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Analysis of genetic diversity and trait correlations among Korean landrace rice (*Oryza sativa* L.)

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ABSTRACT. This study analyzed 394 Korean rice landrace accessions, including 93 waxy varieties, for polymorphisms using 29 simple sequence repeat (SSR) markers. In total, 381 alleles served as raw data for estimating the genetic diversity (GD) and population structure. The number of alleles per locus ranged from 3 to 44 (average = 13.14). The expected heterozygosity and polymorphism information content (PIC) ranged from 0.0341 to 0.9358 (mean = 0.5623) and from 0.0783 to 0.9367 (mean = 0.5839), respectively. The mean GDs in waxy, low amylose content, intermediate amylose content, and high amylose content (HAC) varieties were 0.6014, 0.5922, 0.5858, and 0.7232, respectively,

whereas the mean PIC values for each SSR locus were 0.5701, 0.5594, 0.5550, and 0.6926, respectively. HAC varieties had the highest GD and PIC. Consistent with clustering by genetic distances, a model-based structural analysis revealed 3 subpopulations. Analysis of molecular variance revealed that the between-population component of genetic variance was 22.35%, and that of the within-population component was 77.65%. Significant correlations were observed between eating quality and protein content (r = -0.262), K^+ (r = -0.655), Mg^{2+} (r = -0.680), 1000-GW (r = 0.159), and amylose content (r = -0.134). The overall F_{ST} value was 0.2235, indicating moderate differentiation among the groups. Analysis of variance of the 3 genetic groups (mean of 9 phenotypic and 5 physicochemical traits) by the Duncan multiple range test showed significant differences in 10 traits. This preliminary study represents a first step toward more efficient conservation and greater utilization of rice landraces to broaden the genetic bases of commercially grown varieties.

Key words: *Oryza sativa*; Simple sequence repeat; Landrace; Genetic diversity; Population structure

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, especially in Asian countries such as China, Korea, and Japan. Among these countries, Korea has a long history of rice cultivation and is important in the East Asiatic gene center, in part because of rice seeds that were excavated from a Bronze Age (~3000 years ago) site (Hammer, 2005). Currently, rice is a staple food for more than 40% of the world's population. Thus, improving rice quality has become one of the foremost considerations for rice buyers and breeding programs. Eating and cooking qualities are particularly important because most rice is consumed cooked. Rice-eating quality is strongly related to a number of easily measurable physicochemical characteristics, including the amount of starch, which is determined through indirect indices, namely, the amylose content (AC), gel consistency, and gelatinization temperature. These parameters reflect the starch functionality of the rice grain, and AC is widely recognized as an important determinant for various rice products (Juliano, 1998). Landraces maintained by farmers are endowed with tremendous genetic variability because they have been subjected to subtle selection over long periods of time. This aids in the adaptation of landraces to wide agro-ecological niches, and they have unmatched qualitative traits and medicinal properties. Although rice landraces are not heavily utilized in modern breeding programs, they are considered valuable genetic resources. Therefore, uncovering the unused alleles and qualitative traits of landraces is important to understand their genetic structure and to maximize the conservation and utilization of exotic genetic resources.

Molecular markers are important tools for determining the genetic diversity (GD) in many species and for managing plant genetic resources. In contrast to morphological traits, molecular markers can reveal differences among genotypes based on DNA polymorphisms, providing a direct, reliable, and efficient tool for germplasm characterization, conservation, and management. Many DNA markers have been developed and have become powerful tools for detecting the GD within and between populations, including restriction fragment length

polymorphisms (Sun et al., 2001), amplified fragment length polymorphisms (Bao et al., 2006), random amplified polymorphic DNA (Rabbani et al., 1998), sequence-characterized amplified regions (Li and Park, 2012), simple sequence repeats (SSRs) (Giarrocco et al., 2007), and single nucleotide polymorphisms (McNally et al., 2009). SSRs are the markers of choice for crop improvement in many species because they are reliable and easy to score (Gupta and Varshney, 2000; Moe and Park, 2012). SSR markers are co-dominant, multi-allelic, and require only a small amount of DNA for scoring. To date, more than 2500 SSR primer pairs have been developed in rice (McCouch et al., 2002), offering a tremendous opportunity to gain insight into the genetic structure of the rice genome. In Korea, the GenBank of the Rural Development Administration (RDA) maintains about 1100 rice landrace accessions, and these exotic accessions have not been or are rarely included in breeding programs; thus, genetic characterization is needed to ensure the long-term success of breeding programs and to maximize the conservation and utilization of the rice germplasm in Korea. Studies of the GD of the Korean rice germplasm have been conducted using various molecular markers (Jeong et al., 1999; Song et al., 2002; Lee et al., 2006). These studies have not only provided useful information for understanding the genetic basis of various rice gene pools established in different geographic regions but also facilitated the selection of new gene sources for breeding programs.

In this study, 394 rice landraces collected from South Korea were evaluated for their GD and population structure based on the AC using SSR markers. We also examined the degree of genetic differentiation of morphologically and genetically defined groups. Our findings will promote local rice conservation programs and increase the utilization of the rice germplasm in Korea.

MATERIAL AND METHODS

Plant materials and DNA extraction

The 394 rice landrace accessions, which included waxy (No. 1-93), low AC (LAC) (No. 94-285), intermediate AC (IAC) (No. 286-367), and high AC (HAC) (No. 368-394) varieties, were obtained from the National Agrobiodiversity Center of the RDA (Table 1). Each accession was grown in a greenhouse, and DNA was extracted from the fresh leaves of 15-day-old seedlings using a DNA extraction kit (Qiagen, Valencia, CA, USA). The relative purity and concentration of the extracted DNA were estimated with a NanoDrop ND-1000 spectrophotometer (Qiagen). The final concentration of each DNA sample was adjusted to 20 ng/μL.

Amylose content analysis

AC was measured using the method of Perez and Juliano (1978). Briefly, 100 mg rice flour was placed into a 100-mL volumetric flask, 1 mL 95% ethanol and 9 mL 1 M aqueous sodium hydroxide were added, and the contents were boiled for 8 min. After cooling to room temperature, the volume was made up with distilled water, and 5 mL solution was put into a 100-mL volumetric flask. Subsequently, 1 mL 1 M aqueous acetic acid and 2 mL 2% I_2-KI solution were added, and the volume was raised to 100 mL with distilled water. The absorbance of the solution was measured at 620 nm with a spectrophotometer. A standard curve that was made simultaneously using rice samples of known AC was used to calculate the AC of each sample.

Table 1. Three hundred and ninety-four rice landrace accessions used in this study and their model-based population genetic groups based on 29 SSR markers.

Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]
1	004753	Admixtured	133	005970	Admixtured	265	009243	POP1
2	004770	POP3	134	005980	Admixtured	266	009244	POP1
3	004771	POP3	135	005993	POP1	267	009245	POP1
4	005046	POP1	136	005994	Admixtured	268	009250	POP1
5	005051	POP1	137	006000	POP3	269	009251	Admixtured
6	005126	POP1	138	008438	POP1	270	009264	POP3
7	004688	POP1	139	006084	POP1	271	009265	POP1
8	005736	POP1	140	006087	POP1	272	009267	POP1
9	005743	POP1	141	006103	POP1	273	007442	POP3
10	005756	POP3	142	006114	POP1	274	009797	POP1
11	008732	Admixtured	143	006116	POP1	275	010161	POP1
12	151696	POP3	144	006119	Admixtured	276	010339	POP2
13	005835	POP1	145	006242	Admixtured	277	010340	POP2
14	006100	Admixtured	146	006243	POP1	278	010345	POP2
15	K026146	POP3	147	006247	POP1	279	010374	POP2
16	006620	POP1	148	006258	Admixtured	280	010375	Admixtured
17	K026154	POP1	149	006310	POP3	281	006560	POP1
18	K026181	POP1	150	006328	POP1	282	010417	POP2
19	006078	POP1	151	006354	POP3	283	006735	POP1
20	K026160	POP1	152	006366	POP3	284	010555	POP2
21	006684	POP3	153	006376	Admixtured	285	010577	POP2
22	007460	Admixtured	154	006386	POP3	286	010582	POP2
23	007532	POP1	155	007688	Admixtured	287	010704	POP2
24	007570	POP1	156	006397	POP1	288	009078	POP1
25	007714	POP1	157	006400	POP1	289	010721	POP2
26	007747	Admixtured	158	006404	POP3	290	010726	POP2
27	008196	POP3	159	006410	POP3	291	010727	POP2
28	K026190	POP1	160	006483	POP3	292	010728	POP2
29	008278	POP1	161	006538	POP2	293	007486	Admixtured
30	K026163	Admixtured	162	010627	POP2	294	006380	Admixtured
31	008388	POP1	163	007999	POP1	295	K026192	POP1
32	008453	POP1	164	006577	POP3	296	006622	POP2
33	008725	Admixtured	165	009177	POP1	297	155895	POP3
34	K026155	POP1	166	006663	POP3	298	155896	POP3
35	K026166	POP1	167	006699	POP1	299	007629	POP1
36	005915	POP1	168	006768	POP3	300	005754	POP1
37	008199	POP3	169	006776	POP3	301	173446	Admixtured
38	008798	POP1	170	009077	POP3	302	008992	POP3
39	008883	POP1	171	006818	POP1	303	009117	Admixtured
40	008999	POP3	172	007268	Admixtured	304	006396	POP1
41	K026171	POP3	173	007286	POP3	305	006554	POP1
42	009187	POP1	174	007290	POP1	306	006687	POP1
43	009192	POP3	175	007389	POP3	307	K026148	POP3
44	010151	POP2	176	007436	POP3	308	K026183	POP1
45	010275	POP2	177	007458	POP1	309	K026150	POP3
46	010480	POP2	178	005206	POP3	310	006372	POP1
47	010565	POP2	179	008891	POP1	311	005989	POP1
48	010628	POP2	180	007596	POP1	312	006424	POP3
49	010631	POP2	181	007598	POP3	313	007274	POP1
50	010630	Admixtured	182	007604	POP1	314	K026161	POP1
51	110944	POP2	183	007605	POP1	315	K026162	POP1
52	155897	POP1	184	007660	POP3	316	K026156	POP3
53	008749	POP1	185	007693	POP3	317	K026165	Admixtured
54	009229	POP1	186	007740	POP3	318	K026179	Admixtured
55	K026149	POP1	187	007742	POP3	319	006298	POP3
56	K026180	POP1	188	007792	POP3	320	007254	POP1
57	K026147	POP3	189	007801	POP1	321	007446	Admixtured
58	K026164	POP1	190	010625	POP3	322	007578	POP3
59	006302	Admixtured	191	007981	POP1	323	005693	POP3
60	008710	Admixtured	192	008344	Admixtured	324	005689	POP1
61	K026168	POP3	193	008255	POP1	325	005070	POP3

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Table 1. Continued.

Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]
62	K026186	POP1	194	008268	POP1	326	005681	POP3
63	K026188	Admixtured	195	008267	Admixtured	327	005683	POP1
64	K026157	Admixtured	196	008277	POP1	328	006064	POP1
65	K026158	POP3	197	008286	POP1	329	006112	POP1
66	K026167	POP1	198	008289	POP1	330	006559	POP3
67	K026169	Admixtured	199	008295	POP3	331	007592	POP1
68	K026172	POP1	200	008293	POP1	332	008590	POP3
69	K026173	POP1	201	008296	POP3	333	006005	POP3
70	K026175	Admixtured	202	008310	POP3	334	007746	POP3
71	005052	Admixtured	203	008314	POP1	335	006266	POP3
72	K026182	Admixtured	204	008355	POP1	336	005682	Admixtured
73	K026184	POP1	205	008357	POP3	337	006129	POP3
74	K026185	Admixtured	206	008382	POP3	338	007278	POP1
75	K026195	POP1	207	008385	POP3	339	005718	POP1
76	K026152	POP2	208	008390	POP1	340	004775	POP1
77	009590	POP3	209	005095	POP3	341	008361	Admixtured
78	005205	POP3	210	008401	POP3	342	K026145	POP3
79	007900	POP1	211	008408	POP3	343	008982	POP3
80	K026189	POP1	212	008469	POP3	344	005044	POP1
81	K026187	POP1	213	008471	POP1	345	006657	POP1
82	007903	POP1	214	008528	POP3	346	009172	POP1
83	007585	POP1	215	007633	POP3	347	009173	POP3
84	007270	Admixtured	216	008530	Admixtured	348	007687	POP1
85	K026159	Admixtured	217	008579	POP1	349	006089	POP1
86	007464	POP3	218	008580	POP1	350	005679	Admixtured
87	010707	POP2	219	008951	POP3	351	006066	POP1
88	004760	POP1	220	008591	POP3	352	006260	POP1
89	K026144	POP1	221	008599	POP3	353	007487	Admixtured
90	K026191	POP1	222	008672	POP3	354	005742	POP3
91	007717	POP1	223	008700	POP3	355	007631	POP1
92	006125	POP1	224	008717	POP3	356	006010	POP1
93	K026170	POP1	225	008734	POP1	357	006578	POP1
94	010276	POP2	226	008743	Admixtured	358	007634	POP3
95	004692	POP1	227	008804	Admixtured	359	006596	POP1
96	004694	POP3	228	008816	Admixtured	360	203619	POP1
97	004697	POP3	229	008820	POP1	361	173445	POP1
98	004768	POP1	230	008981	Admixtured	362	006520	POP1
99	004769	Admixtured	231	008831	POP1	363	007975	POP1
100	K026177	POP1	232	008850	POP1	364	006556	POP2
101	004811	POP3	233	008897	Admixtured	365	008799	POP3
102	004839	POP3	234	008895	POP1	366	006138	POP1
103	010612	POP2	235	005678	POP1	367	008983	POP3
104	004861	POP1	236	007721	POP1	368	007684	POP1
105	004899	POP1	237	008986	POP2	369	005762	POP1
106	004914	POP3	238	008996	POP3	370	006385	POP3
107	005040	POP1	239	009023	POP3	371	005508	POP3
108	008413	POP2	240	009056	POP3	372	009142	Admixtured
109	005057	POP1	241	009059	Admixtured	373	005505	POP3
110	005068	POP3	242	009060	Admixtured	374	005893	POP3
111	005076	POP1	243	009065	POP1	375	007630	POP3
112	005133	POP1	244	009069	Admixtured	376	009057	POP3
113	K026178	POP1	245	009073	POP3	377	005660	Admixtured
114	005142	POP1	246	009118	POP2	378	005691	POP1
115	005216	POP1	247	K026174	POP1	379	005509	POP1
116	K026193	Admixtured	248	009123	POP3	380	008741	POP3
117	005223	POP1	249	009128	POP2	381	K026176	Admixtured
118	005500	POP1	250	006772	POP1	382	007245	Admixtured
119	005504	POP1	251	006551	POP1	383	007559	POP2
120	008806	Admixtured	252	009129	POP1	384	006303	POP2
121	005506	POP1	253	009138	POP3	385	006522	POP1
122	007807	POP1	254	009169	POP1	386	008888	POP3

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Table 1. Continued.

Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]	Code	No. IT[a]	Distance-based groups[b]
123	008189	POP3	255	009174	POP1	387	009120	POP1
124	005657	POP1	256	006151	Admixtured	388	009189	POP1
125	010274	POP1	257	005677	POP3	389	010376	POP2
126	005694	Admixtured	258	005987	POP1	390	K026153	POP2
127	005716	POP1	259	009180	POP1	391	005882	Admixtured
128	005908	POP1	260	009182	POP1	392	K026194	POP2
129	005946	POP3	261	009191	POP3	393	007622	POP1
130	K026151	POP3	262	007282	POP1	394	008984	POP1
131	173444	POP3	263	009221	POP2			
132	005948	POP1	264	009233	Admixtured			

[a]No. IT. Introduction number of National Agrobiodiversity Center of RDA (Rural Development Administration) in Republic of Korea. [b]As defined by the STRUCTURE program.

Protein content (PC) analysis

Crude protein was analyzed by the Association of Official Agricultural Chemists method (AOAC, 2005). Briefly, 1 g powdered white rice was mixed with concentrated sulfuric acid, digested for 45 min at 450°C, and cooled to room temperature. Then, total nitrogen contents were measured using an automatic micro Kjeldahl system (FOSS: Kjeltec® 2300 Analyzer Unit, Foss Tecator AB, Höganas, Sweden). PC was calculated from the measured total nitrogen content value using a conversion factor of 5.95.

K^+ and Mg^{2+} analysis

The mineral (K^+ and Mg^{2+}) contents were determined by using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Fisher Scientific, Waltham, USA). Briefly, 0.3 g powdered white rice was combined with HNO_3 and heated at 150°C. After cooling and adding $HClO_4$, the sample was reheated for complete oxidation. Again, after cooling to room temperature, samples were mixed with HNO_3 and dH_2O for digestion in a microwave (model Q 3000; Tektone, Franklin, NC, USA). Digested samples were filtered, and ICP-OES was conducted after adjusting the final volume to 100 mL with dH_2O.

SSR analysis

Markers were chosen according to their location on the rice genetic map and their suitability for high-throughput genotyping. In total, 29 SSR markers distributed across all 12 chromosomes were used (Table 2). All of the markers were obtained from GRAMENE (http://www.gramene.org/). Amplification was performed in a 20-µL volume containing 100 ng template DNA, 1X PCR buffer, 0.2 mM of each dNTP, 1 U Taq DNA polymerase, 8 pmol of each reverse and fluorescently labeled M13 (-21) primer, and 2 pmol forward primer with an M13 (-21) tail at its 5'-end. The conditions for amplification were as described in a previous study (Schuelke, 2000): 94°C for 3 min; 30 cycles of 94°C for 30 s, 55°C (the annealing temperature was changed depending on the primer) for 45 s, and 72°C for 1 min; 10 cycles at 94°C for 30 s, 53°C for 45 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. The SSR alleles were resolved using a 3130xl Genetic Analyzer (Life Technologies Corp., Carlsbad, CA,

USA) with the GeneScan 3.7 software and sized precisely against 6-carboxy-X-rhodamine molecular size standards using the Genotyper 3.7 software (Life Technologies Corp.).

Table 2. Total number of alleles, number of rare alleles, and GD index for 29 SSR loci in the 394 accessions.

Marker	Map	Size range	N_A	N_{RA}	MAF	H_E	GD	PIC
RM021	11	129-197	16	11	0.2944	0.7847	0.8088	0.7836
RM044	8	113-191	15	9	0.4112	0.7431	0.7704	0.7476
RM048	2	121-235	31	28	0.3706	0.7913	0.8213	0.8088
RM206	11	133-237	44	38	0.1193	0.9358	0.9398	0.9367
RM214	7	111-223	27	21	0.2716	0.8590	0.8499	0.8358
RM228	10	95-147	12	8	0.4848	0.6400	0.7009	0.6683
RM231	3	110-192	10	6	0.6954	0.4063	0.4954	0.4748
RM232	3	141-179	20	13	0.2081	0.8632	0.8873	0.8775
RM235	12	89-137	12	10	0.4975	0.2711	0.5790	0.4923
RM241	4	92-134	16	10	0.3426	0.7695	0.8127	0.7929
RM246	1	94-116	10	5	0.3376	0.7409	0.7544	0.7154
RM247	12	135-195	15	11	0.3832	0.7397	0.7312	0.6917
RM249	5	121-207	22	14	0.1447	0.8964	0.9116	0.9050
RM253	6	110-144	12	7	0.4391	0.6710	0.7340	0.7024
RM257	9	136-170	13	9	0.4492	0.6276	0.6972	0.6540
SBE	2	140-218	7	4	0.6168	0.5038	0.5516	0.4991
SSS	6	193-215	6	5	0.9594	0.0593	0.0791	0.0783
WxOligo	6	100-124	9	7	0.4721	0.5545	0.5787	0.4897
RM310	8	138-204	18	13	0.2437	0.8212	0.8474	0.8305
RM3322	5	120-138	7	5	0.8020	0.2792	0.3361	0.3094
RM3718	7	148-166	8	6	0.5076	0.5312	0.5692	0.4793
RM3857	2	116-160	15	9	0.2360	0.8320	0.8571	0.8414
RM6144	10	135-141	3	1	0.8352	0.2425	0.2873	0.2599
RM6165	2	170-194	3	2	0.9492	0.0341	0.0975	0.0948
RM6629	4	71-83	5	3	0.8731	0.1521	0.2323	0.2239
RM12676	2	252-256	3	1	0.4898	0.4987	0.5290	0.4177
RM16427	4	283-289	4	1	0.8629	0.1961	0.2487	0.2388
RM19159	5	159-201	14	10	0.5076	0.5727	0.6855	0.6541
RM23455	8	310-314	4	0	0.7132	0.2904	0.4603	0.4282
Total			381	267				
Mean			13.14	9.21	0.5005	0.5623	0.6156	0.5839

N_A = number alleles; N_{RA} = number of rare alleles; MAF = major allele frequency; H_E = expected heterozygosity; GD = genetic diversity; PIC = polymorphic information content.

Data analysis

Basic statistics, including the total number of alleles, allele frequency, major allele frequency (MAF), and polymorphism information content (PIC), were calculated using Power Marker 3.25 (Liu and Muse, 2005). The variability at each locus was measured in terms of the number of alleles, expected heterozygosity (H_E), and genetic distance between each pair of accessions using the genetic analysis package POPGENE 1.31 (Yeh et al., 1999). The unweighted pair group method with an arithmetic mean (UPGMA) tree from shared allele frequencies was constructed using MEGA 4.0 (Tamura et al., 2007), which is embedded in Power Marker. The possible population was analyzed using the model-based program Structure 2.2 (Pritchard et al., 2000; Falush et al., 2003) without prior assignment of the accessions to populations. In this model, a number of populations (K) are assumed to be present, each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned to populations or jointly to more populations if their genotypes indicate that they are admixed. All loci are assumed to be independent, and each K population is assumed to follow Hardy-Weinberg equilibrium. Posterior probabilities were estimated

using the Markov chain Monte Carlo (MCMC) method. MCMC was run for 100,000 burn-in period lengths at fixed iterations of 5 for each fixed population number, followed by 200,000 iterations using a model allowing for admixture and correlated allele frequencies. At least 3 runs of Structure 2.2 were performed with *K* ranging from 2 to 10, and an average likelihood value, LnP(D), across all runs was calculated for each value of *K*. The model choice criterion to detect the most probable value of *K* was Δ*K*, which is an *ad hoc* quantity related to the second-order change in the log probability of data with respect to the number of clusters inferred by Structure (Evanno et al., 2005). An individual was assigned to a group if >70% of its genome fraction value derived from that group.

Phenotypic trait associations and significance testing for population genetic groups

The Duncan multiple range test (DMRT) was used to determine the significance among 3 groups by a model-based population structure analysis using Structure 2.2 (Pritchard et al., 2000) with 9 phenotypic traits and 5 physicochemical traits, including culm height (cm, average of 20 plants), seed length (mm, average of 20 seeds), seed width (mm, average of 20 seeds), awn number per seed (average of 20 plants), number of panicles (average of 20 plants), panicle length (cm, average of 20 plants), seed coat color, days to flowering (number of days from the date of sowing to the date at which 50% of the plants begin flowering), and 1000-grain weight (g) (Satheeshkumar and Saravanan, 2012). The eating quality traits, as evaluated by the glossiness of cooked rice. In this study, 301 non-waxy varieties were measured using a Toyo-taste meter (Model: MA-90A and 90B) according to manufacturer operation protocols (Toyo Rice Polishing Machine Factory, Tokyo, Japan). To maintain the accuracy and consistency of measurements, rice flour of cv. Ilpoom was used as a reference every 30 samples. These phenotypic traits were tested for correlations using DMRT with the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

SSR polymorphisms and genetic variation in the landrace rice accessions

The 29 SSR markers revealed 381 alleles among the 394 rice landrace accessions (Table 2). The number of observed alleles and rare alleles in the loci varied from 3 (RM6144, RM6165, and RM12676) to 44 (RM206) and from 0 (RM23455) to 38 (RM206), with averages of 13.14 and 9.21 alleles, respectively. The sizes of the alleles ranged from 71 to 314 bp (Table 2). The database of allelic frequencies showed that the rare alleles (frequency <0.05) made up 70.1% of all alleles, whereas intermediate (frequency = 0.05-0.50) and abundant alleles (frequency >0.50) represented 27 and 2.9% of all detected alleles, respectively. These results indicate the presence of a relatively large proportion of rare alleles, and most alleles were present at a low frequency among the rice landrace accessions that were studied (Figure 1). The GD varied from 0.0791 (SSS) to 0.9398 (RM206), with an average value of 0.6156. The frequency of major alleles per locus and the H_E varied from 0.1193 (RM206) to 0.9594 (SSS) and from 0.0341 (RM6165) to 0.9358 (RM206), with averages of 0.5005 and 0.5623, respectively. The PIC values ranged from a low value of 0.0783 (SSS) to a high value of 0.9367 (RM206), with an average of 0.5839 (Table 2).

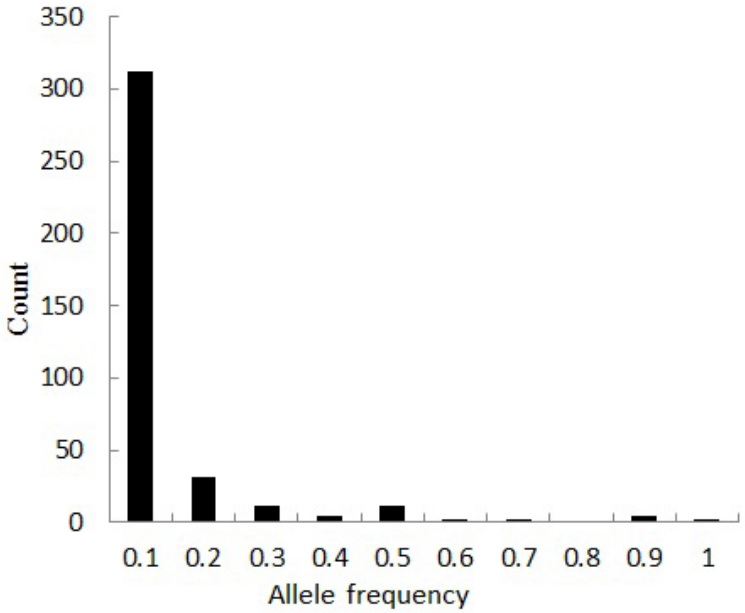

Figure 1. Histogram showing the allele frequencies for all 381 alleles in the 394 rice landrace accessions.

Analysis of GD based on the AC

AC is one of the most important predictors of eating quality in rice. The AC of milled rice can be classified as waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-24%), or high (>24%) (Juliano, 1971). Waxy rice is used in foods such as desserts and snacks. Low-amylose varieties are soft and sticky and include nearly all temperate *japonica* rice varieties. Intermediate-amylose rice is soft but not sticky and is eaten by most consumers. High-amylose varieties are common among *indica* rice and are dry and fluffy when cooked, often becoming hard upon cooling. In this study, 93 waxy, 182 LAC, 82 IAC, and 37 HAC rice varieties were analyzed for GD (Table 1). The GD among rice with the 4 different ACs is summarized in Table 3. The SSR markers revealed totals of 260 and 300 alleles in the waxy and LAC varieties with 8.97 and 10.34 alleles per locus, respectively, whereas the IAC and HAC varieties contained totals of 237 and 218 alleles with averages of 7.97 and 7.52 per locus, respectively. The mean GDs for each SSR locus in the waxy, LAC, IAC, and HAC rice varieties were 0.6014, 0.5922, 0.5858, and 0.7232, respectively, and the mean PIC values for each SSR locus were 0.5701, 0.5594, 0.5550, and 0.6926, respectively. Comparing GD, we found that the LAC varieties had the highest mean number of alleles. However, the values for GD and PIC were the highest in the HAC varieties, and the MAF per locus was the highest in the IAC varieties (0.5320). The MAF decreased in the order IAC > LAC > waxy > HAC, but the order of average GD and PIC value was HAC > waxy > LAC > IAC (Table 3). The distribution of molecular genetic variation among and within the AC-based subgroups was estimated by analysis of molecular variance (AMOVA), which revealed that 4.61% of the total variation was among clusters and 95.39% of the variation was within clusters (Table 4).

Table 3. Average number of alleles, GD, and PIC for different amylose contents (ACs).

	T_A	A_A	T_{RA}	A_{RA}	GD	PIC	MAF
Overall	381	13.14	334	11.52	0.6156	0.5839	0.5005
Waxy	260	8.97	143	4.93	0.6014	0.5701	0.5109
LAC	300	10.34	189	6.52	0.5922	0.5594	0.5235
IAC	237	7.97	126	4.34	0.5858	0.5550	0.5320
HAC	218	7.52	76	2.62	0.7232	0.6926	0.4023

T_A = total alleles; A_A = average alleles; T_{RA} = total rare alleles; A_{RA} = average rare alleles; GD = gene diversity; PIC = polymorphic information content; MAF = major allele frequency; LAC = low AC; IAC = intermediate AC; HAC = high AC.

Table 4. Results of AMOVA for the different populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	P
Amylose content					
Among clusters	3	68.10	0.11	4.61	<0.05
Within	392	1710.18	2.20	95.39	
Model-based population					
Among clusters	2	222.00	0.57	22.35	<0.05
Within	328	1288.96	1.96	77.65	

d.f. = degrees of freedom.

Population structure analysis

The model-based grouping method was performed using all 394 rice landrace accessions and 29 SSR markers (Pritchard et al., 2000). Five runs of Structure 2.2 were conducted by setting the number of populations (K) from 2 to 10. For each run, the burn-in time and replication number were both set to 100,000. We used an *ad hoc* quantity (ΔK) to estimate likelihood values for a given K if the distribution of L(K) did not show a clear mode for the true K (Figure 2A). ΔK was developed to overcome the difficulty of interpreting the real K values when influenced by factors such as inbreeding and departures from Hardy-Weinberg equilibrium, and it was tested under different simulation routines in which a real population structure was present (Falush et al., 2003). According to the second-order statistics developed for Structure (Evanno et al., 2005) to assess the number of subpopulations, the optimal value of K = 3, which had the highest ΔK, was identified (Figure 2B); at K = 3, all 394 rice landrace accessions could be grouped into 3 populations, here designated as POP1, POP2, and POP3. The relatively small value of the alpha parameter (α = 0.0739) revealed that most accessions originated from one primary ancestor, with a few admixed individuals. The degree of admixture, or alpha value, approached zero when calculated using Structure, which indicates that most individuals investigated here were essentially from separate populations. An alpha value >1 would have indicated that most populations were admixtures (Ostrowski et al., 2006). An individual having >70% of its genome fraction value was assigned to a group. As shown in Figure 3, most of the accessions were clearly classified into 1 of the 3 subpopulations. POP1 consisted of 182 accessions, including waxy (48), LAC (87), IAC (38), and HAC (9) varieties; POP2 consisted of 38 accessions, mainly containing LAC (16), waxy (9), and IAC (8) varieties; and POP3 consisted of 111 accessions, which were predominantly LAC (61) and IAC (25) varieties. In total, 332 accessions (84% of 394 accessions) were each clearly assigned to a single subpopulation, whereas 63 accessions (16% of 394 accessions) in the sample were categorized as having admixed ancestry (Figure 3 and Table 1).

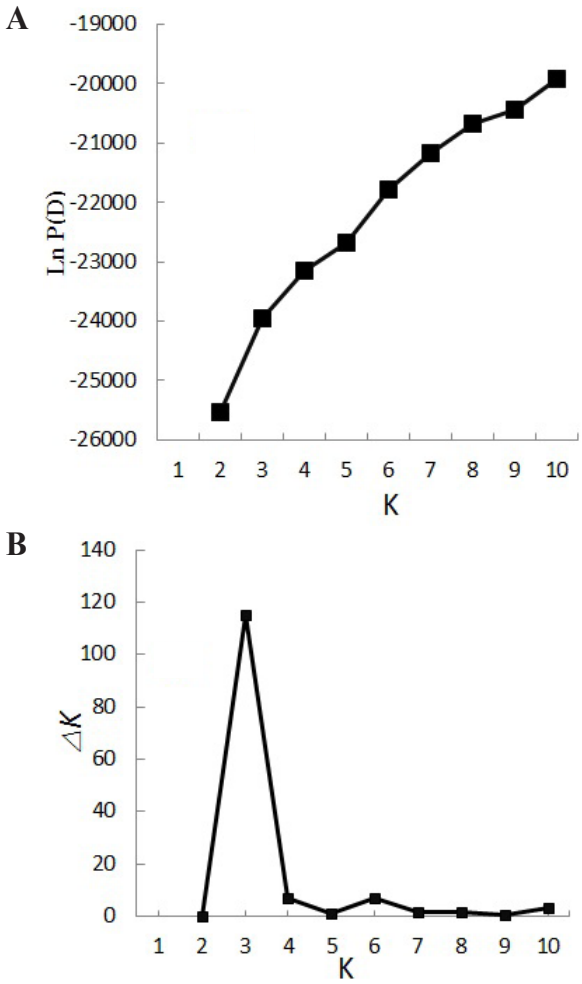

Figure 2. A. Log-likelihood of the data (N = 394) as a function of *K* (the number of groups used to stratify the sample). **B.** Δ*K*, with its modal value detecting a true *K* for the three groups (*K* = 3). For each *K* value, five independent runs were considered; the data were averaged over the replicates.

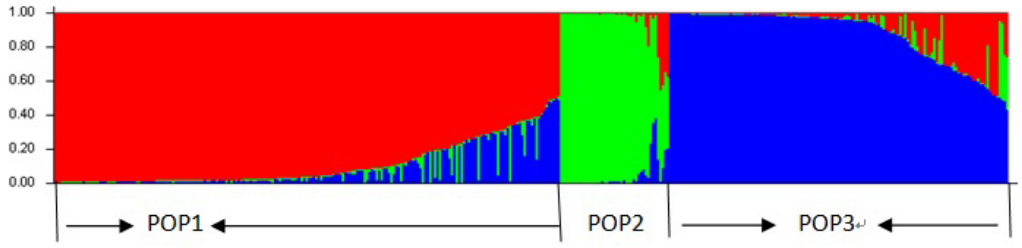

Figure 3. Model-based clustering for each of the 394 rice landrace accessions examined based on the 29 markers used to build the Q-matrix. The groups in each panel are represented by different colors. Each individual bar represents an accession. The y-axis displays the estimated ancestry for each individual in a particular gene pool. POP1-POP3 = populations 1, 2 and 3.

The distribution of molecular genetic variation among and within the 3 clusters of accessions was estimated by AMOVA, which revealed that 22.35% of the total variation was among clusters and 77.65% of the variation was within clusters (Table 4). Wright (1978) suggested qualitative guidelines for the interpretation of F_{ST}. A range from 0 to 0.05 may be considered to indicate little genetic differentiation, whereas ranges from 0.05 to 0.15 and 0.15 to 0.25 and F_{ST} values >0.25 indicate moderate, great, and very great genetic differentiation, respectively. In this study, the overall F_{ST} value was 0.2235, indicating moderate differentiation among the 3 groups (Table 5). A comparison of the GD, PIC, and MAF values in the 3 clusters is shown in Table 5. Among the 3 model-based populations, POP2 showed higher GD and PIC values than POP1 and POP3. In contrast, POP3 had lower GD and PIC values than POP1 and POP2, suggesting that POP3 is more homogeneous than the other POPs. Pairwise F_{ST} values were estimated to explain the genetic differentiation among the subpopulations (i.e., POP1, POP2, and POP3). The subpopulational pairwise F_{ST} values ranged from 0.4197 between POP1 and POP2 to 0.3575 between POP2 and POP3 (Table 5).

Table 5. Comparisons of the rice model-based populations in terms of average GD and population differentiation.

Cluster	Diversity					F_{ST}		
	N	N_A	MAF	GD	PIC	1	2	Overall
POP1	182	9.14	0.5400	0.5751	0.5380			
POP2	38	7.1	0.4855	0.6456	0.6115	0.4197		
POP3	111	7.6	0.6024	0.5120	0.4806	0.1011	0.3575	
								0.2235

N = number of accession; N_A = number of alleles; MAF = major allele frequency; GD = gene diversity; PIC = polymorphic information content; F_{ST}: for AMOVA-based estimates ($P < 0.05$) for 100 permutation for population (POP1-POP3) comparisons.

Phenotypic trait correlations and significance testing for the population genetic groups

The results of our analysis based on 9 phenotypic and 5 physicochemical traits in 301 non-waxy landrace accessions are shown in Table 6. A simple paired *t*-test and an association analysis were carried out to identify varieties and correlations exhibiting significant differences from the checked accessions. Traits having a positive significant correlation and some having a negative significant correlation among the phenotypic traits were identified. A strong association was present between eating quality and other physicochemical traits, including protein content (r = -0.262), K^+ content (r = -0.655), Mg^{2+} content (r = -0.680), 1000-GW (r = 0.159), AC (r = -0.134), and days to flowering (r = -0.123). DMRT was employed to determine whether there was any correlation between phenotypic traits and the 3 subpopulations of Korean rice landraces grouped by Structure. By analysis of variance, 10 of 14 phenotypic traits revealed significant differences ($P < 0.05$). Multiple mean comparisons using DMRT revealed that the 1000-grain weight was significantly different among the 3 subpopulations. Phenotypic characteristics such as seed length, seed width, and 1000-grain weight were smaller in POP1 than in the other 2 POPs, whereas the awn number per seed and culm height were higher. Days to flowering and eating quality were significantly different only in POP2; the phenotypic traits showing a significant difference only in POP3 and POP1 were PC and culm height, respectively (Table 7). As a result, POP2 was discriminated from the other POPs in terms of the eating quality and days to flowering, and POP1 and POP3 differed from each other in terms of culm height, PC, seed width, seed length, and awn number per seed (Table 7).

Table 6. Correlation coefficients among several traits in the 301 non-waxy landrace accessions.

Traits	CH	SL	SW	NOA	NOP	PL	SCC	DF	1000-GW	AC	PC	Mg^{2+}	EQ
SL	-0.025												
SW	-0.041	0.146*											
NOA	0.182**	-0.147**	0.005										
NOP	-0.049	0.004	-0.143**	-0.051									
PL	0.177**	0.095	-0.017	0.017	-0.020								
SCC	0.016	0.096	-0.010	0.027	0.126*	-0.041							
DF	-0.052	0.204**	0.233**	-0.076	-0.069	-0.017	-0.100						
1000-GW	-0.033	0.382**	0.268**	-0.096	-0.136*	0.073	0.014	0.101					
AC	0.079	0.020	-0.133*	-0.064	0.116	0.022	0.232**	-0.116	-0.047				
PC	0.136*	0.064	-0.084	0.059	0.101	-0.052	0.115*	-0.075	-0.059	0.031			
Mg^{2+}	0.084	-0.022	0.050	0.064	0.025	0.018	0.114*	-0.021	-0.156**	-0.008	0.229**		
EQ	-0.062	-0.098	-0.016	0.017	-0.064	0.011	-0.061	-0.123*	0.159**	-0.134*	-0.262**	-0.680**	
K^{+}	0.030	-0.056	0.032	0.009	0.022	-0.020	0.110*	0.009	-0.171**	-0.086	-0.011	0.812**	-0.655**

CH = culm height (mm); SL = seed length (mm); SW = seed width (mm); NOA = No. of awn per seed; NOP = No. of panicle; PL = panicle length (cm); SCC = seed coat color; DF = days to flowering (days); 1000-GW = 1000-grain weight (g); AC = amylose content; PC = protein content, EQ = eating quality. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

Table 7. Comparison of the means for phenotypic traits among the subpopulations (POP1-POP3) by the Duncan multiple range test at $K = 3$.

Traits	POP1	POP2	POP3	F value[a]
PC (%)	7.04[a]	7.28[a]	6.66[b]	7.35***
CH (mm)	98.23[a]	88.56[b]	88.95[b]	15.66***
SL (mm)	6.33[b]	7.32[a]	6.51[b]	19.83***
SW (mm)	3.31[b]	3.39[b]	3.68[a]	5.46*
NOA	2.95[a]	2.34[b]	2.02[b]	13.61***
NOP	9.45[a]	8.47[b]	9.67[a]	3.66*
PL (cm)	21.14[a]	21.27[a]	20.67[a]	2.36
SCC	1.14[a]	1.09[a]	1.03[a]	5.70*
DF	111.87[b]	121.24[a]	111.61[b]	9.31***
1000-GW (g)	23.17[c]	26.41[a]	24.26[b]	12.52***
AC (%)	15.84[a]	16.36[a]	17.02[a]	1.33
EQ	69.40[a]	64.97[b]	69.91[a]	2.28
Mg	23.57[ab]	24.98[a]	22.09[b]	4.18*
K	75.77[a]	76.90[a]	74.54[a]	2.27

PC = protein content; CH = culm height (mm); SL = seed length (mm); SW = seed width (mm); NOA = No. of awn per seed; NOP = No. of panicle; PL = panicle length (cm); SCC = seed coat color; DF = days to flowering (days); 1000-GW = 1000-grain weight (g); AC = amylose content; EQ = eating quality, [a]F value of ANOVA. ***Significant at $P < 0.001$, *$P < 0.05$. [a,b,c]Means along rows with different letters are significantly different ($P < 0.05$).

DISCUSSION

In this study, 381 alleles were detected among 394 Korean rice landrace accessions using 29 SSR markers, with an average of 13.14 alleles per locus. This value is higher than that reported previously for landrace rice (Bajracharya et al., 2006; Pandey et al., 2011), indicating that Korean rice landraces possess strong GD, which may make them an important genetic resource for rice breeding programs and which could increase the utilization of rice landraces. All of the SSR markers were found to be polymorphic, and there was considerable genetic variation among the rice landrace accessions, with a mean $H_E = 0.5623$, MAF = 0.5005, GD = 0.6156, and PIC = 0.5839. Most of the SSR markers differed greatly in the number of alleles, ranging from 3 to 44. Certain SSR markers producing similar numbers of alleles varied greatly in their H_E, GD, and PIC values. For example, 3 alleles were detected at each of the markers

RM6144, RM6165, and RM12676, but there was a significant difference in their H_E, GD, and PIC values. A similar trend was reported previously in strawberry (Yoon et al., 2012) and Italian millet (Zhao et al., 2012a). Among the 29 SSR markers, RM206, RM48, RM214, RM232, and RM249 showed a comparatively high number of alleles and exhibited strong GD in the 394 Korea rice landrace accessions, as reported by Lee et al. (2006) and Zhao et al. (2009). The observed difference in GD and PIC may be attributed to the variation in allele frequency between the datasets for the 2 loci. A similar trend was reported previously in rice (Queller et al., 1993). The significantly high frequency of rare alleles (267 alleles, 70.1%) among landrace accessions indicates that they make a greater contribution to the overall GD of the collection. The GD values for the 4 different AC groups are shown in Table 3. Our results show that the LAC varieties had the highest allele number (300), whereas the HAC varieties had the highest GD (0.7232) and PIC (0.6926). The GD and PIC values for the varieties decreased in the following order: HAC > waxy > LAC > IAC. This preliminary finding suggests that the HAC varieties had the highest GD compared with other groups among Korea landrace rice. Our data will be useful in designing breeding programs and increasing the utilization of rice landraces.

Population genetics deals with variations in allele frequency between and within populations. The model-based method uses a Bayesian grouping approach to probabilistically assign individuals to populations based on their genotypic data and attempts to find a population structure in which the population is in Hardy-Weinberg equilibrium. The model does not assume a particular mutation process, and in most situations, the estimated log probability does not provide a correct estimation of the number of groups (K) (Evanno et al., 2005). In our simulations, as the real K was reached, L(K) at larger Ks plateaued or continued to increase slightly (Pritchard and Wen, 2003), and the variance between runs increased (Figure 2A). The distribution of L(K) did not show a clear mode for the true K, but ΔK did show a clear peak at $K = 3$ (Evanno et al., 2005) (Figure 2B).

Our model-based structural analysis revealed the presence of 3 populations (POP1-POP3) in the Korea rice landraces. When clustering based on the GD and structural analyses based on the model were compared, similar groupings of the accessions were discovered (Figure 3 and **Figure S1**). The degree of admixture, alpha ($\alpha = 0.0739$), was inferred from the data. When alpha is close to zero, most individuals are essentially from one population or another, whereas when alpha is >1, most individuals are admixed (Evanno et al., 2005; Ostrowski et al., 2006). The distribution of the 394 accessions that shared at least 70% ancestry with 1 of the 3 inferred groups is summarized in Table 5. In addition to the groups identified by this analysis, 16% of the accessions showed evidence of mixed population ancestry. The mixture is likely the result of breeding, artificial selection, and domestication history, which have large effects on diversity. The independent population histories of the groups also shaped the gene pools (Garris et al., 2005). In this study, the F_{ST} values of the different groups ranged from 0.4197 between POP1 and POP2 to 0.3575 between POP2 and POP3 based on AMOVA, and the overall F_{ST} value was 0.2235, indicating moderate differentiation within South Korean rice landraces (Table 5). A similar result was observed in Korea soybean landraces (Burnham et al., 2002).

Rice quality is a complex trait that includes many components such as milling, appearance, nutrition, and cooking and eating qualities. Among these qualitative properties, consumers pay most attention to fine appearance and high eating quality (Huang et al., 1998; Zhao et al., 2012b). AC is related to the appearance and texture of rice, and it affects cooking and eating quality (Bao et al., 2006; Lu and Park, 2012). Hence, regulating the AC in rice has been

a major concern of rice breeders. The results of a simple paired *t*-test and correlation testing of 9 phenotypic traits and 5 physicochemical traits, including AC, are shown in Table 6. There were highly significant correlations ($P < 0.05$ or 0.01) among some traits, including PC, K^+, Mg^{2+}, and AC. These results agree with those of Zeng et al. (2005) and Jiang et al. (2007). By DMRT using the same 9 phenotypic and 5 physicochemical traits, significant differences were observed among the 3 groups in 10 qualitative and quantitative traits.

Landraces of rice are thought to be an intermediate stage in the domestication process from the wild ancestor to cultivated rice. Thus, they represent a unique and critical source of genetically variable traits that can serve as a resource for future rice improvement. Assessments of GD are an essential component in germplasm characterization and conservation. This preliminary study could be the first step toward more efficient conservation and utilization of rice landraces to broaden the genetic bases of commercially grown varieties.

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Supplementary material

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