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Research Article
Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) Markers for Genetic Characterization of North Sulawesi Local Rice Varieties, Super Win and Burungan

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Abstract

Background and Objective: The genetic character of North Sulawesi's local rice cultivated by local communities has not been extensively explored. Genetic character can be used as tool for rice varieties identification to complement the morphological characters. This study aimed at developing genetic character of North Sulawesi's local rice varieties Burungan and Super Win using SSR and SNP molecular markers. **Materials and Methods:** Two local North Sulawesi varieties of rice from Bolaang Mongondow Regency, namely Super Win (Tropical Japonica) and Burungan (Japonica), were used. Control varieties included in this research were IR64 (Indica), Nipponbare (Japonica), Super Win Aromatik (Tropical Japonica) and Leukat Hitam (Tropical Japonica). The significant markers associated with agro-morphological characters were used, such as flowering date, panicle length, flag leaf corner, leaf length, panicle branches and the number of productive tillers. The SSR and SNP 384-chip which are mapped in the entire rice genome were employed. **Results:** The study showed that Burungan has alleles' size range 108.7-148.8 with a mean size of alleles 118.97. The frequency of this allele is 33.33%. Alleles' size range of Super Win is 109.2-148.8 with a mean size of alleles 119.50. The frequency of this allele is 22.22%. This diversity variation of allele size cannot be used as a genetic identifier for both varieties. The genetic distance between Burungan and Super Win varieties was quite high (0.461). It was due to they came from different sub species, Burungan was from Japonica while Super Win was from Tropical Japonica. **Conclusion:** Burungan and Super Win varieties are not a local rice of North Sulawesi.

Key words: Burungan , NDUS, North sulawesi, Super Win, rice identity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Local cultivars are traditional crops that have adapted and developed in accordance with local agricultural practice[1]. They act as a source of genetic diversity for a number of important properties, including resistance to disease[2]. Two local varieties that are still widely grown by farmers in North Sulawesi, namely Burungan and Super Win, have been known to be tolerant of drought stress[3-5]. As part of local variety protection, the establishment of varieties' ID can help varieties characterization to meet the criteria of NDUS (novelty, distinctness, uniformity and stability), required by the plant variety protection rules[6].

During this time, differentiation between new and old varieties was conducted morphologically. However, varieties produced generally are characteristically similar to their ancestor, therefore, they are morphologically indistinguishable. Molecular markers can provide unique DNA profiles of protected varieties[7]. The breakthrough that is now being developed and employed are microsatellite based character (Simple Sequence Repeat/SSR) and single nucleotide polymorphisms (SNP)[8]. Development of these markers can be used widely in breeding programs in agriculture[9,10]. Utilization of SNP markers in the form of a chip can be applied in order to determine DNA fingerprinting germplasm[11] for genetic studies and molecular breeding in rice[12] and as a genetic identifier accession (variety identity)[11]. The making of varieties identity (ID) based on DNA fingerprinting profile can be applied to some cases such as duplication and missing/mislabeling of rice germplasm[13]. Moreover, this ID can also be needed in searching seed purity[14], during the maintenance of the collection, varieties seed mixing or duplication can accidentally occur. With their unique fingerprint of each variety, varieties forgery can be easily traced.

Rice crop germplasm characterization using molecular markers SSR can provide results more quickly, effectively and accurately compared with the characterization based on morphological characteristics[15]. Identification of the varieties is carried out in the framework of the registration and protection of plants to confirm the importance and essential derived varieties. For the purposes of registration, evidence is required that these varieties are completely new (novel). In the next step, the result of identification of improved varieties can also be used to gain information regarding the magnitude of genetic diversity between the improved varieties themselves in germplasm collection and compared them with other local varieties.

The aim of this research was to characterize fingerprint of local North Sulawesi varieties, Super Win and Burungan, using SSR markers associated with agro-morphological characters, such as flowering date (UB), panicle length (PM), flag leaf corner (SDB) and leaf length (PD), the number of productive tillers (JAP), panicle branches (CM) and also SNP markers.

MATERIALS AND METHODS

This study was carried out from March-September, 2016 at Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, Indonesia.

Genetic materials: Two local North Sulawesi varieties, Super Win (Tropical Japonica) and Burungan (Japonica), both are from Bolaang Mongondow Regency, were used in this research. Control varieties used were IR64 (Indica), Nipponbare (Japonica), Super Win Aromatik (Tropical Japonica) and Leukat Hitam (Tropical Japonica). Markers used in this study were SSR and SNP 384-chip which is mapped in the entire rice genome. The SSR markers used were flowering date (Rs1, Rs7), panicle length (Rs6, Rs12), panicle branches (Rs26), flag leaf corner (Rs21), the number of productive tillers (Rs24, Rs31) and leaf length (Rs32).

Morphological performance of Burungan and Super Win: Recorded morphological performance of Burungan and Super Win included plant height, the number of productive tillers, stem color and flowering date.

Preparation of total rice genome: Preparation of the total rice genome was performed using the following protocol recommended by Qiagen (Qiagen, West Sussex, UK). Approximately 20-50 mg fresh rice leaves were added into 2 mL microtube containing 2 pieces of stainless steel or tungsten carbide bead with a diameter of 3 mm and placed in tissue lyser adapter set 2×24. The leaves were crushed using Tissue Lyser II at the frequency of 25 Hz for 1 min and repeated at the opposite position. To the mixture, 500 µL of lysis buffer (Thermo Fisher Scientific, Waltham, Massachusetts, United States) containing RNase 0.25 mg mL^{-1} was added. The sample was centrifuged at 1500 rpm for 30 sec and incubated at 56°C for 30 min. Sample was recentrifuged at 6000 rpm for 20 min to separate DNA from other cell debris and contaminants. Purification of total genome DNA was conducted using Thermo Scientific™ KingFisher™ Flex Purification Systems. Five pieces of labelled deep well 96

plates microtitre were prepared. Into the first plate (sample plate), 400 µL supernatant, 30 µL King Fisher Magnetic Beads and 400 µL Binding Buffer were added. On the next plates sequentially, each was added with 600 µL wash buffer 1, 600 µL wash buffer 2, 600 µL ethanol 80% and 600 µL wash buffer 3. Into Thermo Scientific King Fisher Flex 96 KF Plate, 150 µL Elution Buffer was added. The purification process was executed by the program protocol KF_Plant DNA_Flex96 (King Fisher Plant DNA Kit, Thermo Fisher Scientific). Concentration and purity of isolated DNA were furthermore analyzed using spectrophotometer. The DNA concentration was set to 50 ng μL^{-1} for final concentration. The DNA purity ideal limit for molecular analysis has ratio A_{260}/A_{280} = 1, 8-2, 0[16].

SSR markers linked to targeted loci: Molecular markers used in this study were flanking markers to detect QTL loci which governs traits, including flowering date (Rs1, Rs7), panicle length (Rs6, Rs12), panicle branches (Rs26), flag leaf corner (Rs21), the number of productive tillers (Rs24, Rs31) and leaf length (Rs32). Sequences of primers for markers flanking targeted loci are presented in Table 1.

PCR analysis for SSR markers linked to targeted loci: The PCR process was conducted in 20 µL reaction containing buffer 1 µL, dNTPs 100 µM, primer set 0,5 µM, 50-100 ng DNA and 1 unit of Taq DNA polymerase. The PCR condition was set as follows 94°C for 5 min for initial denaturation, followed by 35 cycles comprising of denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec and polymerization at 72°C for 2 min. The final polymerization was conducted at 72°C for 7 min. The PCR products were electrophorized in 8% polyacrylamide gel for 2 h at 80 V. Coloration of DNA was done by soaking the gel in EtBr and documented using ChemiDoc™ MP imaging system (Bio-Rad Laboratories, Inc.).

Genotyping using 384-chip SNP set: The SNP markers are presented in Table 2. Genotyping analysis was conducted using the GoldenGate® Assay (Illumina, San Diego, California, United States) with the following procedure, (1) Pre-amplification and (2) Post-amplification. The pre-amplification process included DNA activation, precipitation, resuspension, ASE (Allele Specific Extension*),* MEL (Master mix Enzymatic Extension and Ligation), preparation of PCR plate and Inoc PCR. The DNA activation process of each sample was conducted by random biotinylation by adding MS1 reagent.

Precipitation was done by adding PS1 reagent and 2-Propanol, followed by resuspension process by adding RS1 reagent. The ASE process took place by the incoporation of biotinylated gDNA with query oligos using hybridization reagent and paramagnetic particles, all contained in ASE plate. The processed was followed by washing out non-specific hybridization and the excess of oligos by adding AM1 and UB1 reagents. The process of enzymatically extention and ligation was conducted by adding MEL master mix into each of DNA samples.

The preparation of PCR plate for DNA amplification was done by adding Titanium Taq DNA polymerase. Each sample then was moved into PCR plate. Extension and the ligation proces was followed by Inoc PCR using three universal primers in MMP reagent: Two fluorescent-labeled primers (Primer 1 and Primer 2) and 1 biotinylated primer (Primer 3). Primer 3 is used to mark PCR product and elute DNA strand containing fluorescent signal. Eluted samples then were transferred from ASE plate into PCR plate.

The post-amplification process included PCR, the binding of PCR, INT plate for BeadChip, hybridization, washing of BeadChip and visualization of BeadChip at Iscan system. The PCR process was done by amplification of the PCR plate with fluorescent label, followed by the binding of PCR with the addition of MPB reagent into PCR plate then the solution was transferred into filter plate. For the binding process, DNA strand was biotinylated with paramagnetic particle thus forming PCR product in the form of immobile double-stranded DNA. In the process of INT plate for BeadChip, the SS fluorescent-labeled PCR products from filter plate were washed with UB1, MH1 and NaOH 0.1 N then diluted inside intermediate (INT) plate.

Next, the BeadChips was hybridized using Hybridization Chamber overnight inside hybridization oven Illumina with with a gradual temperature change from 60-45°C. The BeadsChip then removed from Hybridization Chamber and washed three timws using laser to to excite the fluorine from the product of single-base extension in the beads of BeadChip. Ray emissions of fluorine is then recorded in the form of high-resolution images of the BeadChip parts. Data visualization is then analyzed to determine the genotype of SNP using Illumina's BeadStudio Gene Expression Module.

Statistical data analysis and association mapping: Analysis of phenotype data was conducted using two-way ANOVA test using XLSTAT (Microsoft Excel. Association mapping was conducted entirely in population or subpopulation using software TASSEL 3.0[17]. Based on this analysis, significant SNPs was obtained ($-\log^{10} p>4.0$) which scattered around particular

Table 1: Molecular markers SSR used in the selection of targeted loci

Nama primer	Sequence F	Sequence R	Chr	Coordinate	Gene	Note	SNP
Rs1	ACGTTGGATGCCGTGATGATGATGGTTGTG	ACGTTGGATGGCATATTATCTCCGGCTCTC	12	24.742.069-24.746.544	OS12T0591101	Flowering date	id12008894
Rs6	ACGTTGGATGCTGTGGTCGTAACTTTGTCG	ACGTTGGATGCCTGGAAAGAGGGTCAAATG	8	2.019.190-2.026.643	OS08G0135800	Panicle length	id8000575
Rs7	ACGTTGGATGAGTTGCAACTCGTTTGGCAG	ACGTTGGATGTCAGGAGCTTGAGATCAGAG	1	39.577.048-39.580.184	OS01G0908800	Flowering date	TBGI066976
Rs12	ACGTTGGATGAGCTAACTTTGCCAAACCAC	ACGTTGGATGGTCCCACGTGTCATTAATCC	12	7.665.115-7.669.540	OS12G0238900	Panicle length	id12003066
Rs21	ACGTTGGATGTTTGATAATCTCACGCTACC	ACGTTGGATGGAAGTAGTCGTCCACTGAAG	2	199.223-204.482	OS02G0104100	flag leaf corner	id2000096
Rs24	ACGTTGGATGCAGATCTTCTCTCTGTTGCC	ACGTTGGATGGATTTCGTGTGCGATGAGAG	1	29.447.990-29.450.454	OS01G0708700	the number of productive tillers	TBGI048073
Rs26	ACGTTGGATGTTCGGTTTCTGCACCTTTTC	ACGTTGGATGCTGTTGCTGTTGAATCCTAC	1	14.809.676-14.821.941	OS01G0363900	panicle branches	id1009867
Rs31	ACGTTGGATGAAAACAGCATGGCATCATGG	ACGTTGGATGCAATCTCATGGGTCACGAAC	1	18.259-531-18.267.127	OS01G0516200	the number of productive tillers	id1010973
Rs32	ACGTTGGATGTCTCCATCTGGCATCATAAC	ACGTTGGATGGGTGCTTCATCAACTTCTGC	2	33.476.467-33.483.947	OS02G0787800	Leaf length	TBGI118590

Table 2: Molecular markers SNP used in the selection of targeted loci

ID SNP	ID SNAP	Sequence F	Sequence R	Chromosome	Coordinate	Note
TBGI066976	UB	CAGAGATCAATCAGCACCGGTCCG	CCATCCTCACATCCTTTC CCTTCAGAA	1	39906335	Flowering date
TBGI000738	JAT 1	ATTTAATAGCTTAAAATTCAGGAGGGATGTTACTT	CATATTCATAAAAGAGGAATATATCATCAGATTGCA	1	423329	Total number of tillers
TBGI033096	TT 1	CCTCAGACAAGAATGGCA TGTCAAATGTATATCTA	CGATGGTAACACAATAAAGCAGCATTTTGAA	1	19845250	Plant heigth
TBGI262133	JAT 2	CAAGGGATTGTCAGTGGCTTGGGTATT	CTCGAGTCGATCTTCGATTCACGGTC	5	27125397	Total number of tillers
TBGI264243	TT 2	CCGTCCCTGCAAGCGCCGTA	CCTTATTCCGTTATTGGGCCTCACAAAA	5	28042163	Plant heigth
TBGI275318	TT 3	CCATCAAGGCCGTACCTGGAGGAT	CAAAATGCTATAGTAGAGCCTCATTGACCATATTTT	6	4862717	Plant heigth
TBGI315398	JAT 3	TGGACTGGACTTGACTGCCACCGT	ATATGCTTTTGTGGTTTTGTACTTTCACTGATGC	7	2453894	Total number of tillers
TBGI321694	JAT 4	GCTTGATCAAGGCGTGTGCCAGA	ACCCAGGGGTTGCCCAGGAAC	7	11843395	Total number of tillers
TBGI336735	B1000	GTAGTACGCGGGCACGCCACA	ATATTCCTTTTGCAGTGTGTATATATATGGATGGCC	7	29048897	1000 grains weight
TBGI336564	Fertilitas	CCGAGACTTGACTACAAGTGAGGAAACACC	GAAGCAATGCAACTAAATTGATGCACAAGAATA	7	29048897	Fertility
TBGI111997	JAT 5	GGGAAGATGAGCGGTCCAAGGTTT	GCATTCTTCTGCTCATAGCCAGCAAGTC	2	28687616	Total number of tillers
TBGI367888	Bobot Tajuk	ACATATTGTCCCATGAGTTTTTAGGTCTAGCATAC	ACAAAACCATGGTATTTCTTCACCGTTTGTAATT	9	2397468	Heading weights

LD blocks as a basis of association mapping. Data score was defined as 1 (present) and 0 (absent) for each SSR band in each locus. Genetic variability was measured by Polymorphism Information Content (PIC) value according to Anderson *et al.*[18] Pij is the frequency of jth allele at the ith locus and n is the number of allele in particular locus. The formula is as follow:

$$PICi = 1 - \sum_{j=1}^{n} Pij^2$$

Phylogenetic analysis was calculated following Nei[19] method for the distance and UPGMA (Unweighted Pair Group Mean Arithmatic) for the dendrogram development. The PIC analysis and phylogenetic analysis were done using Power marker version 3.25[20]. Principal Coordinate Analysis (PCoA) is used to visualize proximity matrices, available in Excel using the XLSTAT add-on statistical software.

RESULTS

Morphological performance: Morphological performances of Burungan and Super Win varieties as well as their counterparts are presented in Table 3 and Fig. 1-3. Burungan is a subspecies of Japonica. Its grain is oval and lemma has a tail or feather. Plants have a high posture but easily fall and long-lived. The panicles have medium length (20-30 cm) and are dense. Super Win is one of the Tropical Japonica subspecies. It means that its character is between Indica and

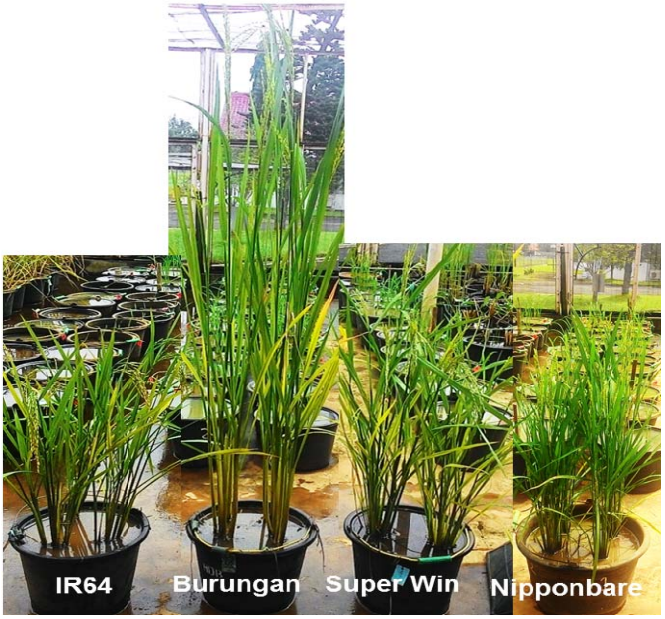

Fig. 1: Plant performance

Fig. 2: Panicle performance

Fig. 3: Grain performance

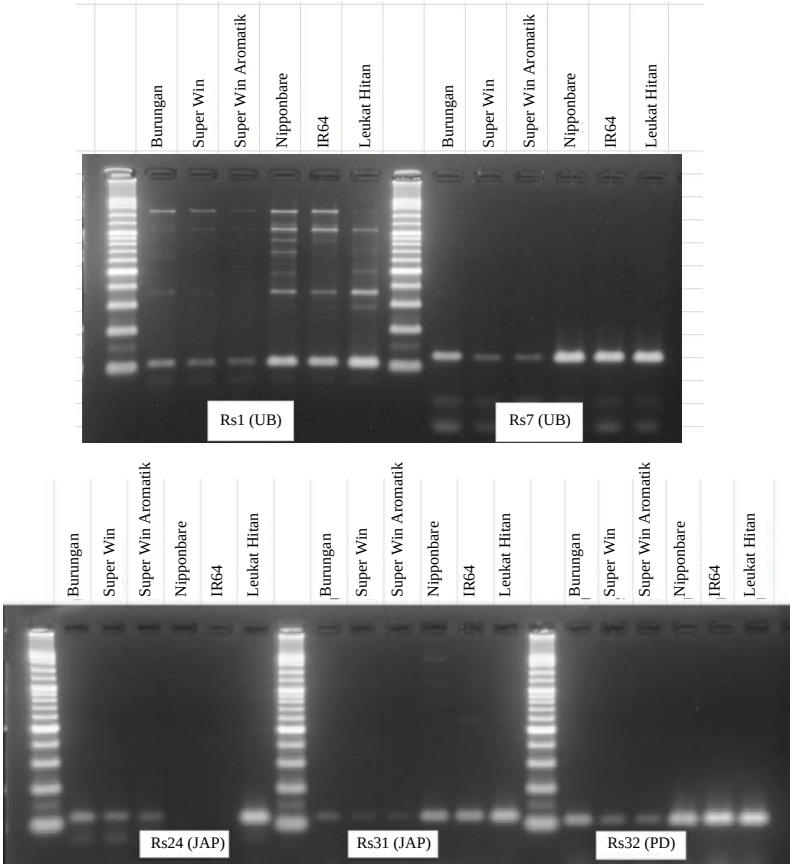

Fig. 4: SSR amplification of Burungan, Super Win and their counterparts

Table 3: Morphological performance of local North Sulawesi varieties, Burungan and Super Win and their counterparts

Varieties	Plant height (cm)	Number of productive tillers	Stem color	Flowering date (days)
Burungan	140.22	4	Green	87
Super Win	100.00	9	Green	87
IR64	80.15	6	Green	83
Nipponbare	63.40	10	Green	80

Japonica. This plant is short-lived, has medium panicle density and long grain shape and its lemma has no tail. Indica including IR64 was introduced from the Philippines. This type of rice is short-lived, has long grain shape and its lemma has no feather. The panicle length is medium and dense. The plant is short.

With the use of SSR markers, alleles of Super Win, Burungan and their allies are seemingly monomorphic (Fig. 4, Table 4), thus those markers cannot be used as identifier of those varieties associated with these markers. By using SSR markers, Burungan variety has an allele size in the range 108.7-148.8, with a mean size of alleles 118.97. The frequency of this allele is 33.33%. Super Win variety has allele size 109.2-148.8, with a mean size of alleles 119.50. The frequency of this allele is 22.22%. Variation in these allele sizes is low, thus cannot be used as a genetic identifier for both varieties.

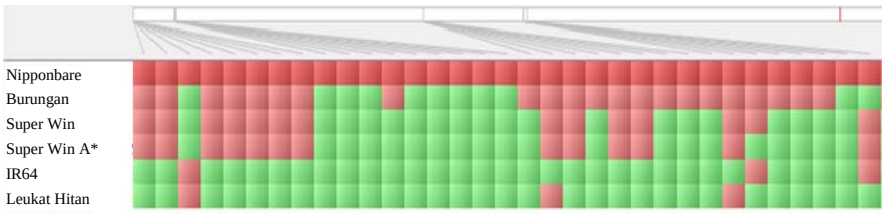

Fig. 5: Genotypic performance using SNP markers
Different colors (red and green) show the different genotypic performance in each locus

Table 4: Diversity of allele size of Super Win (Tropical Japonica) and Burungan (Japonica) with their allied varieties as controls, among others: IR64 (Indica), Nipponbare (Japonica), Super Win Aromatik (Tropical Japonica) and Leukat Hitam (Tropical Japonica)

	Development of SSR markers											
Variety	Rs1 (UB)	Rs 7 (UB)	Rs 6 (PM)	Rs 12 (PM)	Rs 26 (CM)	Rs 21 (SDB)	Rs 24 (JAP)	Rs 31 (JAP)	Rs 32 (PD)	Allele size (bp)	Mean of allele size (bp)	Allele frequency (%)
Burungan	108.7	119.2	108.0	122.7	148.8	119.5	118.2	116.3	109.3	108.7-148.8	118.97	33.33
Super Win	111.4	114.7	109.2	122.7	148.8	119.5	123.4	118.9	106.9	109.2-148.8	119.50	22.22
Super Win A	112.7	116.2	109.2	125.5	146.7	119.5	120.8	118.9	109.3	109.2-146-7	119.87	22.22
Nipponbare	114.1	116.2	106.8	122.7	148.8	116.2	-	118.9	114.4	106.8-148.8	119.76	11.11
IR64	112.7	117.7	102.2	125.5	155.4	117.8	-	118.9	111.8	102.2-155.4	120.25	0.00
Leukat Hitam	111.4	116.2	101.1	125.5	153.2	122.0	118.2	121.6	114.4	101.1-153.2	120.40	11.11

Table 5: Genetic distance between Burungan, Super Win and other control varieties

		Japonica		Tropical Japonica			Indica
Sub species	Variety	Burungan	Nipponbare	Super Win	Super Win A	Leukat Hitam	IR64
Japonica	Burungan	1	0.719	0.471	0.461	0.284	0.331
	Nipponbare	0.719	1	0.281	0.266	0.096	0.195
Tropical Japonica	Super Win	0.471	0.281	1	0.904	0.641	0.625
	Super Win A	0.461	0.266	0.904	1	0.648	0.573
	Leukat Hitam	0.284	0.096	0.641	0.648	1	0.620
Indica	IR64	0.331	0.195	0.625	0.573	0.620	1

Hence, this research was continued using SNP markers to compare the nucleotide difference between the varieties (Fig. 5).

Characterization using SSR markers above is based on the characterization of several genes which are associated with morphological and agronomical characters of crops. In addition to using SSR markers, characterization was also done using the SNP markers, which aims to identify the genetic background in 12 rice chromosomes. Total SNP markers used are 384 markers, which are known, mapped and scattered in all twelve rice chromosomes.

Using SNP markers, as shown in Fig. 5, one genotypic performances of local varieties Super Win and Burungan on chromosome 1 were compared with the genetic performance of their allied varieties Nipponbare (Japonica), IR64 (Indica) and Leukat Hitam (Tropical Japonica). Nipponbare as a control variety of Japonica subspecies has genotypic performance indicated in red color. Among two local varieties analyzed, Burungan variety has genotypic performance closer to Nipponbare. This is shown by more loci with red color than Super Win.

Principal coordinate analysis: Principal coordinate analysis visualizes similarities or dissimilarities of data based on distance matrix. The PCoA (Fig. 6) revealed significant diversity in the rice germplasm collections. The collection was distributed in 3 quadrants based on subspecies and did not show intermixing with each other.

Cluster analysis: Phylogenetic analysis using dendrogram to figure out the genetic distance among genotypes (Fig. 7) showed that the genotypes were divided into 2 groups. Each subspecies is clustered together in their respective clades. Super Win is a rice subspecies of Tropical Japonica. The genetic distance of this local North Sulawesi variety is very similar to Super Win Aromatik deposited in Bogor, with genetic distance 0.904 (Table 5). The genetic distance between Super Win and IR64 is 0.625 (62,5%). Super Win is thought to be local variety from district Maesaan in Minahasa Regency, while Burungan was from Bolaang Mongondow Regency.

Burungan and Nipponbare varieties are subspecies of Japonica. Both has genetic distance 0.719, which means that

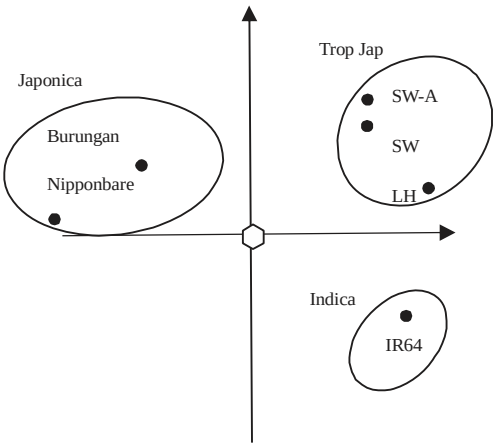

Fig. 6: Principal coordinate analysis (PCoA) of 6 rice varieties based on SNP data
SW-A : Super Win Aromatik, SW: Super Win, LH : Leukat Hitam, Trop Jap: Tropical Japonica

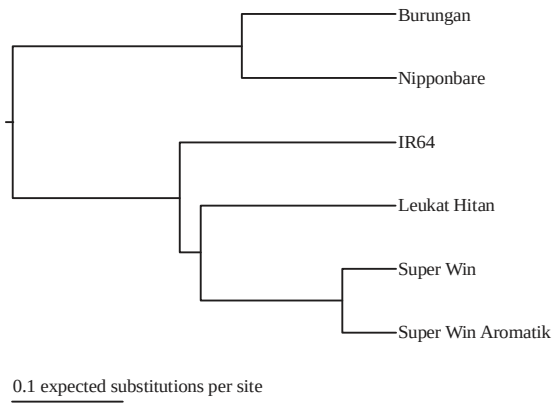

Fig. 7: Dendrogram of 6 varieties resulted from UPGMA cluster analysis based on SNP

the similarity between these 2 varieties is 71.9%, so that they are placed on the same subspecies and in one clade on the phylogenetic tree (Fig. 7). The genetic distance between Super Win and Burungan is 0.471. This supports the position of both, which are at different subspecies.

DISCUSSION

Oryza sativa (*O. sativa*) (Asian cultivated rice) has two major subspecies. The nonsticky, long-grained indica variety and the sticky, short-grained japonica or sinica variety[21]. Using SSR, Garris *et al.*[22] sorted *O. sativa* into 5 groups temperate japonica, tropical japonica and aromatic comprise japonica varieties comprise the japonica varieties, whereas indica and aus comprise the indica varieties.

The use of morphological and chemical parameters have no longer enough to discriminate cultivar of crops. Therefore, more precise techniques are very needed. The DNA fingerprinting can be used to identify specific genes which are linked to agronomic characters[23]. This technology can identify genes with high precision, thus providing useful information for the improvement of variety characters in plant breeding activities. Molecular markers such as SSR can be used as a tool to differentiate inter-genotype, even between varieties with a high phenotypic similarity. The SSR can also be used to prove the authenticity of a variety[24].

In this present study, the use of molecular markers for the assessment of genetic diversity were evaluated. Genetic diversity in each tested variety showed that each variety has a very low heterogenicity, thus SSR markers used in this study are not able to differentiate each variety, therefore cannot be recommended to be used in plant variety protection. The finding of Singh and Sengar[25], however, showed that SSR was able to generate sufficient polymorphisms and hence unique DNA fingerprints to identify each of 30 rice varieties of indica rice (Basmati and Non-Basmati). The different lies in employing different set of primers. They showed that SSR primers RM-263 (Chromosome 2) was highly informative since it recorded high PIC value (0.995). This marker is linked to drought resistance[26].

The analysis was continued using SNP markers to compare the nucleotide difference between the varieties. Based on the genetic distance between Super Win and Burungan, these two varieties were lineal descents from different subspecies. Super Win was a lineage of Tropical Japonica, while Burungan was from Japonica. An interesting finding is that Burungan was from Japonica subspecies, while Japonica rice ecotype is mostly cultivated in temperate[27].

Principal coordinate analysis (PCoA) was able to separate 6 rice varieties based on SNP data into 3 group, Japonica, Tropical Japonica and Indica. This is in accordance with finding of Tang *et al.*[28] which showed that principal component analysis was able to revealed 3 distinct group of rice species. Therefore, this study will be of great use for other groups looking for overcoming the common duplication within rice accession, making varieties identity and seed purity.

CONCLUSION

Microsatellite (SSR) markers with agronomy identity used in this research cannot be used to detect difference each variety of Burungan and Super Win and as a variety identifier. Using SNP markers, this research showed that Super Win and

Burungan belong to different subspecies. Super Win is a variety from subspecies Tropical Japonica, while Burungan belongs to subspecies Japonica. Due to Based on its genetic proximity with Nipponbare, then Burungan variety is thought as not belonging to a local variety of Bolaang Mongondow. Likewise, Super Win variety is thought to be the same variety as Super Win Aromatik.

SIGNIFICANCE STATEMENT

This study discovers that simple sequence repeat (SSR) cannot be used as a genetic identifier for North Sulawesi's local rice varieties Burungan and Super Win. However, single nucleotide polymorphism (SNP) shows that Burungan was from Japonica while Super Win was from Tropical Japonica and both varieties do not belong to local rice of North Sulawesi. This study will help the researcher to establish varieties' ID to help varieties characterization to meet the criteria of NDUS (novelty, distinctness, uniformity and stability).

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