

Genetic Diversity and Population Structure of a Korean Rice Germplasm Based on DNA Profiles

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ABSTRACT Information on the patterns of genetic diversity and population structure is essential for the rational use and efficient management of germplasms; accurate information aids in monitoring germplasms, and can also be used to predict potential genetic gains. In this study, we assessed genetic diversity, focusing on Korean rice accessions for their sustainable conserved diversity. Using DNA profiling with 12 simple sequence repeat (SSR) markers, we detected a total of 333 alleles among 2,016 accessions. The number of alleles ranged from 21 to 53, with an average of 27.8. Average polymorphism information content was 0.797, with the lowest being 0.667 and the highest 0.940. CA cluster analysis and the model-based population structure revealed two main groups that could be subdivided into five subgroups. Analysis of the molecular variance study based on the SSR profile data showed 5% variance among the profiles, whereas we recorded 93% variance among individuals and 2% variance within individuals. Specifically, the utilized diversity for of the breeding program is restricted in that cultivars were located in limited clades. These results revealed that preserving the diversity of Korean landraces could be useful sources for breeding new rice cultivars, and would be the basis for the sustainable conservation and utilization of a Korean rice germplasm.

Keywords : genetic diversity, population structure, rice germplasm, SSR marker

Rice (*Oryza sativa* L.) is an important cereal crop consumed exclusively by humans; it supplies staple food for nearly 50% of the global population (Garris *et al.*, 2005). Researchers in many countries are interested in collecting rice germplasm because genetic rice resources, stored in gene banks throughout the world, could be valuable resources for bioindustry (Rutger *et al.*, 1999). In Korea, the Rural Development Administration (RDA) Gene Bank maintains about 40,000 accessions of rice germplasm (<http://www.genebank.rda.go.kr/>). Although many RDA Gene Bank rice accessions and phenotypic characteristics have already been evaluated in the field of population diversity, there has been insufficient analysis of genetic diversity and relationship at the molecular level. In addition, most of the archived accessions have been utilized rarely or not at all in rice breeding programs.

The loss of genetic diversity in cultivated rice can have

serious consequences for the crop from susceptibility to epidemic disease to the lack of evolutionary potential for adaptation to changing environments (Pusadee *et al.*, 2009). To maintain genetic diversity in a rice germplasm, rice accessions with significant genetic variation should be included. In addition, it is necessary to investigate genetic diversity in the rice germplasm to broaden genetic variation in rice breeding because knowledge of germplasm genetic diversity is critical for utilization in crop improvement (Wang *et al.*, 2013). In many previous researches, the genetic diversity of Korean rice germplasm has been performed using various molecular markers to provide useful guidelines for the strategic conservation and utilization of rice germplasm (Lee *et al.*, 2015; Li *et al.*, 2014; Wang *et al.*, 2013; Zhao *et al.*, 2009).

A clear understanding of genetic diversity is essential for effective conservation and utilization of genetic rice resources

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(Tu *et al.*, 2007). Genetic diversity information provides opportunities for plant breeders to develop new and improved cultivars with desirable characteristics including farmer-preferred and breeder-preferred traits (Govindaraj *et al.*, 2015). Molecular markers are used to assess genetic diversity and population structure to predict hybrid performance and heterosis because these markers are inherited guided by Mendelian rules and are not influenced by environmental factors (Kashiani *et al.*, 2012; Legesse *et al.*, 2007). Among many different molecular markers, simple sequence repeat (SSR) markers are the preferred choice for certain genetic studies because they can easily detect co-dominant alleles, are highly reproducible, and have very high levels of polymorphisms (Litt and Luty, 1989; Weber and May, 1989).

Genetic analysis is very important to ensure the success of preserving and utilizing plant germplasm in Korea's RDA Gene Bank. In order to improve breeding material selection

from the conserved germplasm of the gene bank, we evaluated a total of 2,016 rice accessions including Korean (KOR) landraces and cultivars, landraces from the People's Republic of Korea (PRK), and major International Rice Research Institute (IRRI) rice accessions. Our main objectives were to investigate the genetic diversity of conserved Korean rice accessions using 12 polymorphic SSR markers, evaluate their population structures and clustering patterns, and survey the status of the conserved and utilized genetic diversity. These results will help to improve rice breeding programs and will be helpful for preserving and applying genetic rice resources in Korea.

MATERIALS AND METHODS

Plant materials and Data collection

We obtained a total of 2,016 rice accessions (Table 1 and Supplementary Table 1) from the National Agrobiodiversity

Table 1. Summary of the 2,016 rice accessions used for this study.

Origin	Type	No. of accessions
KOR (Republic of Korea)	Landrace	1,059
	Cultivar	410
PRK (People's Republic of Korea)	Landrace	327
IRRI (International Rice Research Institute)		220
Total		2,016

Table 2. SSR primers used for genotyping the 2,016 rice accessions, No. of alleles amplified, gene diversity, heterozygosity, and PIC.

Marker	Chr.	Forward primer	Reverse primer	MAF ¹	Na	GD	He	PIC
RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	0.484	25	0.701	0.005	0.667
RM249	5	GCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC	0.302	21	0.864	0.012	0.854
RM250	2	GGTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG	0.308	22	0.828	0.002	0.811
RM335	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	0.376	23	0.777	0.002	0.749
RM214	7	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	0.437	28	0.774	0.015	0.760
RM257	9	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	0.210	29	0.889	0.028	0.880
RM418	7	TCGCGTATCGTCATGCATAG	GAGCACATATGCCACGTACG	0.208	30	0.866	0.003	0.853
RM5647	8	ACTCCGACTGCAGTTTTTGC	AACTTGGTCGTGGACAGTGC	0.396	23	0.763	0.003	0.734
RM144	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	0.244	26	0.829	0.003	0.808
RM247	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	0.439	32	0.762	0.004	0.743
RM206	11	CCCATGCGTTAACTATTCT	CGTTCCATCGATCCGTATGG	0.123	53	0.943	0.088	0.940
RM021	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	0.385	21	0.756	0.011	0.725
Mean				0.326	27.8	0.813	0.014	0.797

¹ MAF, major allele frequency; Na, Number of alleles; GD, genetic diversity; He, Heterozygosity; PIC, polymorphism information content.

Table 3. Genetic diversity in the 2,016 rice accessions.

Origin	MAF ¹	Na	GD	He	PIC
KOR Cultivars	0.386	13.4	0.741	0.007	0.705
KOR landraces	0.359	21.5	0.788	0.015	0.765
PRK landraces	0.478	15.2	0.675	0.027	0.641
IRRI accessions	0.222	23.4	0.883	0.010	0.873

¹ MAF, major allele frequency; Na, Number of alleles; GD, genetic diversity; He, Heterozygosity; PIC, polymorphism information content.

Center of the Rural Development Administration (RDA), Republic of Korea (<http://genebank.rda.go.kr>).

DNA extraction

We extracted total genomic DNA from young leaf tissue ground in liquid nitrogen using the modified CTAB method (Dellaporta *et al.*, 1983). We determined DNA concentration and quality using a NanoDrop ND-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA). We diluted DNA stocks to 20 ng/ μ l as working solutions.

SSR analysis

We chose markers according to their location on the rice genetic map and their suitability for high-throughput genotyping, using a total of 12 SSR markers (Table 1). We amplified the alleles in 20- μ l volume containing 100 ng template DNA, 1 \times PCR buffer, and 0.2 mM each of dNTP, 1 U Taq DNA polymerase, and 2 pmol of reverse and forward primer, and we labeled the forward primers with different fluorescence: FAM, HEX, VIC, and NED. The published amplification conditions were: 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C (or other annealing temperature depending on the primer) for 45 s, 72°C for 1 min, and a final extension at 72°C for 10 min. We resolved SSR alleles using an ABI 3130xl Genetic Analyzer (Life Technologies Corp., Carlsbad, CA, USA) with GeneScan 3.7 software. The alleles were sized precisely against 6-carboxy-X-rhodamine molecular size standards using Genotyper 3.7 software (Life Technologies Corp.).

Analysis of population structure

As an indicator of genetic diversity, we calculated major allele frequency (MAF), number of alleles (NA), genetic diversity (GD), expected heterozygosity (He), and polymorphism information content (PIC) using the genetic analysis package PowerMarker V3.25 (Liu and Muse, 2005). We constructed

an unweighted neighbor joining tree using DARwin V6.0 and used structure V2.3.4 to detect possible subpopulations (K=1 to K=10) with a model that allowed for admixture and correlated allele frequencies using a burn-in period of 50,000 and MCMC repeats of 50,000 followed by three iterations (Pritchard *et al.*, 2000). The optimal number of populations corresponds to the highest peak in the ΔK graph (Evanno *et al.*, 2005), and we assigned the landraces with membership probabilities $\geq 70\%$ to each subpopulation.

RESULTS

SSR markers based analysis

We used 12 SSR markers to characterize 2,016 rice accessions and calculated Na (the number of allele), GD (genetic diversity), He (heterozygosity), MAF (major allele frequency), and PIC (polymorphism information content) for all 12 markers. We amplified a total of 333 alleles with an average of 27.8 per locus (Table 2); Na amplified per SSR primer varied from 21 to 53, with the maximum number being amplified by primer, RM205. The PICs for the SSR markers ranged from 0.667 (RM1) to 0.940 (RM206) with a mean of 0.797; GD ranged from 0.701 (RM1) to 0.943 (RM206) with an average of 0.813; He varied from 0.002 (RM250 and RM335) to 0.088 (RM206) with an average of just 0.014; and MAF ranged from 0.123 (RM206) to 0.484 (RM1) with an average of 0.326.

The numbers of alleles were 13.4, 23.4, 21.5, and 15.2 in the Korean cultivars (KC), Korean landraces (KL), PRK landraces (PL) and IRRA accessions (IA), respectively (Table 3). The PICs for KC, KL, PL, and IA were 0.705, 0.765, 0.641, and 0.873, respectively. The GD and He of KC, KL, PL, and IA were 0.741, 0.788, 0.675, and 0.883 and 0.007, 0.015, 0.027, and 0.010, both respectively. The MAFs of KC, KL, PL and IA were 0.386, 0.359, 0.478, and 0.222, also respectively.

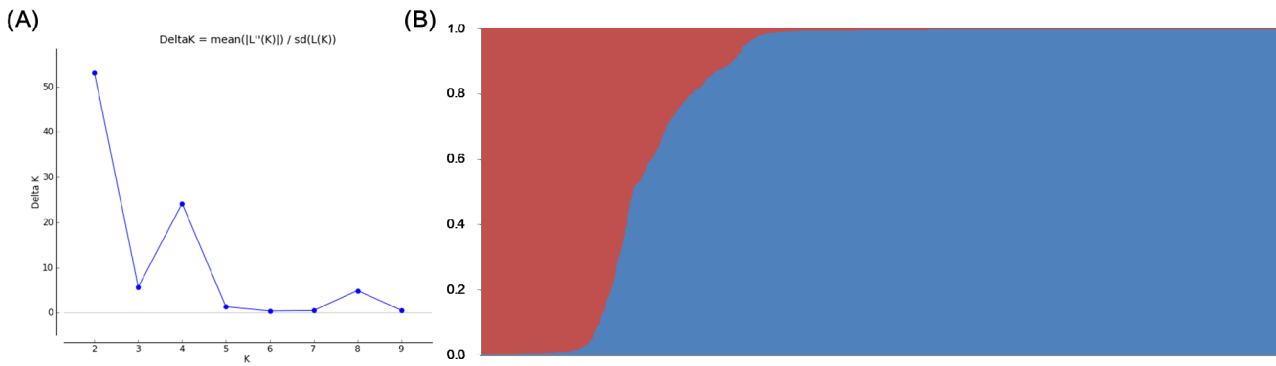


Fig.1. Estimation of population using LnP(D) derived ΔK from 1 to 20 (A) and the population structures of the 2,016 rice accessions (B).

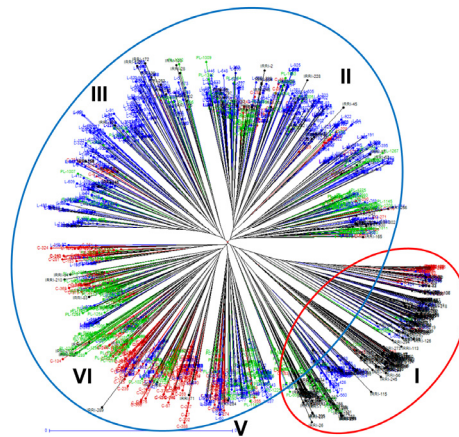


Fig.2. Neighbor-Joining (NJ) tree of the 2,016 rice accessions based on the 12 SSR markers; red and blue circles are the population structures pop1 and pop2, respectively.

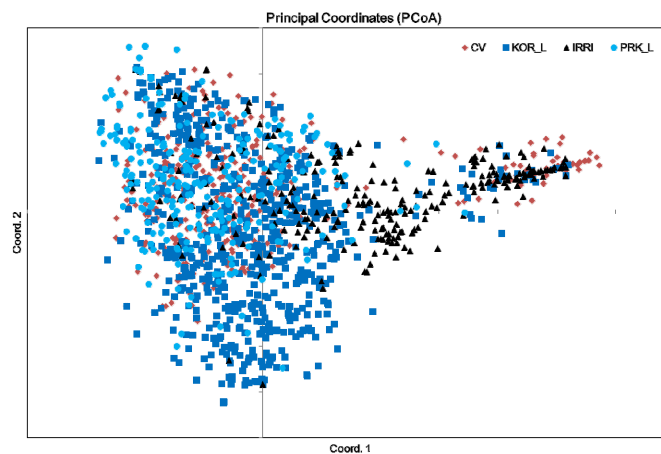


Fig. 3. Principal coordinate analysis of 2,016 rice accessions based on the 12 SSR markers.

Model-based population structure and hierarchical cluster analysis

We used the amplicons generated by 12 SSR markers across 2,016 accessions to analyze model-based population

structure and hierarchical clusters, and the inferred population structure and unrooted neighbor joining (NJ) tree (Fig. 1 and Fig. 2) among the 2,016 accessions revealed that there might be two main group and five groups, respectively, that were divergent.

Based on the inferred population structures (Fig. 1), population1 (pop1) and population2 (pop2) contained 383 and 1,633 accessions, respectively. Pop1 contained 65 Korean cultivars, 205 IRRI accessions, 103 Korean landraces, and 10 PRK landraces, and Pop2 consisted of 339 Korean cultivars, 97 IRRA accessions, 887 Korean landraces, and 310 PRK landraces. Further, based on the membership fractions, we categorized rice accessions under different populations as pure or admixture; we considered the accessions with probability greater than ≥ 0.80 to be pure and less than 0.80 as an admixture. Pop1 showed 326 pure (85.1%) and 57 admixed (14.9%) individuals, and pop2 showed 1,482 pure (90.8%) and 151 admixed (9.2%) individuals.

Among five clusters by cluster analysis, Cluster I comprised of IRRI rice accessions, whereas clusters II and III consisted of almost Korean rice landraces. Clusters IV and V were mixed Korean cultivars and landraces and PRK landraces. All rice accessions in cluster I had population structure pop1, and clusters II, III, IV, and V were in pop2.

AMOVA and PCoA of 2,016 rice accessions

We performed analysis of molecular variance (AMOVA) for the 2,016 rice accessions based on the 12 SSR markers. The four populations (origins KC, KL, PL and IA) showed 5% variance among themselves, but we recorded 93% variance among individuals and 2% variance within individuals (Table 4). Principal coordinate analysis (PCoA) based on the 12 SSR markers showed intermixing of four groups across the coordinates (Fig. 3). The first three axes explained 17.3% of

the cumulative variance (Table 5).

DISCUSSION

Erosion of plant genetic diversity is a very serious problem caused by modernization and replacement of wild plants or landraces with a few elite varieties (van de Wouw *et al.*, 2010; van Heerwaarden *et al.*, 2009). Therefore, collecting and preserving genetic plant resources is increasingly important for crop breeding to support the demands of a growing human population. Effective management and utilization of these resources requires information about strain origins, phenotypic traits, and genetic background, which can be identified by molecular techniques (Kim *et al.*, 2017). In this study, we analyzed genetic diversity among rice accessions collected from several regions and conserved in Korea's RDA Gene Bank; genetic diversity is the raw material that spurs genetic improvement, both to increase yield potential per se (i.e., production per unit area) and to decrease reliance on production inputs. Genetic crop diversity ensures future genetic progress and is insurance against unforeseen threats to agricultural production such as disease epidemics or climate changes. Thus, the fate of genetic diversity in these gene pools is of great important if plant breeding will continue to address the pressing needs of society such as increased yield, genetic resistance to diseases and pests, improved nutritional and processing quality of crop products, and fewer environmental effects (Gepts, 2006).

Our results revealed a high mean number of alleles per

Table 4. Summary of analysis of molecular variance (AMOVA).

Source	df ¹	SS	MS	Est. Var.	%	Fst	P value
Among Populations	3	660.646	220.215	0.234	5%	0.048	0.001
Among Individuals	2012	18649.052	9.269	4.592	94%		
Within Individuals	2016	170.500	0.085	0.085	2%		
Total	4031	19480.198		4.911	100%		

¹ df, degrees of freedom; SS, sum of squares; MS, mean sum of squares; Est. Var., estimate of variance; %, percentage of total variation; Fst, genetic differentiation among populations.

Table 5. Percentage of variation explained by the first three axes using principal coordinate analysis.

Axis	1	2	3
%	7.46	5.50	4.30
Cum %	7.46	12.96	17.26

locus (27.8) and a high PIC (0.797; Table 1), and these values were higher than those from other studies of genetic rice diversity. Previous research reported on numbers of alleles: 3.11 alleles per locus and 0.29 PICs for 729 rice varieties (Singh *et al.*, 2016), 4.80 alleles per locus and 0.50 PICs for 82 rice genotypes collected from different parts of Asian countries (Babu *et al.*, 2014), 3 alleles per locus and 0.468 in a set of 192 Indian rice germplasm (Nachimuthu *et al.*, 2015), and 9.65 alleles per locus and 0.58 PICs for 76 Korean rice germplasm (Lee *et al.*, 2015). Although the numbers of individuals, markers, and material types differed among the other studies, we concluded that the conserved Korean accessions in Korea's RDA Gene Bank are relatively diverse; in particular, the Korean landraces and major IRRI accessions showed more genetic variability than did the Korean cultivars and PRK landraces in a view of alleles, genetic diversity, and PICs (Table 2).

Analyzing genetic diversity and population structure in germplasm is very important for identifying potentially valuable resources (Wang *et al.*, 2008). In this study, we used two methods to evaluate population structure, model-based clustering and neighbor joining. The model-based clustering using STRUCTURE revealed a maximum ΔK of $K=2$ based on the statistical method described by Evanno *et al.* (2005). In addition, we phylogenetically analyzed the used rice accessions by NJ (Fig. 1 & Fig. 2). Overall, we detected two main groups in population structure and cluster analysis that could be subdivided into five clades; however, the patterns of population structure calculated by the two methods did not clearly distinguish among four origins. In general, population structures and clustering patterns are influenced by many different factors, such as the natural history of a population, breeding systems, complexity of breeding practices, and human selection (Xie *et al.*, 2008). We found Korean cultivars located in narrow clades on the NJ tree among the diversity of South and North Korean landraces, and these revealed alleles that were being utilized for a breeding program and were restricted to limited accessions.

Analysis of molecular variance showed more variability, 93%, among the individuals than among populations or within individuals (Fig. 2), and because the variation among the individuals was greater, it is important to conserve and utilize as many rice accessions population as possible. This result

was similar to the findings from previous reports by (Hassan *et al.*, 2012; Saini *et al.*, 2004), who obtained 91.2% and 91% variation among the individuals and within their study populations, respectively.

In conclusion, for this study, we surveyed the genetic diversity and analyzed the population structures and phylogenetic relationships primarily among the Korean rice accessions conserved in the country's RDA Gene Bank. The average numbers of alleles per locus and gene diversity indicated the existence of a broad genetic base in this rice germplasm, and the STRUCTURE analysis results accorded with the neighbor joining tree and principal coordinate analysis clustering methods. These results revealed that the preserved diversity in Korean landraces could be useful for breeding new rice cultivars and the basis for the sustainable conservation and utilization of Korean rice germplasm.

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