GENETIC DIVERSITY OF UPLAND RICE LANDRACES FROM JAVA ISLAND AS REVEALED BY SSR MARKERS

Keragaman Genetik Padi Gogo Lokal dari Pulau Jawa Berdasarkan Marka SSR

Sutoro, Puji Lestari, Reflinur and Hakim Kurniawan

[1]*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development*
Jalan Tentara Pelajar No. 3A Bogor 16111, Indonesia
Phone +62 251, 8337975, Fax. +62 251, 8338820, E-mail: bb_biogen@litbang.deptan.go.id
*Corresponding author: storo8@gmail.com

ABSTRACT

Java Island is one of origins of a large number of indigenous upland rice accessions, which may serve as valuable plant genetic resources for future crop improvement in Indonesia. However, these landraces especially non-glutinous and glutinous rice are rapidly being lost because of land-use, agricultural practices and other factors. A better understanding of genetic diversity of local upland rice is important for crop improvement program, crop management and conservation strategy. This study aimed to evaluate the genetic diversity of upland rice landraces originating from Java Island. A total of 82 upland rice accessions comprising of 55 non-glutinous rice and 27 glutinous type were genotyped using the 16 simple sequence repeat (SSR) markers. The result showed that a total of 74 alleles were found with major allele frequency found on RM431 (0.96). Most of the SSR markers (56.3%) showed high discriminating power as represented by polymorphic information content (PIC) value higher than 0.5. A moderate genetic diversity index was detected in all landraces, which was 0.55. Genetic diversity index of non-glutinous and glutinous rice were 0.54 and 0.53, respectively. Their genetic distance was about 0.057. The phylogenetic tree generated two main clusters that demonstrated discrimination among landraces according to the individual genetic properties rather than their geographical origins and grain types (non-glutinous and glutinous type). The levels of genetic diversity were varied across rice types and geographical origins. According to the regions, the closest genetic distance was found between upland rice landraces from Central Java and West Java (0.040). The information derived from this study is important, in combination with phenotypic data, to identify desired useful traits came from different origins of the gene pool to be used for breeding purposes.

[**Keyword**: Upland rice, genetic diversity, SSR markers, Java]

ABSTRAK

Pulau Jawa merupakan salah satu asal plasma nutfah padi gogo asli Indonesia yang berperan penting sebagai sumber genetik untuk perbaikan tanaman. Namun, plasma nutfah khususnya beras dan ketan ini cepat hilang karena alih fungsi penggunaan lahan, praktik budi daya, dan faktor lainnya. Pemahaman yang lebih baik tentang keragaman genetik padi gogo lokal penting untuk program pengembangan varietas, pengelolaan tanaman, dan strategi konservasi. Penelitian ini bertujuan mengevaluasi keragaman genetik padi gogo lokal yang berasal dari Pulau Jawa. Total 82 aksesi padi gogo yang terdiri atas 55 aksesi padi beras dan 27 aksesi ketan telah dikarakterisasi menggunakan 16 marka simple sequence repeat (SSR). Sebanyak 74 alel ditemukan dengan frekuensi alel mayor pada RM431 (0,96). Sebagian besar marka SSR (56,3%) menunjukkan kekuatan diskriminatif yang tinggi, yang diwakili oleh polymorphism information content (PIC) dengan nilai lebih tinggi dari 0,5. Indeks keragaman genetik terdeteksi moderat sekitar 0,55. Indeks keragaman genetik beras dan ketan berturut-turut 0,54 dan 0,53 dengan jarak genetik 0,057. Analisis filogeni menghasilkan dua klaster utama yang menunjukkan diferensiasi antaraksesi lebih berdasarkan pada sifat genetik individu daripada asal geografis dan tipe beras (beras dan ketan). Keragaman genetik berdasarkan grup juga bervariasi pada tipe beras maupun asal daerahnya. Menurut wilayah, jarak genetik terdekat ditemukan pada padi gogo dari Jawa Tengah dan Jawa Barat (0,040). Informasi yang dihasilkan dari penelitian ini penting dalam program pemuliaan, dengan menggabungkan data molekuler dan fenotipik untuk mencari sumber gen dari plasma nutfah yang diinginkan sesuai tujuan pemuliaan tanaman.

[*Kata kunci*: Padi gogo, keragaman genetik, marka SSR, Jawa]

INTRODUCTION

Nearly 2/3 of the upland rice area is located in Asia, including Bangladesh, China, India, Thailand, Vietnam, Cambodia, Myanmar and Indonesia, which are well known as important rice producers (Gupta and O'Toole 1986). Upland rice is usually grown in rain-fed fields and the ecosystem is extremely diverse. Upland rice usually has the ability to turn in a poor soil and however, is prone to pests and diseases. The absence of flooded field allows weeds to thrive, and the presence of stress soils could be considered as a perfect breeding aspect of biotic and

abiotic stress (Dhakal *et al.* 2006; Bernier *et al.* 2008). Despite of the development of high yielding varieties benefited irrigated lowland rice since years ago due to climate change, such varieties have remained increasingly unsuitable because of its high input of water and nutrient. Interestingly, with less management practices, some local upland rice (landraces) are well adapted to their environments and produce high yield to meet the local demands (Joshi *et al.* 2001).

In Indonesia, rice production is mainly concentrated on the islands of Java and Sumatra, with nearly 60% of total production emanating from Java. Java is dominated by about 6.35 million hectares of paddy with a productivity of 5620 kg/ha in the same year (BPS 2014). While, the upland rice area is only approximately 10% of the total rice areas in Indonesia. Upland rice is tolerant to dry land with lower fertilizer requirements; therefore, upland rice has been developed in various regions in Indonesia including Java.

A large number of rice germplasm is originating from Java, either upland rice or irrigated rice which are scattered in various areas. Upland rice landraces in Java may represent a unique and critical genetic resource that can be used for rice improvement program. Even though upland rice production is lower than irrigated rice, upland rice is accustomed to the harsh upland conditions and so it could be a serious option for farmer in the upland areas (Harahap *et al.*1995; BB Padi 2005). Landraces of upland rice or high yielding varieties which are adapted to environmental conditions area preferentially able to cope with climate change (Warda 2011).

To support national food security, many upland rice varieties have been released by the Indonesian Agency for Agricultural Research and Development (IAARD). During 1999-2002, seven upland rice varieties had been released (Cirata, Towuti, Limboto, Danau Gaung, Batutegi, Situpatenggang and Situbagendit). In general, those varieties showed early maturity, tolerant to aluminum toxicity, drought tolerant, and resistant to several blast races (Suwito 2005). While within the last five years (2010-2014), seven upland rice varieties have also been released by IAARD, i.e. Inpago 4-Inpago 9 (IAARD 2015). The varieties need to be adapted to determine their suitability to be cultivated in the various upland areas.

In line with the rice breeding program, upland rice landraces are important genetic materials as breeding parents (Suwito 2005). However, many upland rice landraces are being lost and under threat. To race

genetic erosion, local upland rice germplasm needs to be collected, preserved and characterized. The overall goal of characterization of upland rice germplasm represents one of the main sources of parents in the development of new populations that can be exploited in the breeding program. The magnitudes of genetic variability that it has for the traits of interest, depend on the genetic divergence of the parents for crossings (Rathi *et al.* 2014).

A better method for characterization of such group of rice is important to be available to facilitate their use in breeding programs. Molecular characterization is one of the efficient methods for characterizing the genetic variability available for breeding program (Lu *et al.* 2005). In recent years, high accuracy molecular markers like the single nucleotide polymorphism (SNP), which can detect variation at the DNA level are increasingly used for efficient characterization and evaluation of germplasm. Among the various classes of molecular markers available in rice, microsatellite markers (SSR markers) are the most popular markers due to their abundance, high discriminatory power, co-dominance, and economic use in both manual and automated systems (McCouch *et al.* 2001). Thousands SSR markers for rice are already available (McCouch *et al.* 2002). SSR markers are known to have more information in comparison to other molecular markers and can be performed using polymerase chain reaction (PCR) (Powell *et al.* 1995). Thus, SSR marker is a convenient technique for genetic study and improvement of upland rice for yield, quality and tolerance to abiotic/ biotic stresses and any other desired traits.

A number of studies have been conducted to evaluate the genetic variability of upland rice in Asian countries. Genetic variation in root morphology and SSR loci on upland rice from Vietnam gave useful information for mapping quantitative trait loci (QTL) related to drought resistance (Thanh *et al.* 1999). Genetic variation using molecular markers has been studied on several upland rice landraces in Assam, India (Rathi *et al.* 2014), upland rice accessions from southwest China using SSR markers (Tang *et al.* 2010), and upland drought-tolerant of the germplasms from India and Bangladesh using RFLP, RAPD and SSLP markers (Bautista *et al.* 2001). A current study reported a genetic difference between upland and lowland rice, which were explored by 47 SSR located in drought responding expressed sequence tags (ESTs) among 377 rice landraces (Xia *et al.* 2014). In addition, high levels of genetic diversity in the Chinese 221 upland rice germplasm were identified using 28 SSR markers and could provide invaluable

genetic resources for improving economically important traits in rice (Zeng *et al.* 2010). So far, there have no available such reports about genetic variation in upland rice landraces including the glutinous type of Java by using the benefit of molecular markers like SSR.

Because Indonesia has an abundant genetic resource of upland rice, a study on the evaluation of genetic diversity is necessary for conservation purposes and to assist the selection process in rice breeding program. This study aimed to evaluate the genetic diversity of local upland rice genetic resources, mostly originating from Java Island based on SSR markers.

MATERIALS AND METHODS

Plant Materials

A total of 82 accessions of local upland indica rice (landaraces) collected in the gene banks of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Indonesian Agency for Agricultural Research and Development (IAARD) was used in the present study. These rice collections were selected from a diverse geographical area of rice growing in Java. The majority of rice accessions were collected from West Java (44 accessions) and Central Java (29 accessions) and the remaining 9 accessions were from East Java. Fifty five accessions out of 82 rice accessions assessed in this study were classified into non-glutinous rice and the remaining were glutinous type accessions. Rice accessions were grown in the greenhouse of ICABIOGRAD in August 2014. After 5 weeks, young leaf samples were taken for DNA extraction. PCR analysis was done in Molecular Biology Laboratory of ICABIOGRAD. List of 82 upland rice landraces with their local origins is presented in Table 1.

SSR Amplification Procedure

For DNA isolation, all accessions were grown in a well-controlled greenhouse until tillering stage. The young leaf tissue of each rice accession was harvested, collected and extracted its total genomic DNA based on the cetyl trimethyl ammonium bromide (CTAB) procedure (Murray and Thompson 1980). The concentration and quality of isolated DNA were determined using a NanoDrop1000 spectrophotometer

(Thermo Scientific Co). The concentration of DNA samples was further equalized among all samples at a final concentration of 20 ng μl^{-1} before performing PCR analysis.

A total of 16 simple sequence repeat (SSR) markers was used for analysis in this study. The list of SSR primers and their sequences is presented in Table 2. PCR reaction was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc.) machine under the following conditions: 5 minutes at 94°C; 35 cycles of 30 second at 95°C, 30 second at 55°C, 1 minute at 72°C; and 7 minute of 72°C. PCR amplification of DNA was performed in a total volume of 20 μl with the following PCR reagents: 2 μl of DNA sample at 20 ng μl^{-1}, 2 μl of 10x buffer containing 25 mM $MgCl_2$, 1 μl of 2.5 mM dNTPs, 1 U of Taq Polymerase (Genetica Science), and 1 μl each of forward and reverse primers (10 μM). The amplicons were analyzed by non-denaturing electrophoresis on 8% polyacrylamide gels stained with ethidium bromide (Model MGV, CBG Scientific Co.). The banding pattern was visualized under UV light by using a digital system and image scanning Gel Documentation EQ system (Bio-Rad). A 100 bp ladder was used for sizing the SSR banding profile.

Data Analysis

The alleles were scored based on the profiling of total alleles of each primer. Statistical summary, including values of Polymorphic Information Content (PIC), heterozygosity and allele number was calculated for the total population and SSR markers (Liu 2001). Genetic diversity index and cluster analysis were analyzed based on estimates of Nei genetic distance by using the neighbor-joining method. The genetic variation was estimated inter- and intra-population according to rice types (non-glutinous and glutinous type) and geographical origins (West, Central and East Java). To support the feasible cluster formation, bootstrap analysis at 1000 permutation was performed by using Power Marker V3.25 (Felsenstein 1985; Liu and Muse 2005).

RESULTS AND DISCUSSION

Polymorphism Analysis

The result revealed that all SSR markers well amplified the genomic DNA of both non-glutinous and glutinous rice. All markers showed clearly polymorphic bands

Table 1. List of upland rice landraces from Java used in this study.

Local name	Origin	Type
Ketan Gajih	Majalengka, West Java	Glutinous rice
Segon Darat	Sukabumi, West Java	Non-glutinous rice
Ketan Hitam	Subang, West Java	Glutinous rice
Padi Ner	Majalengka, West Java	Non-glutinous rice
Mesir	Sumedang, West Java	Non-glutinous rice
Ketan Beureum	Kuningan, West Java	Glutinous rice
Pare Odeng	Lebak, Banten, West Java	Non-glutinous rice
Pare Sampai	Lebak, Banten, West Java	Non-glutinous rice
Pare Petey	Lebak, Banten, West Java	Non-glutinous rice
Bulu Roma	Sukabumi, West Java	Non-glutinous rice
Markoti	Sukabumi, West Java	Non-glutinous rice
Mantare	Sukabumi, West Java	Non-glutinous rice
Ketan Hitam	Subang, West Java	Glutinous rice
Leri	Subang, West Java	Non-glutinous rice
Koproj	Subang, West Java	Non-glutinous rice
Tjere Beton	Indramayu, West Java	Non-glutinous rice
Sempor	Indramayu, West Java	Non-glutinous rice
Gempol	Bogor, West Java	Non-glutinous rice
Bengawan	Cilangkap, West Java	Non-glutinous rice
Pare Merah	Lebak, West Java	Non-glutinous rice
Segon	Bandung, West Java	Non-glutinous rice
Gombal	Bandung, West Java	Non-glutinous rice
Sirung Amis	Garut, West Java	Non-glutinous rice
Badigul	Sukabumi, West Java	Non-glutinous rice
Cingir Putri	Tasikmalaya, West Java	Non-glutinous rice
Cikapundung	Cilegon Ilir, Banten, West Java	Non-glutinous rice
Pare Koneng	Sangkanwangi, Leuwidamar, West Java	Non-glutinous rice
Pare Bentik	Sangkanwangi, Leuwidamar, West Java	Non-glutinous rice
Buntut Kuda	Sangkanwangi, Leuwidamar, West Java	Non-glutinous rice
Ketan Kasumba	Sangkanwangi, Leuwidamar, West Java	Glutinous rice
Ketan Hideung	Sangkanwangi, Leuwidamar, West Java	Glutinous rice
Ketan Super	Muncang, Banten, West Java	Glutinous rice
Ketan Lason	Muncang, Banten, West Java	Glutinous rice
Ketan Hideung	Muncang, Banten, West Java	Glutinous rice
Cokrom	Muncang, Banten, West Java	Non-glutinous rice
Ketan Nangka	Ujung Tebu, Banten, West Java	Glutinous rice
Ketan Kesumba	Cemplang, Banten. West Java	Glutinous rice
Segon	Sumedang and Kuningan, West Java	Non-glutinous rice
Ketan Langgarsari	Lebak, Banten, West Java	Glutinous rice
Ketan Bayong	Serang, Banten, West Java	Glutinous rice
Ketan Boyong	Cianjur, West Java	Glutinous rice
Ketan Hitam	Subang, West Java	Glutinous rice
Ketan Garut	Subang, West Java	Glutinous rice
Ketan Wadas	Subang, West Java	Glutinous rice
Ketan Gudel	Gunung Kidul, Yogyakarta	Glutinous rice
Papah Aren	Gunung Kidul, Yogyakarta	Non-glutinous rice
Brontok	Rembang, Central Java	Non-glutinous rice
Gondil	Boyolali, Central Java	Non-glutinous rice
Genjah Mayangan	Gunung Kidul, Yogyakarta	Non-glutinous rice
Molog	Gunung Kidul, Yogyakarta	Non-glutinous rice
Ketan Lumbu	Gunung Kidul, Yogyakarta	Glutinous rice
Lok.B.(Bj.panjang)	Banyumas, Central Java	Non-glutinous rice
T L	Pesantun, Brebes, Central Java	Non-glutinous rice
Pudat A	Menganti, Central Java	Non-glutinous rice
Pudat B	Menganti, Central Java	Non-glutinous rice
Melati	Menganti, Central Java	Non-glutinous rice
Marus A	Jalatunda, Banjarnegara, Central Java	Non-glutinous rice
Marus B	Jalatunda, Banjarnegara, Central Java	Non-glutinous rice
Segreng	Jalatunda, Banjarnegara, Central Java	Non-glutinous rice

Table 1. (continued).

Local name	Origin	Type
Slegreng	Setra,Wonogiri, Central Java	Non-glutinous rice
Padi Umbul-umbul	Setra,Wonogiri, Central Java	Non-glutinous rice
Dodokan Abang	Ngasem, Wonogiri, Central Java	Non-glutinous rice
Ketan Salome	Ngasinan, Wonogiri, Central Java	Glutinous rice
Ketan Putih	Kebo Kuning, Bantul, Yogyakarta	Glutinous rice
Ketan Salome	Kebo Kuning, Bantul, Yogyakarta	Glutinous rice
Ketan Serang	Kebo Kuning, Bantul, Yogyakarta	Glutinous rice
Pandanwangi	Candi 3, Sleman, Yogyakarta	Non-glutinous rice
Mentik	Pati, Central Java	Non-glutinous rice
Indramaju B	Pati, Central Java	Non-glutinous rice
Jenar	Kebumen, Central Java	Non-glutinous rice
Perak	Kebumen, Central Java	Non-glutinous rice
Poloman A	Kebumen, Central Java	Non-glutinous rice
Tjempo Brondol	Purwokerto, Central Java	Non-glutinous rice
Ridjal	Magetan, East Java	Non-glutinous rice
Ketan Cikut	Sidoarjo, East Java	Glutinous rice
Ketan Lanbok	Sidoarjo, East Java	Glutinous rice
Makmur	Surabaya, East Java	Non-glutinous rice
Gedangan	Surabaya, East Java	Non-glutinous rice
Gedangan Lulut	Surabaya, East Java	Non-glutinous rice
Ketan Hitam	Gunggung, Sumenep, Madura, East Java	Glutinous rice
Ketan Putih	Gunggung, Sumenep, Madura, East Java	Glutinous rice
Ketan Putih	Tumajah Timur, Bangkalan, Madura, East Java	Glutinous rice

Table 2. List of SSR primers and their sequences used in this study.

Primer	Chr[1)]	Repeat motif	Forward sequence (5'—3')	Reverse sequence (5'—3')	References
RM105	9	(CCT)6	gtcgtcgacccatcggagccac	tggtcgaggtggggatcgggtc	Temnykh *et al.* (2000)
RM11	7	(GA)17	tctcctcttccccccgatc	atagcgggcgaggcttag	Panaud *et al.* (1996)
RM124	4	(TC)10	atcgtctgcgttgcggctgctg	catggatcaccgagctcccccc	Temnykh *et al.* (2000)
RM144	11	(ATT)11	tgccctggcgcaaatttgatcc	gctagaggagatcagatggtagtgcatg	Temnykh *et al.* (2000)
RM413	5	(AG)11	ggcgattcttggatgaagag	tccccaccaatcttgtcttc	Temnykh *et al.* (2001)
RM223	8	(CT)25	gagtgagcttgggctgaaac	gaaggcaagtcttggcactg	Chen *et al.* (1997)
RM19	12	(ATC)10	caaaaacagagcagatgac	ctcaagatggacgccaaga	Panaud *et al.* (1996)
RM215	9	(CT)16	caaaatggagcagcaagagc	tgagcacctccttctctgtag	Chen *et al.* (1997)
RM259	1	(CT)17	tggagtttgagaggaggg	cttgttgcatggtgccatgt	Chen *et al.* (1997)
RM287	11	(GA)21	ttccctgttaagagagaaatc	gtgtatttggtgaaagcaac	Temnykh *et al.* (2000)
RM431	1	(AG)16	tcctgcgaactgaagagttg	agagcaaaaccctggttcac	Temnykh *et al.* (2001)
RM474	10	(AT)13	aagatgtacgggtggcattc	tatgagctggtgagcaatgg	Temnykh *et al.* (2001)
RM5	1	(GA)14	tgcaacttctagctgctcga	gcatccgatcttgatggg	Panaud *et al.* (1996)
RM514	3	(AC)12	agattgatctcccattcccc	cacgagcatattactagtgg	Temnykh *et al.* (2001)
RM536	11	(CT)16	tctctcctcttgtttggctc	acacaccaacacgaccacac	Temnykh *et al.* (2001)
RM541	6	(TC)16	tataaccgacctcagtgccc	ccttactcccatgccatgag	Temnykh *et al.* (2001)

[1)]Chr: chromosome (mapping position).

along the landraces tested. The example of SSR markers banding pattern on polyacrylamide 8% is presented in Figure 1. Statistical summary of 82 upland rice accessions using 16 SSR markers according to the polymorphic level and genetic variability is presented in Table 3. The number of alleles per marker varied from 2 to 7 with an average of 4.6, making up a total of 74 alleles. Major alleles existed in these landraces as demonstrated by the major allele frequency found on RM431 (0.96), indicating that the common allele existed in this rice collection.

Most SSR markers (56.3%) showed high discriminating power and reasonably informative as represented by the PIC values of higher than 0.5 (Table 2). The PIC values reflecting allele diversity of a particular marker, ranged from 0.08 (RM431) to 0.69 (RM105) with a mean of 0.49. These results seem to
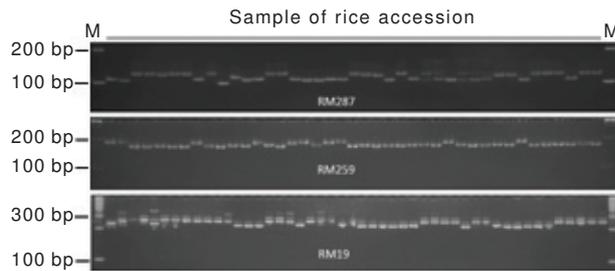
Fig. 1. Banding pattern of SSR markers showing polymorphism observed on a number of represented upland rice landraces from Java. M: DNA ladder 100 bp, SSR markers: RM287, RM259, RM541.

Table 3. Statistical summary of 16 SSR markers observed on 82 upland rice landraces from Java.

Marker	Major allele frequency	Allele no.	Gene diversity	Heterozygosity	PIC
RM259	0.80	3	0.33	0.012	0.28
RM431	0.96	3	0.08	0.012	0.08
RM514	0.75	5	0.40	0.085	0.37
RM19	0.53	6	0.59	0.061	0.52
RM11	0.44	5	0.70	0.000	0.65
RM413	0.45	7	0.69	0.000	0.64
RM287	0.49	4	0.53	0.085	0.43
RM105	0.35	6	0.73	0.000	0.68
RM144	0.44	5	0.65	0.000	0.58
RM215	0.52	2	0.50	0.000	0.37
RM474	0.62	6	0.55	0.012	0.51
RM541	0.38	5	0.72	0.000	0.67
RM5	0.33	4	0.71	0.000	0.66
RM223	0.49	6	0.66	0.000	0.62
RM536	0.67	4	0.47	0.037	0.40
RM124	0.53	3	0.50	0.012	0.38
Mean	0.55	4.62	0.55	0.019	0.49

be lower than those of upland rice landraces from Assam, India (Rathi *et al*. 2014) and Embrapa, Brazil (Brondani *et al*. 2006). It was probably influenced by the low number of markers used and less diverse genetic backgrounds which came from the same subspecies, indica (Ni *et al*. 2002). An average heterozygosity was recorded for eight markers and found to be as low as 2% as expected because rice is a self-pollinating crop. The heterozygosity appeared is likely due to residual heterozygosity in the collection as well as outcrossing in some landrace accessions (Blair *et al*. 2009). Moreover, the markers which identified loci in heterozygosis could be used in the selection of homozygous plants for the most frequent allele of each marker. Such polymorphic SSR markers could be applied in quantitative trait loci (QTL) mapping and genetic analyses (Chuang *et al*.

2011). Overall, these total markers provided sufficient informative polymorphism to evaluate genetic diversity of these 82 upland rice accessions.

Genetic Diversity among Accessions

Genetic diversity of upland glutinous and non-glutinous rice landraces based on SSR was presented as dendrogram or phylogenetic tree which constructed based on a neighbor-joining tree method (Fig. 2). The rice landrace accessions were grouped into two main clusters with a cutoff of 0.73. The phylogenetic tree demonstrated the discrimination among landraces which preferentially grouped according to the individual genetic variability rather than their geographical origins. The two main clusters consisted of a mixed landraces from different local origins and rice types. Most of accessions belonged to cluster I (59 accessions) and the remaining accessions were grouped in cluster II.

A number of upland rice accessions had close distanced individually with the other glutinous rice accessions (for example Bulu Roma, Gedangan Lulut, Ridjal, Padi Umbul-Umbul, Molog etc.). While as comparison using popular accession, Pandanwangi known as rice with stickiness shared higher genetic similarity with several glutinous rice landraces than non-glutinous rice. Unlike Pandanwangi, a rice accession which is popular with its taste (Mentik) was genetically closer with other upland rice accession than glutinous type. Furthermore, these SSR markers were also able to distinguish upland rice accessions with similar initial names such as between Marus A and Marus B, Pudat A and Pudat B, and between Pare Sampai, Pare Petay and Pare Bentik, which indicate their close genetic relationships.

The majority of glutinous rice accessions which locally known as 'ketan', 21 out of total 28 upland glutinous rice accessions were placed in cluster 1. Glutinous rice accession tended to be grouped in the same sub-cluster representing their close genetic relationships. This was represented by a close relatedness of glutinous rice based on grain colors (for example, Ketan Hideung from Sangkanwangi, West Java and Ketan Hideung from Muncang, West Java; Ketan Putih from Madura and Ketan Cikut from East Java). The close relationships among glutinous rice were also shown by Ketan Lason (West Java), Ketan Nangka (West Java) and Ketan Salome (Central Java); and between three glutinous type of West Java (Ketan Bayong from Serang, Ketan Hitam from Sankanwangi and Ketan Garut from Subang). No clear
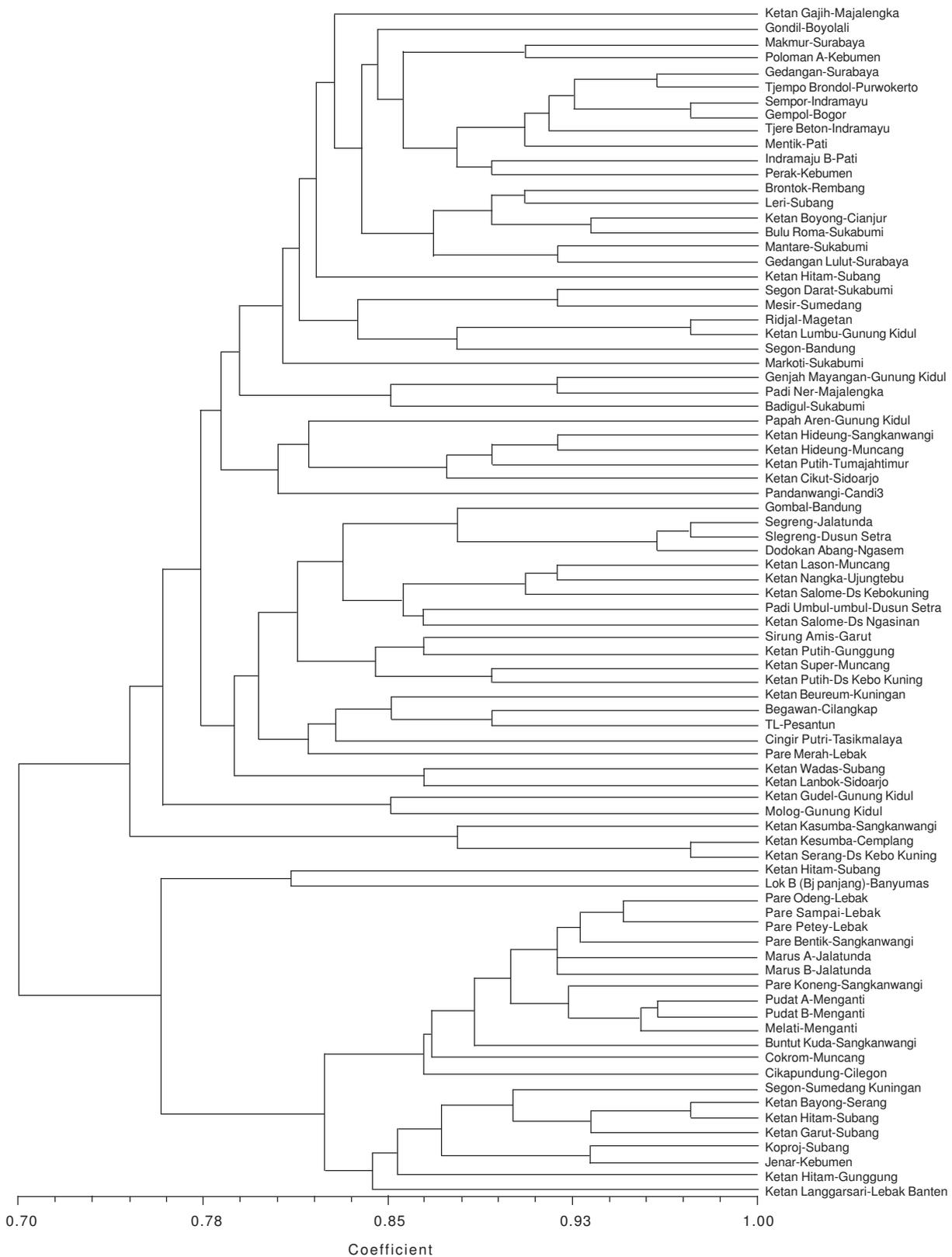
Fig. 2. Phylogenetic tree generated from neighbor-joining analysis depicting the genetic relationship among 82 upland rice landraces from Java based on 16 SSR markers. I and II are two generated clusters.

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difference among upland rice and glutinous type may be due to partial sharing of their genetic polymorphism and/or recent gene flow (Gao and Innan 2008).

These SSR markers could be useful for evaluating genetic diversity that allows for identification of loci responsible for important agronomical traits. The phylogenetic clusters derived from the rice germplasm provided a diversity analysis of local genetic resources. Notably, some of local cultivars with superior agronomical traits in the specific region become popular and majorly cultivated by farmer in Indonesia. The pretty high diversity of upland rice in Java reflects the importance of traditional knowledge in conservation of indigenous rice genetic resources. Therefore, appropriate conservation measures should be taken to promote the cultivation of local varieties with local knowledge (Brush and Meng 1998; Choudhury *et al*. 2013).

Genetic Diversity Within and Between Groups

In this study, we divided the germplasm into several groups to evaluate genetic diversity and genetic distance based on rice types and provincial origins. The levels of genetic diversity were varied across rice types and geographical origins (Table 4). The genetic diversity index within non-glutinous rice group (0.54) was relatively comparable with that of the glutinous rice group (0.53) without considering their local origins (Table 4). While based on the geographical area, upland rice landraces from West Java did not so differ from that of the Central Java accessions, accounting 0.54 and 0.55, respectively. Low genetic diversity was found in upland rice collected from East Java (0.39) probably due to intensive artificial selection by human. Since accessions from Madura only few, thus we only compared with that from East Java, its nearby region and showed higher (0.46) than that from East Java. Thus, the genetic diversity of Java upland rice landraces population was narrow.

To know the genetic relatedness among groups, Nei's genetic distance was calculated as presented in Table 4 and Table 5. All Java upland non-glutinous rice and glutinous landraces showed a low level of genetic distance of 0.057 with the SSR markers. Interestingly, upland rice landraces in Central Java were much closer to that of West Java (0.040) than that of East Java (0.139). The far geographical distance seems to influence the genetic distance of rice collection of West Java and East Java, with the

Table 4. Gene diversity index of non-glutinous and glutinous upland rice from Java and their genetic distance.

Rice type	Genetic diversity index	Genetic distance
Non glutinous	0.54	0.057
Glutinous	0.53	

Table 5. Genetic distance between upland rice landraces group based on their local origin.

Province	Genetic distance		
	East Java	Madura	West Java
Central Java	0.139	0.144	0.040
East Java		0.258	0.145
Madura			0.162

value of 0.145. However, far distance separated by the ocean has no positive correlation with high genetic distance, as demonstrated by quite high genetic distance value of upland landraces (0.258) from Madura than that from East Java, the closer province compared to other provinces in Java. This result is in good agreement with previous studies that the benefit of SSR markers having high allelic polymorphism and abundant in the genome, allowed to be used for genetic diversity evaluation of diverse rice germplasm (Thomson *et al*. 2007; Pusadee *et al*. 2009; Lin *et al*. 2012).

The genetic diversity maintained in a plant species is probably considered as a function of its ecological and evolutionary history (Hamrick and Godt 1996). In Java, many mountains and plateaus are far apart and several isolated areas could be suitable for paddy. In the north coastal area is usually hotter, while the southern coastal area is generally cooler. Rainfall in West Java is higher than that of East Java, consequently, the mountainous areas receive higher rainfall. Considering the geography and environment of Java, thus, the genetic diversity among upland rice landraces in Java region could be attributable to the accumulated effect of eco-geographical conditions, various agro-ecosystems associated with rice farming practices and human cultural preferences (Choudhury *et al*. 2013). Ultimately, exploiting the genetic diversity of upland rice is needed to assist breeders in parental selection for developing the high yielding variety and to tag gene for desired characters. SSR markers have been applied in molecular analysis to characterize plant genetic resources in order to support gene bank management and administration (Yang *et al*. 1994; Brondani *et al*. 2006). Therefore, in the present study the SSR markers were chosen to assess the genetic variation of upland rice landraces

originating from Java Island. Estimation of genetic variation in crop germplasm collection is useful for their effective conservation, management and for crop improvement (Lu *et al.* 2005; Mondini *et al.* 2009).

The genetic diversity index of 0.55 for total population obtained in the present study (Table 2) indicated their moderate genetic diversity, referring to a previous study (Bangi and Aquino 2014) which is consistent with the PIC value of total population. In comparison to previous studies, the genetic diversity of total upland rice populations from Java is lower than that of Assam, India (Rathi *et al.* 2014), but still comparable with the collection in Arakan Valley, Philippine (Bangi and Aquino 2014). In our study, the genetic diversity index and genetic distance values indicated diverse upland rice landraces which are distributed in Java Island (East, Central and West Java).

To support this molecular characterization, proper morpho-agronomical data in further study are necessary to identify various useful traits of the upland rice land races. These molecular marker data could be utilized in a breeding program for effective utilization of diverse upland glutinous and non-glutinous rice germplasm of Java Island. To sum up, sufficient polymorphism revealed by these SSR markers among the landraces in our study would enable their proper characterization. Given the importance of upland rice germplasm for crop improvement, the essential regulation of farmer's practices in maintaining the variation within upland rice landraces, *in situ* conservation is an essential strategy for future rice crop breeding efforts. The results of the genetic diversity will be useful for the selection of the parents for developing rice breeding variety not only in Java but also in Indonesia.

CONCLUSION

Sufficient polymorphism revealed by SSR markers among 82 rice accession, including both glutinous and non-glutinous upland rice accessions in this study indicated the power of SSR markers to differentiate closely genetic relatedness among the upland rice accessions from Java. Genetic diversity index of non-glutinous rice and glutinous rice were 0.54 and 0.53, respectively. Their genetic distance was about 0.057. Upland rice from West Java was closer to that of Central Java (0.04) than that of West Java and East Java (0.145) and between upland rice from East Java and Central Java (0.139).

A moderate genetic diversity was found in upland rice landraces in Java and was comparable to genetic diversity in other upland rice population in various parts of the world. The information derived from this study is important, in combination with phenotypic data, to identify desired useful traits came from different origins of the gene pool to be used for breeding purposes. The genetic diversity among upland rice landraces in Java region could be attributable to the accumulated effect of eco-geographical conditions, various agro-ecosystems associated with rice farming practices and human cultural preferences.

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