GENETIC DIVERSITY AND TRAIT ASSOCIATION ANALYSIS OF INDONESIAN RICE (*Oryza sativa* L.) GERMPLASM USING SSR MARKERS

FATIMAH*, LINA HERLINA and TIUR SUDIATY SILITONGA

*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development,
Bogor 16111, Indonesia*

ABSTRACT

Generating a new crop variety with certain desirable traits requires germplasm collection with wide genetic diversity to facilitate accelerated development of improved cultivars. This study was conducted to characterize genetic diversity of Indonesian rice accessions using SSR markers and their agro-morphological characters to allow the identification of Marker Trait Association (MTA). A survey of genetic diversity among 72 traditional landraces of 15 provinces in Indonesia and 24 improved varieties was conducted using four multiplex panels of 14 fluorescent-labeled SSR markers and 22 agro-morphological characters. A total of 140 alleles with an average of 10 alleles per locus and Polymorphism Information Content (PIC) values at 0.73. The population structure identified five subgroups. A total of 4 highly significant MTA were identified. One character with highly significant MTA was number of panicle associated with RM277. Based on SSR marker, RM105 had two MTAs for auricle color and ligule shape. After validation, these MTAs may be deployed to improve rice productivity.

Keywords: Genetic diversity, Indonesian rice, Marker-Trait Association, SSR

INTRODUCTION

Through various exploration missions, more than 4,000 rice germplasms consisted of landraces, elite lines, high yielding varieties, introduced variety/lines as well as 88 accessions of wild rice species are kept in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD). They have very important role as genetic resources. In breeding program to create high yielding varieties of food crops, it is important to carry out characterization and evaluation of germplasms to increase utilization of genetic resources (Silitonga *et al.* 2001, Silitonga 2010).

Agro-morphological characterization of germplasm accessions is fundamental in order to provide information for plant breeding programs (Lin 1991, Nascimento *et al.* 2011). Systematic study and characterization of collected germplasms are not only important for utilizing

the appropriate attribute based on donors, but also essential in the present era for protecting the unique rice. Thus, there is a need to collect, exploit and evaluate the untapped germplasm (Parikh *et al.* 2012). Identification of cultivars with distinct morphological traits facilitates high resolution QTL mapping and validates candidate genes responsible for quantitative agronomical characters (Li *et al.* 2010).

The advantage of using fluorescent-labeled microsatellite markers for genetic diversity analysis in rice with multiple dyes allows efficient genotyping using multiplex panels of markers (Blair *et al.* 2002, Coburn *et al.* 2002, Jain *et al.* 2004, Thomson *et al.* 2007). The use of capillary electrophoresis provides additional benefits, including greater automation, ease of use and precision in fragment sizing, which makes it the preferred technology for Simple Sequence Repeat (SSR) genotyping in rice (Lu *et al.* 2004, Garris *et al.* 2005, Thomson *et al.* 2007). Previous study carried out on genetic diversity and population structure of Indonesian germplasm characterized 330 rice

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* Corresponding author: fatimahsuw@gmail.com

accessions, including 246 geographically-diverse Indonesian landraces and 63 Indonesian improved cultivars, using capillary electrophoresis with 30 fluorescent-labeled microsatellite markers (Thomson *et al.* 2007).

Several researchers reported the use of agro-morphological markers combined with SSR markers in the characterization and study of rice (*Oryza sativa* L.) germplasm diversity. Using 42 agro-morphological characters associated with 39 SSR markers, Bajracharya *et al.* (2006) examined genetic relationships among 147 high altitude rice landraces from Nepal. Agrama *et al.* (2007) identified SSR markers associated with agronomically important phenotypic traits using 92 rice germplasm accessions evaluated for yield and kernel characteristics; when being genotyped with 123 SSR markers it was shown that many of the associated markers were located in regions where QTL had previously been identified. Seetharam *et al.* (2009) characterized 30 rice genotypes comprising landraces, pure lines, somaclones, breeding lines and varieties specifically adapted to costal saline environments using SSR markers and morphological characters.

The objectives of the present study were to characterize genetic variation of 96 rice accessions using SSR markers and agro-morphological characters allowing identification of marker trait association and to provide useful information to facilitate rice plant breeding programs.

MATERIALS AND METHODS

Seeds of 72 Indonesian rice landrace accessions originated from 15 provinces were obtained from Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD). Twenty-four cultivated varieties were added from Indonesian Center for Rice Research, Agency for Agricultural Research and Development (IAARD), Indonesia Ministry of Agriculture. The control variety used in this study was Inpari 1 for *indica*. Inpari 1 was improved variety originated from IR64 and IRBB7 which developed for resistance to leaf blight disease. It was developed by conventional breeding method, while rice variety code was developed using marker assisted selection (Bustamam *et al.* 2002). The control variety for *tropical japonica* rice was Kalimutu. It was

rice variety introduced from Kenya having drought resistance characteristic (Silitonga 2010). These rice paddy varieties were first sown in five plastic boxes which contains 20 rice varieties each. Fourteen days later seedlings were transplanted into a set of boxes containing natural paddy soil. Rice plants were grown under greenhouse maintenance.

Molecular Analysis

DNA was extracted using Dellaporta (1983) method. Individual Polymerase Chain Reaction was performed in 20.0 μL of a mixture containing 5 μL (10 ng/μL) DNA template of each sample, 1.2 μL (5 M) CEQ-compatible fluorescent-labeled primer pairs, 2 μL of dNTP mix (2.5 mM), 2 μL 10X PCR buffer and 0.2 μL Roche FastStart Taq DNA Polymerase kit 5 units/μL. The PCR machine of DNA Engine Tetrad2 Peltier Thermal Cycler MJ Research was run in program following touchdown procedure which consisted of 4 minutes initial denaturation step at 95 °C, 13 cycles touchdown at -0.5°C increments, 45 seconds at 95 °C, 45 seconds at 61.5 °C decreased by 0.5 °C every cycle, 30 seconds at 72 °C, followed by 27 cycles at 45 seconds at 95 °C, 45 seconds at 55 °C, 30 seconds at 72 °C and a final extention step for 5 minutes at 72 °C. The automatic capillary electrophoresis was used to separate and visualize the PCR products following procedures recommended by the manufacturer (Beckman 2004).

Data Analysis

The SSR fragment analysis was based on the allele sizes generated and implemented in PowerMarker V3.25 (Liu & Muse 2005) that calculated the allele frequency, gene diversity and Polymorphism Information Content (PIC). The population structure of the rice accessions was calculated using Structure v3.2 (Pritchard *et al.* 2000). The posterior probabilities were estimated assuming prior values of k between 1 and 8 using the Markov Chain Monte Carlo method (MCMC). The results of this study were based on burn-in period length of 10,000; MCMC iteration after burn-in of 100,000; with twenty replications. The degree of admixture, alpha, was inferred from the data. When alpha is close to zero, most individuals are essentially from one population or another; whereas when alpha >1, this means that

most individuals are admixed (Falush *et al*. 2003). The k value was determined by LnP(D) in STRUCTURE output and delta k based on the rate of change in LnP(D) between successive k (Evanno *et al*. 2005). Both LnP(D) in STRUCTURE output and its derived delta k were used to determine the k value. To determine the putative association between agro-morphological characterization and the SSR markers it was run on Mixed Linear Model (MLM) method in TASSEL (Bradbury *et al*. 2007). The p value calculated by TASSEL determined whether a QTL was associated with the marker. Of the several methods suggested to correct false positive in association analysis, even keeping stringent p value benchmark, the most stringent correction method called "Bonferroni Correction" was used in the present study. The threshold was found to be 7.1 x 10^{-4} at significance level of 1% after Bonferroni multiple test correction (0.01/14).

Agro-Morphological Characteristic

The accessions were characterized according to descriptors established by Silitonga *et al*. (2003). A total of 13 qualitative and 9 quantitative descriptors were used. The qualitative descriptors were: auricle color, ligule colour, ligule shape, leaf type, flag leaf attitude, collar color, culm color, culm habit, panicle exertion, panicle type, apiculus color, lemma and palea color and thresh ability. The quantitative descriptors were: plant height, total number of tiller, number of effective tiller,

panicle length, number of panicle, number of fertile grain, number of sterile grain, grain percentage of sterility and 1,000 grain weight. Descriptive analysis was used to calculate the quantitative descriptors. Genetic variability of observed characters was calculated using Anderson and Bancroft (1952) formula. Genetic variability is broad when $\sigma^2_G \geq 2(\sigma_G)$ and narrow when $\sigma^2_G < 2(\sigma_G)$. The qualitative descriptors analyzed were determined by Principal Coordinate Analysis (PCoA) generated from NTSYSpc 2.11p to create a scatter plot showing genetic relationships among accessions.

RESULTS AND DISCUSSION

SSR Fragment Analysis

A total of 140 alleles, with an average of 10 alleles per locus were detected at the 14 SSR loci. The number of alleles per locus ranged from 4 i.e. RM484 and RM124 to as many as 17 i.e. RM474. RM484 presented the smallest allele size range (4 bp), while RM250 had the largest allele size range (74 bp). Polymorphism Information Content (PIC) values were high for most of the microsatellites, averaging 0.73 and ranging from a low of 0.54 i.e. RM484 to a high of 0.89 i.e. RM541. RM250 presented the highest frequency allele up to 62%. RM541 and RM144 showed the lowest frequency alleles, 17% and 19%, respectively (Table 1).

Table 1 List of Indonesian rice landraces and improved varieties used in this study

No	Reg.	Accession	Source	No.	Reg.	Accession	Source
1	3303	Slamet	Lampung	49	20628	Kalimutu	Introduction from Kenya
2	3981	Loneng	West Kalimantan	50	20743	Mama Wangi Kero	East Nusa Tenggara
3	3983	Reli	West Kalimantan	51	20747	Madha Kedhi	East Nusa Tenggara
4	4355	Ase Djambe	South Sulawesi	52	21377	Padai Pulut Melayang	East Kalimantan
5	4363	Ase Bolong A	South Sulawesi	53	20773	Nalu Lepu	East Nusa Tenggara
6	4365	Ase Puteh	South Sulawesi	54	20798	Dinu Toru	East Nusa Tenggara
7	5575	Bulan Sabit	East Nusa Tenggara	55	20831	Toliwang IV	Maluku
8	5634	Gedangan	East Java	56	20919	Cirata	Improved variety
9	5652	Pelangan	Central Java	57	21313	Batutegi	Improved variety
10	19999	Padai Pulut Ayang	East Kalimantan	58	21002	Sibuyung Pendek	East Kalimantan
11	5665	Gotera	Central Java	59	21003	Padi Merah	East Kalimantan
12	5720	Tholo	Central Java	60	21021	Pulut olan	East Kalimantan

Table 1 Continued

No	Reg.	Accession	Source	No.	Reg.	Accession	Source
13	6293	Si Hadap	West Sumatera	61	21415	Padai Oko Bat	East Kalimantan
14	6768	Serendeh	Lampung	62	21050	Pait	East Kalimantan
15	6790	Palembang Kuning	Lampung	63	19871	Padi Ketumbar	West Kalimantan
16	7885	Umbang Bilis	Central Kalimantan	64	21057	Bawi	East Kalimantan
17	8000	Serlak	South Sumatera	65	19936	Gropak	Central Java
18	8052	Kemandi Pance	South Sulawesi	66	21075	Ketan Mayang	East Kalimantan
19	8065	Padi Kuda	Jambi	67	21087	P. Ajatn	East Kalimantan
20	8081	Si Bujang Putih	South Sumatera	68	21157	G.02	Improved variety
21	8116	Gando	South Sumatera	69	21357	Padai Mahak	East Kalimantan
22	8125	Padi Agai Pandak	South Sumatera	70	21191	Pemangkat	West Kalimantan
23	8126	Padi Amir	South Sumatera	71	21152	Sintanur	Improved variety
24	21266	Buntut Kasar	West Kalimantan	72	21225	Padi Dewi	West Kalimantan
25	8192	Si Cantik	NAD	73	21228	Tayan	West Kalimantan
26	8656	Padi Aceh	Riau	74	21230	Segeret	West Kalimantan
27	15153	Pare Lambem	East Nusa Tenggara	75	21328	Konawe	Improved variety
28	15200	Padi Jerming	West Nusa Tenggara	76	21260	Bilak	West Kalimantan
29	19066	B57C-Md-10-2	Improved variety	77	21261	Ketupat	West Kalimantan
30	19193	BPI-76	Improved variety	78	21262	Pance	West Kalimantan
31	19670	Singkarak	Improved variety	79	21265	Ringkak Siman	West Kalimantan
32	19907c	Padi Lima Bulan	West Kalimantan	80	21267	Ringka Kasir	West Kalimantan
33	20306	Meulaboh	NAD	81	21268	Ketumbar	West Kalimantan
34	20377	Misik A	Central Kalimantan	82	21325	IR Kebo	West Java
35	20387	Unggul Sawah	Central Kalimantan	83	21338	Silugonggo	Improved variety
36	20389	Jaka	Central Kalimantan	84		INPARI-1	Improved variety
37	20390	Gading Garu	Central Kalimantan	85		INPARI-2	Improved variety
38	20394	Kumpang Emas	Central Kalimantan	86		INPARI-3	Improved variety
39	20410	Talun Bajang	Central Kalimantan	87		INPARI-4	Improved variety
40	20413	K.Sampi Hiling	Central Kalimantan	88		INPARI-8	Improved variety
41	20442	Talun Bura	Central Kalimantan	89		INPARI-9	Improved variety
42	20445	Pontianak C	Central Kalimantan	90		INPARI-10	Improved variety
43	20448	Satum	Central Kalimantan	91		INPARI-11	Improved variety
44	21272	Ringkak Sa'dan	West Kalimantan	92		INPARI-12	Improved variety
45	20523	Umbang Pulai	Central Kalimantan	93		INPARI-13	Improved variety
46	20528	Pulut Silu	Central Kalimantan	94		INPAGO-4	Improved variety
47	20556	Sebaung	Central Kalimantan	95		INPAGO-5	Improved variety
48	20627	Jatiluhur	Improved variety	96		INPAGO-6	Improved variety

SSR markers generate multi allelic data revealing genetic structure of a natural population consisting of diverse genotypes (Remington *et al.* 2001). This study, using 96 Indonesian rice accessions, revealed a total of 140 alleles detected from 14 SSR loci. This finding was lower compared to the study results obtained by Thomson *et al.* (2007) who detected 394 alleles at 30 SSR loci across 330 rice accessions. However, abundant genetic variation in this study was identified with an overall gene diversity of 0.75 and average of 10 alleles per locus (Tabel 2) compared to an overall gene diversity of 0.68 and average of 11.9 alleles per locus (Thomson *et al.* 2007).

Polymorphism Information Content (PIC) values, a reflection of allele diversity and frequency among the varieties, averaged 0.73 and ranged from a low of 0.54 to a high of 0.89 compared to results obtained from the study carried out by Thomson et al. (2007). The PIC values ranged from 0.34 to 0.88 (RM154 and RM474), with an average of 0.66. Different results of this study and the study conducted by Thomson *et al.* (2007) might be caused by different

sampling strategies, due to different provinces, different numbers of provinces and different numbers of SSR markers used by the two studies).

Population Structure

Fourteen SSR markers were distributed on the rice genome to assess genetic architecture and population structure. It was observed that delta-K declined after $k = 5$ was continuously significant, suggesting the presence of five subgroups (Fig. 1).

At $k = 5$, most accessions were classified into five groups, which corresponded to *indica*-1 with 20 consisted of improved varieties, traditional rice sub-species *japonica* with 13 accessions, *indica*-2 with 21 germplasm accessions, *indica*-3 with 24 germplasm accessions and *indica*-4 with 18 germplasm accessions and no admixture population was found. The degree of admixture (alpha) generated in this study was close to zero (0.04) which means most individuals are essentially from one population.

Analysis of population structure identified the highest log likelihood and delta *k* with the number

Table 2   Data summary for 14 fluorescent-labeled microsatellite markers across 96 rice accessions

Panel	Dye[a]	Marker	Chr	Motif[b]	No.of Allele	Range	Gene diversity	Major Allele[c] Size (bp)	Major Allele[c] Frequency (%)	PIC[d]
1	D3	RM484	10	(AT)9	4	292-296	0.59	294	58	0.54
1	D4	RM105	9	(CCT)6	7	100-137	0.79	133	30	0.76
1	D4	RM536	11	(CT)16	10	218-259	0.60	232	60	0.58
2	D3	RM124	4	(TC)10	4	266-272	0.63	268	51	0.56
2	D4	RM162	6	(AC)20	12	204-250	0.84	210	31	0.83
2	D4	RM277	12	(GA)11	8	112-137	0.72	121	45	0.68
3	D3	RM287	11	(GA)21	11	93-157	0.83	113	26	0.80
3	D3	RM259	1	(CT)17	14	119-179	0.79	154	34	0.77
3	D4	RM250	2	(CT)17	10	119-193	0.59	171	62	0.57
3	D4	RM514	3	(AC)12	12	240-274	0.80	249	29	0.77
4	D3	RM541	6	(TC)16	14	131-198	0.90	182	17	0.89
4	D3	RM144	11	(ATT)11	11	220-286	0.88	220	19	0.87
4	D4	RM474	10	(AT)13	17	228-299	0.88	229	25	0.87
4	D4	RM171	10	(GATG)5	6	321-345	0.70	341	48	0.67

[a]Fluorescent dyes for CEQ Beckman Genetic Analyzer: D2 = Black, D3 = Green, and D4 = Blue
[b]Motif of the SSR and number of repeats as previously published (http://www.gramene.org)
[c]Major allele is defined as the allele with the highest frequency
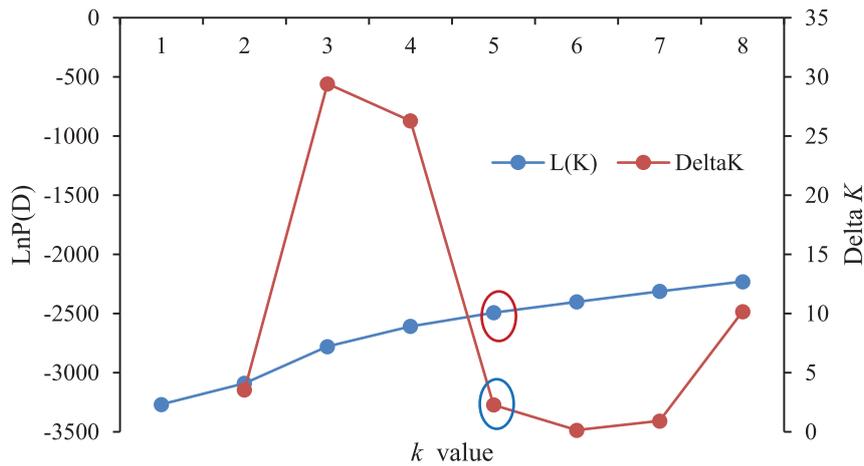[d]PIC = Polymorphism Information Content

Figure 1 The presence of $k = 5$ based on posterior probability, L(K) and delta K where k ranged from 1 - 8
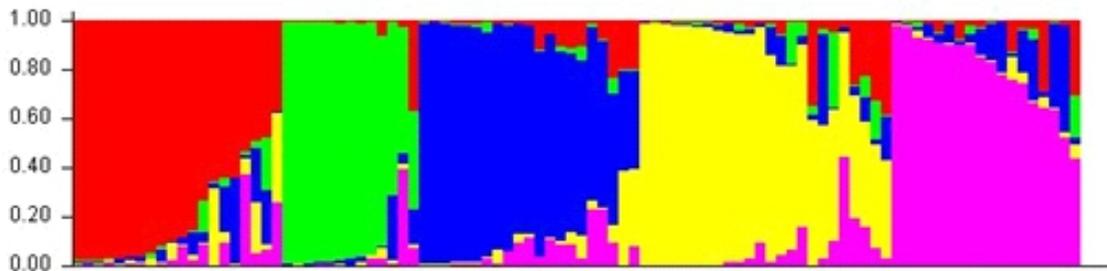

Figure 2 Bar plot representing population structure of five subgroups of Indonesian rice in this study. Red=*Indica*-1 (Improved varieties), Green= *Japonica*, Blue=*Indica*-2 (germplasm), Yellow=*Indica*-3 (germplasm), and Pink=*Indica*-4 (germplasm)

of populations set at five ($k = 5$). These five populations corresponded to one *tropical japonica* population (13.5%) and four *indica* populations and no admixture population was identified. These four subgroups corresponded to Indonesian improved varieties in subgroup 1 (20.8%), rice landraces in subgroup 3 (21.8%) and subgroup 4 (25%) and subgroup 5 (18.7%) (Fig. 2).

These results were quite different from previously analyzed population structure at $k = 2$ of 330 Indonesian rice varieties with 30 microsatellite markers that divided Indonesian germplasms into *indica* and *tropical japonica* (Thomson *et al*. 2007). The $k = 2$ setting forced all accessions into two populations, causing the more distant related groups, such as the *O. rufipogon* and *aus* accessions, to appear as admixtures between *indica* and *japonica*. Khush *et al*. (2003) analyzed 3,670 Indonesian varieties with 11 isozymes and found out that 69% of the sampled Indonesian germplasms as *indica*, 28% as *japonica* and 3% as intermediates. However, it was difficult to make

any distinctions between landraces and improved varieties.

Agro-Morphological Characteristic

PCoA analysis classified the 13 qualitative agro-morphological characteristics into three groups, which corresponded to the traditional rice subspecies *tropical japonica* with 19 accessions, *indica*-1 with 23 germplasm accessions and *indica*-2 with 54 improved varieties and germplasm accessions (Fig. 3).

This result was different from the SSR analysis which classified the characteristics into five groups. The SSR results divided the third group in agro-morphological characteristic into three groups which separated the *indica* (group 1) from improved varieties and *indica* (group 3 and 5) from germplasm accessions. The descriptive analysis of 9 quantitative descriptors of 96 Indonesian rice accessions is presented in Table 3. The broad variability of quantitative morphological character was found in all 9 quantitative descriptors. Genetic variability is considered
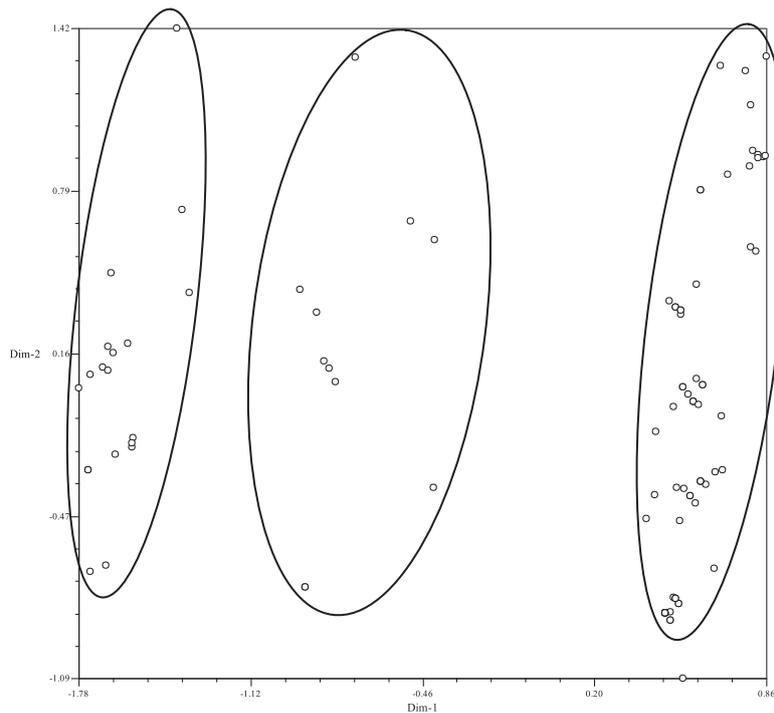
Figure 3   The PCoA analysis based on 13 qualitative agro-morphological characteristics classified into three groups, which corresponded to the traditional rice sub-species *japonica* (19 accessions), *indica*-1 (23 accessions) consisted of germplasm accessions and *indica*-2 (54 accessions) consisted of improved varieties and germplasm accessions

broad when $\sigma^2_G \geq 2(\sigma_G)$ and narrow when $\sigma^2_G < 2(\sigma_G)$ (Anderson & Brancoft 1952).

Based on the culm habit there were 5 types of characteristics however we found 3 types in these rice germplasm i.e.: erect (15°), semi erect/intermediate (20°), and open (40°). In this study, the 96 accessions were divided into 3 characteristics i.e. erect (78%), semi-erect (21%) and open (1%). From these, the cultivated rice varieties were divided into 2 characteristics i.e.

erect (83%) and semi-erect (17%) while Indonesian rice landraces were divided into 3 characteristics i.e. erect (76.4%), semi-erect (22.2%) and open (1.4%). Similar with Rabara *et al.* (2014) mentioned that most of the traditional rice varieties in Philippines had thick culms and an erect culm habit. Regarding for improving yield, Wu *et al.* (2011) shown that large culm rice varieties have a higher number of grains per panicle and a longer spike length. Gealy *et al.*

Table 3 Descriptive analysis of quantitative descriptors of 96 Indonesian rice

Character	Min	Max	Range	Means	SD	SE	Variance	Skewness	Kurtosis
PH	84.7	195.3	110.6	129.87	28.46	2.90	809.80	0.00	-0.99
TT	4	16	12	7.95	2.49	0.25	6.20	1.33	1.72
NET	4	13	9	6.82	2.08	0.21	4.32	1.10	0.99
PL	14.8	35.7	20.9	23.33	3.89	0.40	15.17	0.44	0.75
NP	2	23	21	5.46	2.50	0.26	6.26	3.82	24.69
NGF	1.3	482	480.7	100.36	67.86	6.96	4,604.61	2.17	9.46
NSG	4.7	258	253.3	52.32	39.47	4.05	1,557.70	2.15	7.40
PcS	6	97.3	91.3	34.86	17.50	1.80	306.09	0.90	1.02
ToGW	12	37.9	25.9	21.00	4.31	0.44	18.60	0.64	1.93

Notes: PH=Plant Height, TT= Total Number of Tiller, NET=Number of Effective Tiller, PL=Panicle Length, NP=, Number of Panicle, NGF= Number of Fertile Grain, NSG= Number of Sterile Grain, PcS= Percentage of Sterility, ToGW=Total 1,000 Grain Weight

(2006) and Hussain *et al.* (2010) reported that the presence of erect plant types could be due to back-crossing between accessions or with commercial rice cultivar.

The culm color was characterized as light gold (92%) and green (8%), while the cultivated accessions were characterized as light gold. The collar color from the 96 accessions was characterized as light green (95%) and purple (5% i.e. Bulan Sabit, Gedangan, Loneng, Padi Kuda, and Pulut Silu). The cultivated accessions were characterized as light green. The ligule color from the 96 accessions was characterized as absent (93%), purple lines (5%) and purple (2%), while the cultivated accessions were characterized as absent. The auricle color from the 96 accessions were characterized as absent (83%), purple lines (16%) and purple (1% i.e. Gedangan and Toliwang IV), while the cultivated accessions were characterized as absent.

The flag leaf attitude from the 96 accessions were characterized as 28% erect, 49% semi erect, 3% horizontal and 20% descending, in which the cultivated accessions were in erect category. Dewi *et al.* (2009) mentioned that the erect flag leaf was more desirable than the horizontal one due to the higher sunlight intercept and might have a positive correlation with the high yield. Narayanan *et al.* (2007) reported that flag leaf as the major source of phloem-delivered photo assimilates during the grain-filling stage in rice and Khalifa *et al.* (2008) showed that cutting of flag leaves result to 45% grain yield loss.

There were 5 panicle types (attitude) i.e. erect (compact panicle), semi-erect (semi-compact panicle), spread (open panicle), horizontal and drooping. The 96 accessions category were 93% semi-erect and 7% spreading, in which the cultivated accessions were in semi-erect category. The means for panicle number per plant was 5.4 from 96 accessions (Table 3). For the cultivated varieties, the mean panicle number was 5.1, in which Inpari 11 had the highest panicle number (23). The mean panicle number for Indonesian rice landraces was 5.6, in which Padai Agak Pandak had the highest panicle number (11). The mean for panicle length was 23.3 cm from 96 accessions (Table 3). For the cultivated varieties, the mean panicle length was 21, in which Inpago 4 had the longest panicle length (34.7 cm). The mean panicle length for Indonesian rice landraces was 24.1, in which Ketan Mayang had the longest panicle length (35.7 cm). Peng *et al.* (2014) reported that panicle structure is one of the most important factors for rice yield and panicle branches and their number affects spikelet number. Dense panicle structure has been a major target for improvement of rice grain yield because of its relationship with grain number.

The recorded mean for plant height in 96 accessions was 179.9 cm, with a minimum height of 84.7 cm for Inpari 3, 85.7 cm for Madha Kedhi and a maximum of 195.3 cm for Bulan Sabit accession (Table 3). From the 96 accessions 31.3% was dwarf (80-110 cm), 17.7% was semi-dwarf (110-130 cm) and 51% was tall (130-200 cm) (Fig. 4). For the cultivated varieties, the mean height was 102 cm, which the distribution were dwarf (31.3%), semi-dwarf (17.7%) and tall (51%). The differences in the heights of the accessions were possibly due to various internodal lengths. The varieties having longer internodes produced
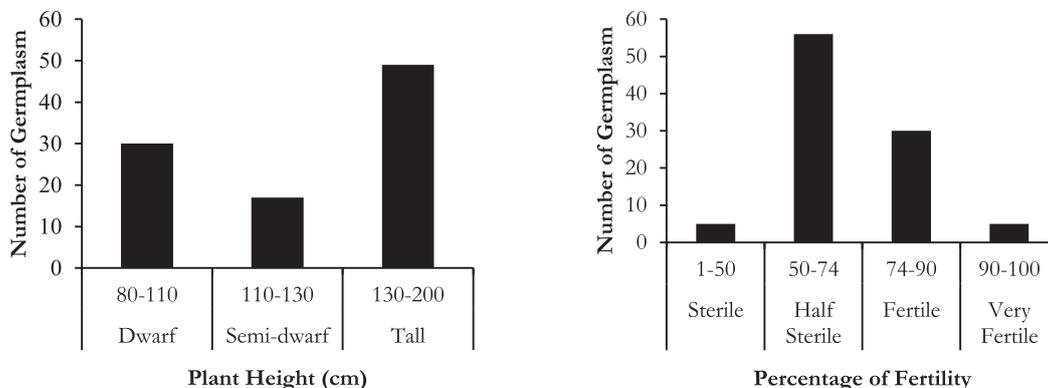

Figure 4 The agro-morphological characteristics based on plant height (left) and percentage of fertility (right) on 96 accessions

Table 4 Marker-trait associations (MTAs) identified for select agronomic traits

No	Trait	Marker	F Value	P Value
1	Auricle color	RM105	4.4963	8.89E^{-05}
2	Collar color	RM287	6.3796	1.09E^{-06}
3	Ligule shape	RM105	4.8616	3.70E^{-05}
		RM536	5.0657	1.61E^{-05}
		RM514	5.3648	3.91E^{-06}
4	Number of panicle	RM277	13.779	7.50E^{-10}

taller plants (Ashrafuzzaman *et al.* 2009; Hussain *et al.* 2014) and Mohammad *et al.* (2002) reported that plant height depends on genetic makeup of a plant and environmental conditions.

There were 4 types of fertility based on the percentage of fertility of the 96 accessions i.e. sterile (5.2%), half sterile (58. 3%), fertile (31.3%) and very fertile (5. 2%) (Fig. 4). The cultivated rice varieties were categorized as sterile (0%), half sterile (75%), fertile (20.8%) and very fertile (4.2% i.e. Sintanur). Indonesian rice landraces were categorized as sterile (6.9%), half sterile (47.2%), fertile (40.4%) and very fertile (5.5% i.e. Gando, Padi Dewi, Talun Bajang and Ringkak Siman). Kwon *et al.* (2015) found that a deficiency of SLR1 activity causes different spikelet fertility phenotypes in two different cultivars, Koshihikari and H143 which H143 exhibits partial sterility of spikelets while Koshihikari has normal fertility. Liu *et al.* (2013) mentioned that spikelet fertility is a critical yield-determining trait that is influenced by genetic background and environmental factors including rain, wind, and temperature. Anther development and pollen viability particularly affect spikelet fertility.

Association Analysis

A total of 4 marker-trait associations were identified with 14 different SSR markers (Table 4).

Based on agro-morphological characters, one character with highly significant MTA was number of panicle associated with RM277 on chromosome 12. Liu *et al.* (2008) identified QTL for primary branch number in panicle (*qPBN-6*) on chromosome 6 in the interval of RM3 - RM162. Mu *et al.* (2005) and Liu *et al.* (2008) explained that the primary branch number per panicle was determined by the initial size of the reproductive apex. The floret number per primary

branch was determined by the cell division activity. Thicker panicle neck development and larger panicle came from the parallel development during the panicle initiation, or floret developing. Grain filling were enhanced by stronger transporting process of water and nutrients based on thicker panicle neck. Spikelet number was mostly determined during the panicle initiation, while the number and weight of filled grain were largely affected by the conductive tissue together with the source strength. Wickneswari *et al.* (2012) reported that RM259 and RM208 were used to identify QTL for grains per panicle as peak markers for *qGPP-1* and *qGPP-2*, respectively.

Among the MTAs obtained in this study, some were similar as previously reported, indicating that these MTAs were reliable. These reliable MTAs were repeatable in different studies with various segregation population. Other MTAs had been probed for the first time, indicating that more information on QTLs could be found using populations of different genetic backgrounds. This is important for an in-depth understanding of the genetic control of yield traits in rice and for future rice production using molecular marker–assisted selection in rice breeding (Iqbal *et al.* 2003).

CONCLUSIONS

High resolution DNA banding pattern of the CEQ capillary gel electrophoresis system measured genetic variation of 96 rice varieties using 14 SSR markers, through population structure analysis. Taken together, the SSR markers identified in this study could be used for Marker-Trait Association (MTA) analysis of agro-morphological characters. This study resulted to a

total of 140 alleles with an average of 10 alleles per locus and Polymorphism Information Content (PIC) value at 0.73. The population structure analysis identified five subgroups. A total of 4 highly significant MTA were identified. One character with highly significant MTA was number of panicle associated with RM277. Based on SSR marker, RM105 had two MTAs for auricle color and ligule shape. These MTAs may be deployed in improving rice productivity, after being validated.

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