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Research Article

GENETIC DIVERSITY OF SABAH RICE CULTIVARS USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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ABSTRACT

Rice is the most important staple crop in Malaysia and is cultivated all over the country, including the state of Sabah. The uniqueness of rice cultivation in Sabah lies in the type of rice itself, deriving mainly from local or non-commercial cultivars but with distinctive characteristics including long grains, aromatic properties, and drought tolerance. However, despite having these important agricultural traits, information on the genetic diversity of Sabah rice remains limited. Hence, the purpose of this study was to determine the genetic polymorphisms of Sabah rice using random amplification of polymorphic DNA (RAPD) markers. A total of 101 alleles were profiled, from which 94% were identified as polymorphic. Phylogenetic analysis grouped the rice samples into three clusters, with two clusters classifying the ability of rice to grow under different planting conditions, suitable for growth irrigate and upland condition. The first cluster was dominated by cultivars that could survive in wet (irrigated) areas, while the other featured those that were found in dry (upland) areas. Furthermore, two alleles, OPA-05-B2 and OPA-01-B11, were found to be unique to cultivars within the upland cluster and were thus proposed to be involved in dry environmental adaptation. The results of the present study provide an insight into the genetic relationships and diversity of Sabah rice.

Keywords: rice, upland, irrigated, genetic diversity, RAPD

INTRODUCTION

Rice is a major staple food globally and is consumed by almost 70% of the world's population, especially in Asian countries (Delseny et al., 2001; Razak et al., 2016). According to the Food and Agriculture Organization of the United Nations (FAOSTAT), the total global rice production reached 984 million tons in 2017, higher than that of other staple crops such as potatoes and wheat with a total production of 906 million and 487 million, respectively.

To date, more than 120,000 rice accessions and wild relatives have been identified and deposited to the International Rice GeneBank by the International Rice Research Institute (IRRI). Rice cultivars have different genetic and biochemical properties, and this diversity enables various types of rice to be grown all over the world across different types of geographical area and climate. For example, *Oryza glaberrima* species are widely cultivated in Africa (Vaughan, Lu, & Tomooka, 2008) while *Oryza sativa* species are more versatile, with one subspecies (*Indica*) widely cultivated in temperate, low-latitude and -altitude regions and in warm climates in tropical and sub-tropical countries while the other subspecies (*Japonica*) grows in more temperate regions at high altitude and latitude under cooler climate conditions (Lal et al., 2013; Delseny et al., 2001).

To determine the type of rice for cultivation under different environmental conditions, rice has been categorised into different types including deep-water rice, irrigated rice, rain-feed lowland rice, and upland rice (Poehlman & Sleper, 1995). In Malaysia, a hot and humid climate with variations in rainfall trends often followed by a drier season necessitates that rice is cultivated using irrigation methods (Toriman & Mokhtar, 2012). Therefore, 85.5% of the total rice production in Malaysia is from the states of Peninsular Malaysia, which is dominated by lowland regions suitable for irrigation systems (Vaghefi, Shamsudin, Radam, & Rahim, 2016). In the states of Sabah, however, rice production is lower as the geographical topography with a hilly terrain is unsuitable for large scale cultivation using irrigation systems. Due to this, rice cultivation in Sabah has undertaken upland rice cultivation. However, since both irrigated and upland rice in Sabah is grown on a small scale, limited research has been carried out to profile its genetic diversity. In the present study, the genetic diversity of both irrigated and upland rice in Sabah, from the districts of Kota Belud and Tepulid, was investigated.

MATERIALS AND METHODS

Plant Materials

A total of 29 samples were collected from four cities in the districts of Kota Belud (Tenghilan and Kota Belud towns) and Telupid (Tongod and Telupid towns), including irrigated and upland areas. Samples were collected as peduncles and stored at −20°C until use.

Extraction of DNA from Rice Seeds

DNA was extracted from the rice seeds of all samples following the procedure of Kang, Cho, Yoon and Eun (1998) with modifications. Rice seeds were de-hulled and crushed using a mortar and pestle and DNA was extracted in a buffer [200 mM Tris-HCl (pH 8.0), 200 mM NaCl, 25 mM EDTA, and 0.5% SDS] with the addition of 50 mg of proteinase K. The samples were incubated at 37°C for 1 hour and an equal volume of chloroform was then added. DNA was precipitated from the sample by the addition of two thirds (2/3) of the volume of isopropanol. The DNA pellet was washed with 70% ethanol and suspended in sterilised MiliQ water. Ribonuclease A (RNase; 10 mg) was added to remove RNA from the samples. The quality of DNA was evaluated by gel electrophoresis, and the DNA samples were stored at −20°C until use.

PCR Amplification Using RAPD Markers

DNA extracted from rice samples was used as a template for PCR amplification using a set of 9 decamers of arbitrary oligonucleotide primers. Amplification reactions were carried out in a volume of 25 µl containing 1 × Promega GoTaq® Flexi Buffer, 2.5 mM of $MgCl_2$, 200 µM of each deoxynucleotide triphosphate (dNTP), 10 pmole of primer, 2.5 U of GoTaq® DNA polymerase, and 2 µg of bovine serum albumin (BSA). The PCR reaction protocol modified from Rabbani, Pervaiz, and Masood (2008) and consisted of 1 cycle of 4 minutes at 94°C for initial strand separation followed by 35 cycles of 1 minute at 94°C, 1 minute at 37°C, and 1 minute at 72°C. Finally, 1 cycle of 10 minutes at 72°C was used for the final extension. PCR amplification patterns were profiled based on bands produced following gel electrophoresis in a 2.0% agarose gel stained with ethidium bromide and visualised under UV light in a gel documentation system.

Data Analysis

The banding patterns of agarose gel electropherograms were scored using a binary scoring system where the presence of a band was recorded as "1" and the absence recorded as "0". Only clear bands that were consistently amplified were scored, whereas very faint bands were excluded to avoid false-positive results (REF). The similarity and distance matrices were generated using Nei's (1978) formula in POPGENE 1.31 software based on the shared bands (alleles). The resulting similarity and distance coefficients were subsequently used to evaluate the relationships between samples with a cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) software. The POPGENE programme used to construct the dendrograms was an adaptation of the NEIGHBOR of PHYLIP programme, version 3.5c.

RESULTS AND DISCUSSION

Genetic Polymorphism in Sabah Rice

This study was conducted using 29 rice samples collected from two major cultivation area in Sabah, the Kota Belud and Telupid districts. Rice is generally categorised based on the environmental conditions of the cultivation site and can include deep-water rice, irrigated rice, rain-feed lowland rice, and upland rice (Poehlman & Sleper, 1995). In the present study, 24 samples were collected from irrigated paddy fields and grouped under eight cultivars, namely Baragang (4 samples), Pahu (3 samples), Pilit (2 samples), Purak (2 samples), Sarawak (2 samples), Sibor (5 samples), Silia (3 samples), and Sompug (3 samples). Furthermore, five samples were collected from upland fields and locally named as padi wangi (1 sample), upland-1 (1 sample), upland-2 (1 sample), purak-upland (1 sample), and telangkai (1-sample) cultivars. Different cultivars were represented by different numbers of samples, ranging from one to five, with each representative collected from different farms. The number of representatives for each cultivar was by the availability of the cultivars in the area of collection.

The genetic diversity of the study samples was profiled using 11 primers (OPA-01, OPA-02, OPA-03, OPA-05, OPF-09, OPF-17, OPF-19, OPK-12, OPG-17, OPG-18, and OPG-19), of which 2 primers (OPF-17 and OPF-19) showed no amplification in any sample while 9 primers (OPA-01, OPA-02, OPA-03, OPA-05, OPF-09, OPK-12, OPG-17, OPG-18, and OPG-19) showed clear and consistent amplification patterns. The number of alleles identified from each primer ranged from 8 to 17 (see Table 1).

Table 1 The total amplified alleles from nine RAPD markers in Sabah rice

RAPD markers (Name)	Number of alleles amplified
OPA-01	13
OPA-02	17
OPA-03	10
OPA-05	9
OPF-09	7
OPK-12	13
OPG-17	10
OPG-18	8
OPG-19	14
Total	101

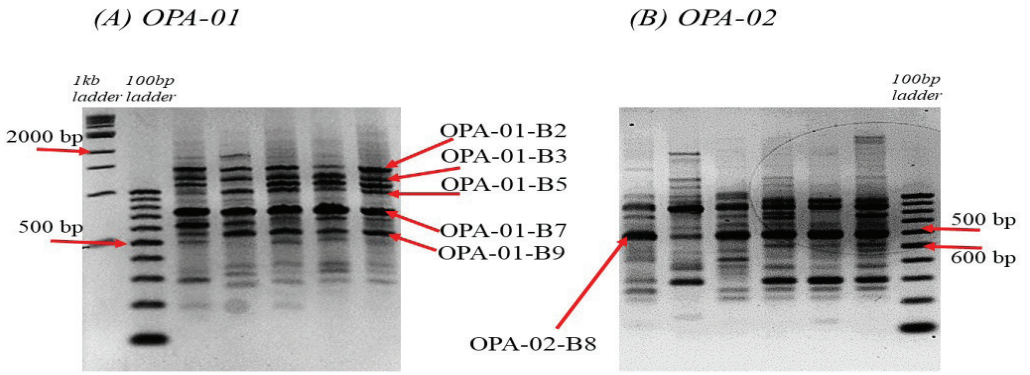

Figure 1 Monomorphic alleles found in all samples (irrigated and upland rice). Alleles OPA-01-B2, OPA-01-B3, OPA-01-B5, OPA-01-B7, OPA-01-B9 were amplified from RAPD marker OPA-01, while allele OPA-02-B8 was amplified from OPA-02.

Using RAPD markers, 101 alleles were identified, from which 94% were polymorphic. Only 6 alleles (out of 101) showed monomorphism, of which five were generated from primer OPA-01 (allele OPA-01-B2, -B3, -B5, -B7, and -B9) and one from OPA-02 (allele OPA-02-B8) (Figure 1). It is suggested that the monomorphic alleles identified in this study may represent beneficial alleles that become fixed during the rice domestication process in Sabah (Payseur & Nachman, 2002). Furthermore, the presence of these common alleles might be attributable to the continuous usage of same-genotype parents for cultivation purposes, which over time may narrow the genetic variation in Sabah rice, as is the case for rice cultivation in Brazil (Rabelo, Guimaraes, Pinheiro, & da Silva, 2015).

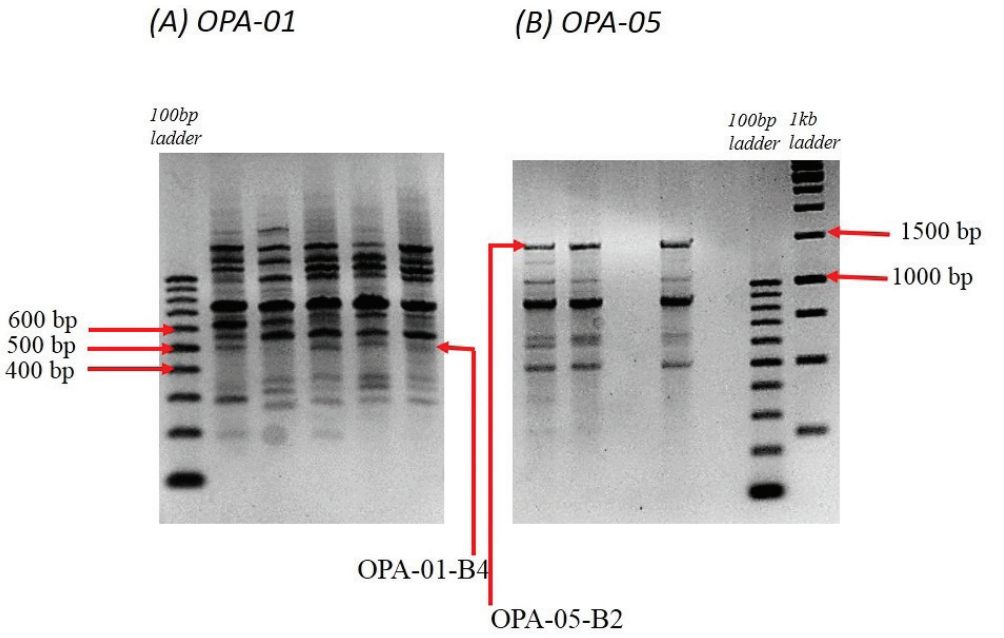

Figure 2 Two alleles unique to upland rice namely OPA-01-B11 and OPA-05-B2 unique to upland rice

RAPD marker analysis also revealed that several alleles were shared between rice cultivars collected from upland areas alone. These alleles were OPA-05-B2 (present in the upland-1, upland-2, and telangkai samples) and OPA-01-B11 (present in the padi wangi, upland-2, purak-upland, and telangkai samples) (Figure 2). Therefore, these alleles are postulated to be unique to upland rice and may be involved in adaptation (drought tolerance), enabling upland rice to survive in upland ecosystems that are exposed to a higher risk of drought (Zhou et al., 2016).

Genetic Diversity of Sabah Rice

In the cluster analysis, the 29 samples were grouped into three main clusters, Cluster I, II, and III (see Figure 3). Cluster I was dominated by samples collected from irrigated areas, as such 22 out of 24 samples were grouped under this cluster. Samples collected from upland areas were found scattered in Clusters II and III. As such, Cluster II comprised upland samples alone, while Cluster III was a mixture of upland and irrigated rice samples. The categorisation of upland and irrigated rice into three clusters was according to a study conducted by Coelho et al. (2017), whereby the genetic diversity of upland and irrigated Brazilian rice was found to be divided into three main clusters. As such, one cluster was formed by irrigated rice, one cluster by upland rice, and a third by a combination of upland and irrigated rice.

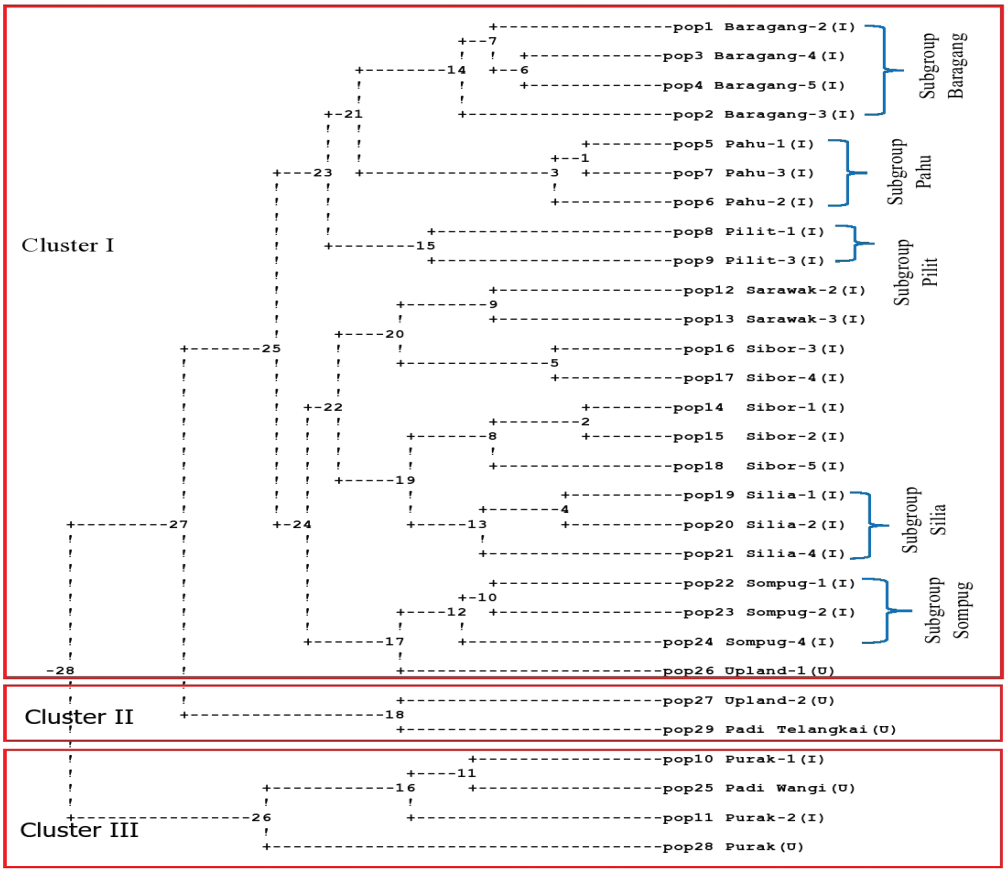

Figure 3 Dendogram based on UPGMA method consisting of 29 Sabah rice. Three boxes represent three cluster namely Cluster I (dominated by irrigated rice), Cluster II (consists of upland rice), and Cluster III (consists of both irrigated and upland rice). Five main subgroups were fund including the Baragang, Pahu, Pilit, Silia, and Sompug subgroup.

Some patterns of clusters indicate various genetic postulation of Sabah rice. As such, the separation between samples collected from irrigated areas into Cluster I and upland areas into Cluster II and III marks the genetic differences of rice of different types; that is, irrigated and upland rice. This separation indicates that Sabah rice may have a polyphyletic origin, whereby rice cultivars may have originated from more than one common ancestor (Wunna et al., 2016). Furthermore, since the clustering of samples in the study pointed towards separation based on cultivation area, the separation of irrigated rice in Cluster I against upland rice suggests that samples under this cluster are of the *Indica* subspecies or are of *Indica* origin. *Indica* rice is mainly cultivated in tropical and subtropical environments at lower latitudes or altitudes (Delseny et al., 2001), similar to the Kota Belud area from which most of the rice from Cluster I originated. Extending from observation, samples grouped under Cluster II and III,

which were dominated by rice collected from upland areas, may be categorised under the *Japonica* subspecies, which are mainly cultivated in more temperate environments at higher latitudes or altitudes (Delseny et al., 2001), similar to the Telupid area from which these samples were collected. Therefore, the distribution of different alleles between irrigated and upland rice cultivars maybe be associated with environment adaptation behaviour, especially with regards to response to drought and water stress conditions (Lyu et al., 2014; Wang, Zhang, Gao, Li, & Li, 2007).

The mixture of samples collected from irrigated and upland areas in Cluster II and III indicates genetic admixture in Sabah rice. Genetic admixture can occur when two or more genetically differentiated plants begin interbreeding, resulting in the introduction of a new genetic lineage (Wang et al., 2017), and may have occurred with the samples in the present study as they were cultivated together in the same area. A similar observation was reported in a study conducted in West Africa, whereby 67% of *Oryza glaberrima* were found to carry some level of admixture with *Oryza sativa* due to natural breeding between these two species, which are frequently grown together or in combination (Chen et al., 2017).

Another observation from this study is that the rice varieties of similar cultivars were clustered under the same subgroup. As such, five distinct subgroups were found, the Baragang, Pahu, Pilit, Silia, Sarawak and Sompug subgroups, indicating that members of these subgroups derive from a common ancestor.

CONCLUSIONS

The results obtained from this study provide additional insight into the genetic polymorphisms, genetic relationships, and diversity of Sabah rice, which has been limited to date. Although rice samples could be classified as irrigated or upland rice, future studies in a larger number of samples collected from the entire state of Sabah and using higher numbers of RAPD markers may obtain more precise information on the genetic polymorphisms and diversity of Sabah rice.

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