of rice is round, hollow, jointed and hairless. Culm is the stem of a rice plant. A mature rice plant has a main culm and a number of tillers depending on the variety and cultural conditions. Each culm has a certain number of nodes (generally ranging from 13 to 16 nodes) and internodes under a certain condition of environment for a variety of rice (Jules, 2002). The culm of the rice is used as a measurement of the plant height. The height of rice plant can range from 0.4 m to more than 6 m (in floating rice) depending on the variety of rice or environmental conditions (Jules, 2002).

2.3.3 Leaves

The leaves are borne on the culm in two ranks with one at each node. Leaves of rice are rather flat. They are long and green in colour. The first rudimentary leaf or prophyllum is found at the tiller base and, with a two-keeled bract only, and has no blade (Jules, 2002).

The uppermost leaf immediately below the panicle is known as the flag leaf. The leaf consists of sheath and blade which are attached at the node, where there is

an auricle, and the ligule immediately above the auricle (Jules, 2002). The leaf sheath is continuous with the blade. It envelopes the culm above the node which varies in length, form, and tightness (Chang *et al.*, 1965).

2.3.4 Panicle

The panicle is borne on the uppermost internode of the culm that is always misst- termed as a peduncle. Rice has a terminate panicle. The major structures which can be found in the panicle are the axis, base, primary and secondary branches, pedicel, rudimentary glumes, and spikelets. Each panicle generally bears from 50 to 300 flowers or spikelets (Jules, 2002).

2.3.5 Flowers or Spikelets

The rice panicle is a perfect and determinate inflorescence. A spikelet of rice has two sterile lemmas, the rachilla and the floret. A rice floret includes six stamens and a pistil having stigmas, styles, and ovary, which is enclosed by the lemma and palea, sometimes with an awn (Jules, 2002). The awn is a filiform extension of the keel of the lemma. Generally, the sterile lemmas are shorter than the lemma and palea, seldom exceeding one-third the length of the later. Sterile lemmas may be equal or unequal in size, the upper one generally being larger (Chang *et al.*, 1965). The stigma, a plumose structure has a function in catching the pollen for fertilization purpose.

2.3.6 Grain

Generally, the rice grain refers to rough rice or paddy consisting of brown rice (or caryopsis) and the hull (Figure 2.1). Brown rice consists of the endosperm, embryo, and several thin layers of differentiated tissues-the-pericarp (the wall of ovary), the seed coat, and the nucellus (Jules, 2002). The seed coat consists of six layers of cells, with the aleurone layer which is the innermost. The embryo of rice is small and contains the embryonic leaves (plumule) that enclosed by a sheath (coleoptiles), embryonic primary root (radicle) ensheathed by the coleorhizae, and the joining part (mesocotyl) (Jules, 2002).

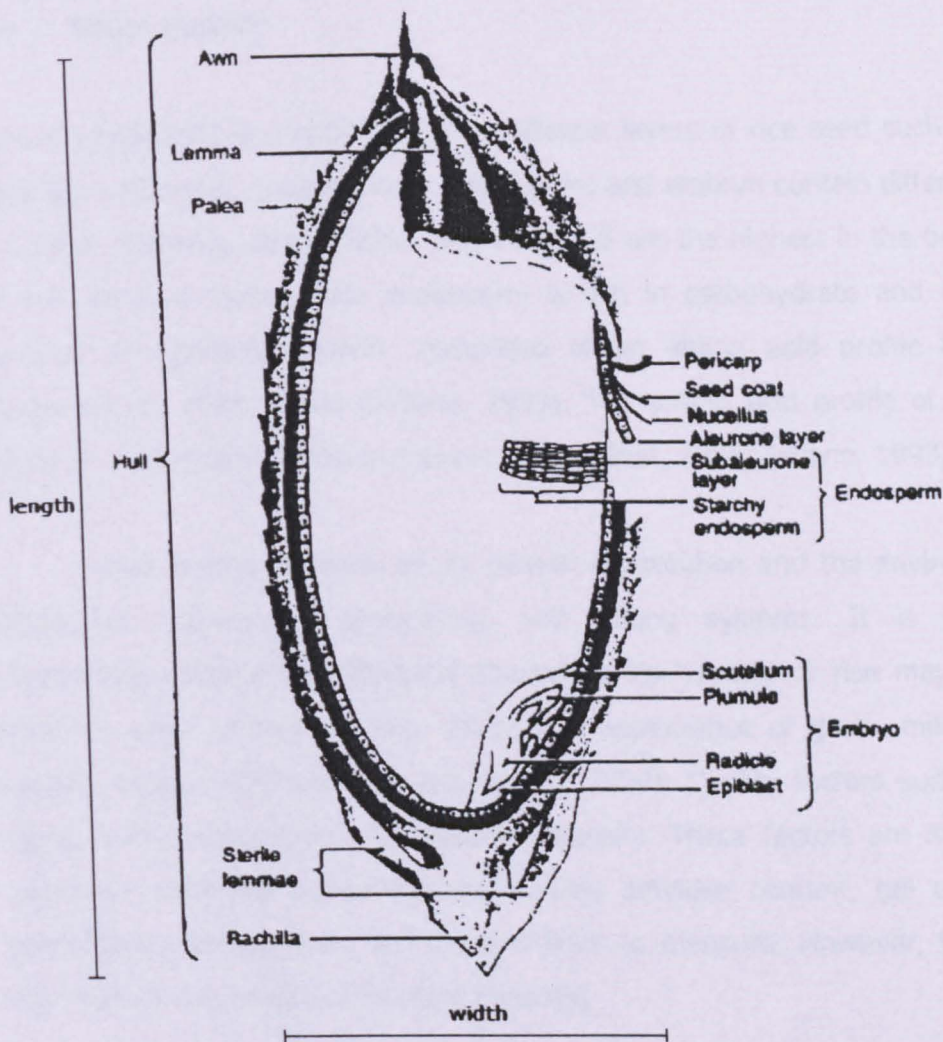


Figure 2.1: Structure of rice grain

Source: Juliano, 1993

The endosperm of rice is enclosed by the aleurone layer which lies beneath the tegmen. Starch granules embedded in a proteinaceous matrix can be found in the white starchy endosperm. The starch fraction is composed almost entirely of amylopectin and turns reddish-brown when stained with weak potassium iodide-iodine solution in the waxy (glutinous) varieties (Chang *et al.*, 1965). As for the common non-waxy (non-glutinous) type, the starch fraction contains amylase in addition to amylopectin. It will turn into dark blue colour when stained with potassium iodide-iodine solution. Besides that, the starchy endosperm also contains sugars, fats, organic matter, and crude fiber (Chang *et al.*, 1965).

2.4 Grain Quality

Rice is wholesome and nutritious. The different layers of rice seed such as outer hull, caryopsis, aleurone, sub-aleurone, endosperm; and embryo contain differing amount of nutrients. Minerals, dietary fiber and Vitamin B are the highest in the bran and lowest in the aleurone layers. Rice endosperm is rich in carbohydrate and contains a fair amount of digestible protein, composed of an amino acid profile that compares favourably to other grains (Juliano, 1993). The amino acid profile of rice is high in glutamic and aspartic acids, but low in lysine (Grist, 1986; Juliano, 1993).

Grain quality depends on its genetic constitution and the environment of crop production, harvesting, processing, and milling systems. It is determined by measurable physical and chemical characteristics. Quality of rice may be considered from the point of view in size, shape and appearance of grain, milling quality and cooking properties (Dela Cruz and Khush, 2000). Quality factors such as grain size, shape, and chalkiness can be assessed visually. These factors are the easiest to be measured. Chemical characteristics, namely amylose content, gel consistency, and gelatinization temperature are more difficult to measure. However, they are just as important to the programs of plant breeding.

2.4.1 Grain Length and Shape

The length of rice grains is determined after the grains are being hulled. The sizes of the grains are classified into short, medium, long, and extra long as described by Graham (2002; Table 2.1):

Table 2.1 Classification of grain size

Grain Size	Length (mm)
Extra long	Greater than 7.50
Long	6.61 – 7.50
Medium	5.51 – 6.60
Short	Less than 5.51

Source: Graham, 2002

The ratio of length to width of brown rice grains is used to determine the grain shape. The major classifications are round, bold, medium, and slender (Graham, 2002; Table 2.2):



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