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Analysis of genetic diversity and population structure of rice cultivars from Africa, Asia, Europe, South America, and Oceania using SSR markers

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ABSTRACT In this study, 29 simple sequence repeat (SSR) markers were used to analyze the genetic diversity and population structure of 125 rice accessions from 40 different origins in Africa, Asia, Europe, South America, and Oceania. A total of 333 alleles were detected, with an average of 11.5 per locus. The mean values of major allele frequency, expected heterozygosity, and polymorphism information content (PIC) for each SSR locus were 0.39, 0.73, and 0.70, respectively. The highest mean PIC was 0.71 for Asia, followed by 0.66 for Africa, 0.59 for South America, 0.53 for Europe, and 0.47 for Oceania. Model-based structure analysis revealed the presence of five subpopulations, which was basically consistent with clustering based on genetic distance. Some accessions were clearly assigned to a single population in which >70% of their inferred ancestry was derived from one of the model-based populations. In addition, 12 accessions (9.6%) were categorized as having admixed ancestry. The results could be used to understanding the genetic structure of rice cultivars from these regions and to support effective breeding programs to broaden the genetic basis of rice varieties.

Keywords : gene diversity, population structure, SSR, rice

As a staple cereal crop, rice (*Oryza sativa* L.) feeds more than 50% of the world's population (Mather *et al*., 2007) and is the most important component of human diet in many regions of the world (Zhao *et al*., 2009a). African cultivated rice was domesticated in the Niger River Delta about 3,500 years ago (Viguier, 1939) and is widely grown in West Africa today. New high-yielding varieties suitable for cultivation in West Africa (NERICA varieties) have recently been developed from interspecific crosses between *Oryza glaberrima* and *O. sativa* (http://www.warda.org). Three centers of domestication for *O. glaberrima*-Mali, Sene-Gambia, and Guinea (Portères, 1970)-may have contributed to the broad ecological adaptation of African rice cultivars today. Estimates of genetic diversity in *O. glaberrima* based on RFLP and isozyme markers are significantly lower than those in Asian cultivated rice, *O. sativa* (Second, 1982 & 1986; Wang *et al*., 1992). Asian cultivated rice (*O. sativa* L.) holds a unique position among domesticated crop species in that it is both a critical food staple and the first fully sequenced crop genome. It was domesticated from its wild ancestor about 11,500 years ago (Normile, 1997), and two centers of domestication have been identified: the *japonica* variety group and the *indica* variety group (Crawford & Chen, 1998; Garris *et al*., 2005). Recent evidence based on nuclear genome sequence comparisons suggests an ancient *indica/japonica* divide dating between 200,000 and 440,000 years ago; evidence based on chloroplast sequences suggests a divide dating between 86,000 and 200,000 years ago. Both of these estimates significantly predate the domestication of rice (Ma & Bennetzen, 2004; Tang *et al*., 2004; Vitte *et al*., 2004; Zhu & Ge, 2005). This deep population structure is readily apparent in rice landraces and improved varieties grown around the world. Representative samples of the global rice gene pool have been extensively studied using molecular markers, beginning with Glaszmann's pioneering study that used isozyme markers to identify six groups from within 1,688 accessions of *O. sativa* (Glaszmann, 1987). In Asian nations, people's lives revolve around the production of rice. Rice originated from Asia, making it more popular as a food source among Asian nations. It then spread through

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Europe and later into the Americas. Even today, many aspects of European culture have their basis on the cultivation of rice. In the Middle East and Mediterranean Europe, rice growing started around 800 BC and spread throughout Italy and then France after the middle of the 15th century, later propagating to all continents during the great age of European exploration. In 1694, rice arrived in South Carolina, probably originating from Madagascar. The Spanish brought rice to South America at the beginning of the 18th century.

Molecular markers, such as simple sequence repeats (SSRs) and simple sequence repeat polymorphisms (SSRPs) (Park *et al*., 2009) were valuable for developing markers in many kinds of plants, such as *Lycium barbarum* (Kwon *et al*., 2009), garlic (*Allium sativum* L.) (Ma *et al*., 2009), *Perilla frutescens* Britt (Park *et al*., 2008), *Amaranthus hypochondriacus* (Lee *et al*., 2008) and rice (Yang *et al*., 1994; McCouch *et al*., 1997; Ishii & McCouch, 2000; Ishii *et al*., 2001). SSRs provide much greater resolution than other markers in terms of interpreting population structure because of the high level of polymorphism at SSR loci (Cho *et al*., 2000; Akkaya *et al*., 1992). SSR markers for cultivated rice have been developed (McCouch *et al*., 2001), and studies have demonstrated the applicability of SSR alleles for determining both intra- and interpopulation genetic structures (Kuroda *et al*., 2003).

Studies based on the genetic diversity of rice cultivars from Asian and African countries have demonstrated that the genetic diversity of *O. glaberrima* is significantly lower than that of *O. sativa* (Second, 1982 & 1986; Wang *et al*. 1992). But analyses of the genetic diversity and population structure of rice cultivars from Africa, Asia, Europe, South America, and Oceania have not been reported. The objective of this study was to compare genetic diversity and analyze population structure among cultivars from the five continents.

MATERIALS AND METHODS

Plant materials

A total of 125 rice accessions were selected by their origins including Africa, Asia, Europe, South America, and Oceania (Table 1). All accessions were obtained from the National Agrobiodiversity Center of Rural Development Administration (http://genebank.rda.go.kr), Republic of Korea.

Table 1. The 125 rice accessions used in this study.

No.	Code	IT*	Variety	Country	Region
1	1/DZA	202338	Harra	Algeria	Africa
2	2/CMR	202359	M-52	Cameroon	Africa
3	3/CAF	212389	TT2	Central African Republic	Africa
4	4/CIV	152693	Col1/M312A	Cote D'ivoire	Africa
5	5/EGY	000314	Bellardone	Egypt	Africa
6	6/EGY	003110	Nahda	Egypt	Africa
7	7/EGY	003686	Sabinu	Egypt	Africa
8	8/GIN	002869	La-Plate-Gualeyan F.A	Guinea	Africa
9	9/LBR	152647	Yupul	Liberia	Africa
10	10/MDG	000093	Alicombo	Madagascar	Africa
11	11/MWI	213535	FRX92F3B-14F4BF5	Malawi	Africa
12	12/MLI	003498	IRRI9011	Mali	Africa
13	13/NGA	000041	Agbede	Nigeria	Africa
14	14/NGA	000343	Bikom	Nigeria	Africa
15	15/NGA	212310	Upland	Nigeria	Africa
16	16/SEN	001828	Iguape Cateto	Senegal	Africa
17	17/SEN	003010	Miro-Miro	Senegal	Africa
18	18/SLE	152691	Ngovie	Sierra Leone	Africa
19	19/AFG	000342	Bicol	Afghanistan	Asia
20	20/AFG	001142	Dondunikunluz	Afghanistan	Asia
21	21/AFG	002420	Kalakho Shakunday	Afghanistan	Asia
22	22/AFG	152642	Spin Mere	Afghanistan	Asia
23	23/AZE	207403	Bakshish-Ali	Azerbaijan	Asia

Table 1. The 125 rice accessions used in this study(Continued).

No.	Code	IT*	Variety	Country	Region
24	24/AZE	207419	Akula	Azerbaijan	Asia
25	25/IRN	000103	Amber-Bau	Iran	Asia
26	26/IRN	001418	Gardeh Shal Shal	Iran	Asia
27	27/IRN	001524	Had Saduri	Iran	Asia
28	28/IRN	003553	Red Rice	Iran	Asia
29	29/IRN	212352	Hassany	Iran	Asia
30	30/IRQ	000101	Ambarbau	Iraq	Asia
31	31/KAZ	202322	Coyenye	Kazakhstan	Asia
32	32/KAZ	207407	Xi-Muke	Kazakhstan	Asia
33	33/KGZ	207408		Kyrgyzstan	Asia
34	34/PAK	000666	Charnock	Pakistan	Asia
35	35/PAK	000672	Chen Chu Yai	Pakistan	Asia
36	36/PAK	001082	Dharial	Pakistan	Asia
37	37/PAK	002627	Kataktara	Pakistan	Asia
38	38/PAK	003068	Mushkan	Pakistan	Asia
39	39/PAK	003482	Pukhi	Pakistan	Asia
40	40/PAK	003490	Pusur	Pakistan	Asia
41	41/PAK	212357	Lateefy	Pakistan	Asia
42	42/PHL	001883	IR24	Philippines	Asia
43	43/PHL	001894	IR29	Philippines	Asia
44	44/PHL	001898	IR30	Philippines	Asia
45	45/PHL	001902	IR34	Philippines	Asia
46	46/PHL	001904	IR36	Philippines	Asia
47	47/PHL	001907	IR38	Philippines	Asia
48	48/PHL	123531	IR54	Philippines	Asia
49	49/PHL	123532	IR56	Philippines	Asia
50	50/PHL	123533	IR58	Philippines	Asia
51	51/PHL	123537	IR6	Philippines	Asia
52	52/PHL	191695	IR60	Philippines	Asia
53	53/PHL	191696	IR64	Philippines	Asia
54	54/PHL	192045	IR65	Philippines	Asia
55	55/TWN	000349	Bir-Co-Chin-Yu	Taiwan	Asia
56	56/TJK	207417	N 721	Tajikistan	Asia
57	57/ARE	000132	Araby	United Arab Emirates	Asia
58	58/ARE	003683	Sabieny	United Arab Emirates	Asia
59	59/UZB	216884	Avangard	Uzbekistan	Asia
60	60/UZB	216430	Uzrose	Uzbekistan	Asia
61	61/UZB	217508	Debzera	Uzbekistan	Asia
62	62/UZB	217518	Akurook	Uzbekistan	Asia
63	63/BGR	004056	Sukhuwell	Bulgaria	Europe
64	64/FRA	000056	Ailorio Lambda	France	Europe
65	65/FRA	000108	Americano 1600	France	Europe
66	66/FRA	000631	Cesariot	France	Europe
67	67/FRA	000924	Cigalon	France	Europe
68	68/FRA	003595	Rinaldo Bersano	France	Europe
69	69/HUN	000182	Arpa	Hungary	Europe
70	70/HUN	000465	Bulgare	Hungary	Europe
71	71/HUN	001242	Eiko	Hungary	Europe
72	72/HUN	004686	Zoeow Shani	Hungary	Europe
73	73/HUN	202330	Cgi-Csing	Hungary	Europe
74	74/ITA	000141	Arborio	Italy	Europe
75	75/ITA	000165	Ardizzone	Italy	Europe

Table 1. The 125 rice accessions used in this study(Continued).

No.	Code	IT*	Variety	Country	Region
76	76/ITA	000279	Balilla	Italy	Europe
77	77/ITA	000555	Carnaroli	Italy	Europe
78	78/ITA	003435	Pierrot	Italy	Europe
79	79/ITA	003521	Rafaello	Italy	Europe
80	80/ITA	003584	Rialto	Italy	Europe
81	81/ITA	003587	Ribe	Italy	Europe
82	82/ITA	003597	Rinenosso	Italy	Europe
83	83/ITA	003599	Ringo	Italy	Europe
84	84/ITA	003610	Rizzoto	Italy	Europe
85	85/ITA	003631	Rove Sbella	Italy	Europe
86	86/ITA	003835	Sasia	Italy	Europe
87	87/ITA	004320	Tgostano	Italy	Europe
88	88/PRT	000094	Allorio	Portugal	Europe
89	89/PRT	000098	Alorna	Portugal	Europe
90	90/PRT	002899	Lusitano	Portugal	Europe
91	91/PRT	003451	Portuguse	Portugal	Europe
92	92/PRT	195123	Magos	Portugal	Europe
93	93/PRT	195124	Lusito	Portugal	Europe
94	94/RUS	179824	Novosel Skii	Russian Federation	Europe
95	95/RUS	179825	Spal Chik	Russian Federation	Europe
96	96/RUS	179826	Solnechnyi	Russian Federation	Europe
97	97/RUS	179827	Solyaris	Russian Federation	Europe
98	98/RUS	179828	Kulon	Russian Federation	Europe
99	99/RUS	179829	Liman	Russian Federation	Europe
100	100/RUS	195079	Liman Belozernij	Russian Federation	Europe
101	101/RUS	207414	Xokkaido	Russian Federation	Europe
102	102/RUS	207438	Novoselskii	Russian Federation	Europe
103	103/ESP	000326	Benlloch	Spain	Europe
104	104/ESP	000411	Bombilla	Spain	Europe
105	105/ESP	000413	Bombon	Spain	Europe
106	106/ESP	000946	Colina	Spain	Europe
107	107/ESP	003995	Sollana	Spain	Europe
108	108/TUR	152676	Rikuki	Turkey	Europe
109	109/UKR	801877	WIR6299	Ukraine	Europe
110	110/DOM	002345	JUMA 1	Dominican Republic	South America
111	111/HTI	001825	Iguape Cateto	Haiti	South America
112	112/SUR	001550	Hashikalmi	Suriname	South America
113	113/SUR	152695	Ceysvoni	Suriname	South America
114	114/SUN	000102	Ambariru Belyi	Suriname	South America
115	115/SUN	002298	Jahanov	Suriname	South America
116	116/SUN	002621	Kasaki-Shalo	Suriname	South America
117	117/SUN	004682	Zeravschanica Karatalski	Suriname	South America
118	118/SUN	004683	Zilanica	Suriname	South America
119	119/ZAR	152689	OS 6	Suriname	South America
120	120/AUS	000116	Anbar Y 4704	Australia	Oceania
121	121/AUS	207636	Goolarah	Australia	Oceania
122	122/AUS	207660	Kyeema	Australia	Oceania
123	123/AUS	210125	Illabong	Australia	Oceania
124	124/AUS	808160	Amaroo	Australia	Oceania
125	125/FJI	003512	Rameajara	Fiji	Oceania

*: Introduction No. in the National Agrobiodiversity Center of Rural Development Administration (RDA).

DNA extraction and SSR assay

DNA was extracted from freeze-dried leaves of 15-day-old seedlings of each accession using a DNA extraction kit (Qiagen). The relative purity and concentration of extracted DNA was then checked using NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). Finally, DNA concentration was adjusted to 20 ng/μl.

In total, 29 microsatellite markers were chosen according to their location on the rice genetic map and their suitability for high-throughput genotyping (Table 2). All SSR marker information is available in GRAMENE (http://www.gramene.org/). Two different marker types were used for SSR assays. A three-primer system was used that included a universal M13 oligo-nucleotide (TGTAAAACGACGGCCAGT) labeled with the fluorescent dye 6-FAM (blue), NED (green), or HEX (yellow), which allows PCR products to be triplexed during electrophoresis. A special forward primer composed of the concatenation of the M13 oligo-nucleotide and the specific forward primer was used with the normal reverse primer for SSR PCR amplification.

Table 2. Size range, total number of alleles, major allele frequency, expected heterozygosity, and polymorphism information content index for 29 simple sequence repeat loci in 125 accessions from five continents.

Marker	Size Range	N_A[a]	M_{AF}[b]	H_E[c]	PIC[d]
RM21	125-169	18.0	0.17	0.90	0.88
RM44	97-145	14.0	0.20	0.87	0.85
RM48	179-205	5.0	0.45	0.65	0.58
RM206	123-229	36.0	0.11	0.95	0.95
RM214	109-181	20.0	0.25	0.89	0.87
RM228	103-187	19.0	0.26	0.86	0.84
RM231	164-192	9.0	0.32	0.80	0.76
RM232	119-187	24.0	0.16	0.92	0.91
RM235	89-133	12.0	0.41	0.76	0.73
RM241	90-130	14.0	0.21	0.87	0.85
RM246	90-114	9.0	0.23	0.83	0.81
RM247	125-197	24.0	0.21	0.90	0.89
RM249	117-145	11.0	0.26	0.82	0.79
RM253	112-140	9.0	0.33	0.81	0.78
RM257	140-170	12.0	0.32	0.83	0.81
SBE	196-218	5.0	0.46	0.63	0.55
SSS	193-215	5.0	0.73	0.45	0.42
WxOligo	100-218	9.0	0.40	0.77	0.73
RM310	146-188	14.0	0.24	0.88	0.86
RM3322	118-128	5.0	0.67	0.51	0.48
RM3718	148-166	8.0	0.51	0.67	0.64
RM3857	120-160	14.0	0.18	0.87	0.86
RM6144	135-141	2.0	0.65	0.45	0.35
RM6165	170-194	2.0	0.73	0.40	0.32
RM6629	59-83	7.0	0.53	0.66	0.62
RM12676	252-256	2.0	0.73	0.40	0.32
RM16427	271-289	5.0	0.37	0.70	0.64
RM19159	159-193	12.0	0.44	0.77	0.75
RM23455	290-314	7.0	0.70	0.47	0.42
Mean		11.5	0.39	0.73	0.70
Total		333.0			

[a] Number of alleles.
[b] Major allele frequency.
[c] Expected heterozygosity.
[d] Polymorphism information content.

The fluorescent-labeled microsatellite markers were subjected to PCR amplification in a total volume of 20 μl containing 20 ng/μl genomic DNA, 4 μl of each primer, 10 μl 1×PCR buffer, 0.1 μl dNTP, and 0.5 μl *Taq* polymerase. The PCR profile used was 1 cycle at 94°C for 3min, followed by 30 cycles at 94°C for 30s, annealing temperature at 72°C for 45s, and a final extension at 72°C for 15 min. In the case of the M13 tail PCR method, PCR amplification was performed in a total volume of 20 μl containing 20ng genomic DNA, 2 μl of the specific primer, 4 μl M13 universal primer, 6 μl normal reverse primer, 1×PCR buffer, 0.2 μl dNTP, and 0.5 μl *Taq* polymerase. Processes of the PCR amplification were as follows: 94°C for 3 min; 30 cycles at 94°C for 30s, the appropriate annealing temperature for 45s, and 72°C for 45s; followed by 20 cycles at 94°C for 30s, 53°C for 45s, 72°C for 45s; and a final extension at 72°C for 20 min. The PCR products of the three microsatellites were mixed together in a ratio of FAM:HEX:NED = 1:3:4, which was varied depending on the amplification intensity for individual markers as determined on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR products labeled with HEX and NED were added in higher amounts and those labeled with FAM were added in lower amounts because of the different signal intensities of these fluorescent dyes. The mixed PCR product of 1.5 μl was combined with 9.2 μl Hi-Di formamide and 0.3 μl of an internal size standard, Genescan-500 ROX. All samples were denatured at 94°C for 3 min and analyzed in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Molecular weights, in base pairs, of microsatellite products were estimated using Genescan software version 3.7 (Applied Biosystems) using the local Southern method. The individual fragments were assigned as alleles of the appropriate microsatellite loci with Genotyper software version 3.7 (Applied Biosystems).

Data analysis

Basic statistics for diversity measurements at each microsatellite locus, including the total number of alleles (N_A), allele frequency, major allele frequency, gene diversity (GD), and polymorphism information content (PIC), were calculated using the genetic analysis package PowerMarker V3.25 (Liu & Muse, 2005). Genetic distances between each pair of accessions were measured by calculating the shared allele frequencies using PowerMarker V3.25. The neighbor-joining algorithm was used to construct an unrooted phylogram from a distance matrix using MEGA4 software (Tamura *et al*., 2007).

Population structure was determined and identification of admixed individuals performed using the model-based software program STRUCTURE (Pritchard *et al*., 2000). 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 (clusters) 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 a Markov Chain Monte Carlo method. The Markov Chain Monte Carlo chains were run for 100,000 burn-in period lengths, followed by 200,000 iterations using a model allowing for admixture and correlated allele frequencies. An individual was assigned to a group if more than 70% of its genome fraction value derived from that group.

RESULTS

SSR polymorphism

The 29 SSR markers revealed 333 alleles among the 125 rice accessions representing the five continents (Table 1). The accessions were collected from 40 different countries throughout Africa, Asia, Europe, South America, and Oceania, representing a variety of ecological zones. The allelic richness per locus varied widely among the markers, ranging from 2 (RM6144, RM6165, RM12676) to 36 (RM206) alleles, with an average of 11.5 alleles. The database of allelic frequencies showed that rare alleles (frequency <0.05) composed 57.1% of all alleles, whereas intermediate (frequency =0.05–0.50) and abundant (frequency >0.50) alleles composed 40.5% and 2.4% of all alleles, respectively. This demonstrated that there was a large proportion of rare alleles among all detected alleles and that most alleles were of low frequency (Table 2, Fig. 1). The frequency of major alleles per locus and the expected heterozygosity varied from 0.11 (RM206) to 0.73 (SSS) and from 0.34 (RM6165) to 0.95 (RM206), with an average of 0.39 and 0.73, respectively. PIC values ranged from 0.32 (RM6165) to 0.95 (RM206), with an average of 0.70 (Table 2).

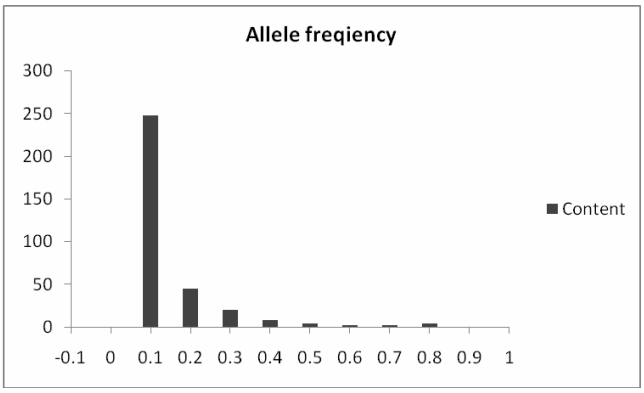


Fig. 1. Histogram of allele frequencies for all 333 alleles in the 125 rice accessions.

Table 3. Number of accessions, number of alleles, major allele frequency, and polymorphism information content of the regions.

Region	N_A[a]	N_A[b]	M_{AF}[c]	GD[d]	PIC[e]
Africa	18	6.2	0.41	0.70	0.66
Asian	44	8.7	0.38	0.74	0.71
Europe	47	6.2	0.55	0.57	0.53
South America	10	4.3	0.47	0.63	0.59
Oceania	6	3.0	0.57	0.52	0.47

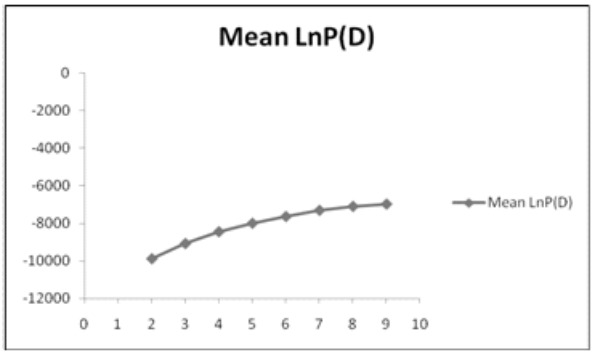
[a] Number of accessions.
[b] Number of alleles.
[c] Major allele frequency.
[d] Genetic diversity.
[e] Polymorphism information content.

Geographical analysis of diversity

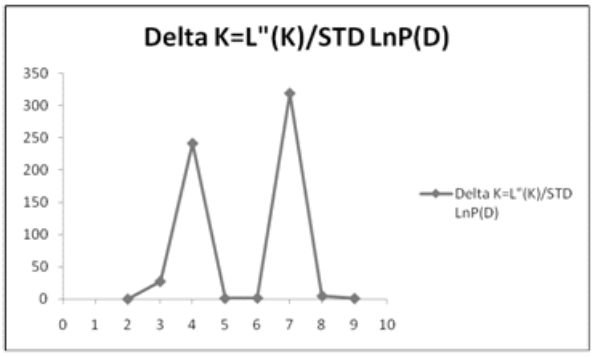
The number of accessions, number of alleles, major allele frequency, genetic diversity, and PIC of the five regions are shown in Table 3. The mean PIC values for each SSR locus in Africa, Asia, Europe, South America, and Oceania were 0.66, 0.71, 0.53, 0.59, and 0.47, respectively. The average number of alleles for each region was 6.2, 8.7, 6.2, 4.3, and 3.0, respectively. The tendencies of PIC and average number of alleles were in the order Asia > Africa > South America > Europe > Oceania and Asia > Africa = Europe > South America > Oceania, respectively. It was found that Asian rice cultivars had the highest level of PIC, gene diversity and average number of allele, but lowest level of major allele frequency. While Oceanian rice cultivars was the opposite.

Population structure analysis

When the 125 accessions were analyzed for population



(a)



(b)

Fig. 2. (a) Log-likelihood of the data (n =125), L(K), as a function of K (number of groups used to stratify the sample). (b) Values of ΔK, with its modal value used to detect the true K of the four groups (K=4)

structure using a model-based approach (Pritchard *et al*., 2000), the estimated likelihood values for a given K in three independent runs yielded consistent results, but the inference of the exact value of K (gene pool) was not straightforward. Thus, another ad hoc quantity (ΔK) was used (Evanno *et al*., 2005) to overcome the difficulty of interpreting the real K value. The relatively high value of ΔK for 125 accessions were for K = 4 and K= 7 (Fig. 2). Clustering bar plots with K = 4 and K = 7 were shown in Fig. 3. At K = 4, all 150 accessions were divided into 4 clusters. At K = 7, accessions belonging to S2 and S3 divided into 3 and 2 groups, respectively. However, the highest number of accessions assigned to one specific cluster with a probability higher than 90% was obtained with k = 4, while with K = 7 this percentage dropped to 84% of the total number of accessions, thus indicating the presence of complex relationships among accessions. Analysis of these data identified the major substructure groups when the number of clusters was set at 4 with the relatively high value of ΔK and high probability of accessions assigned to one

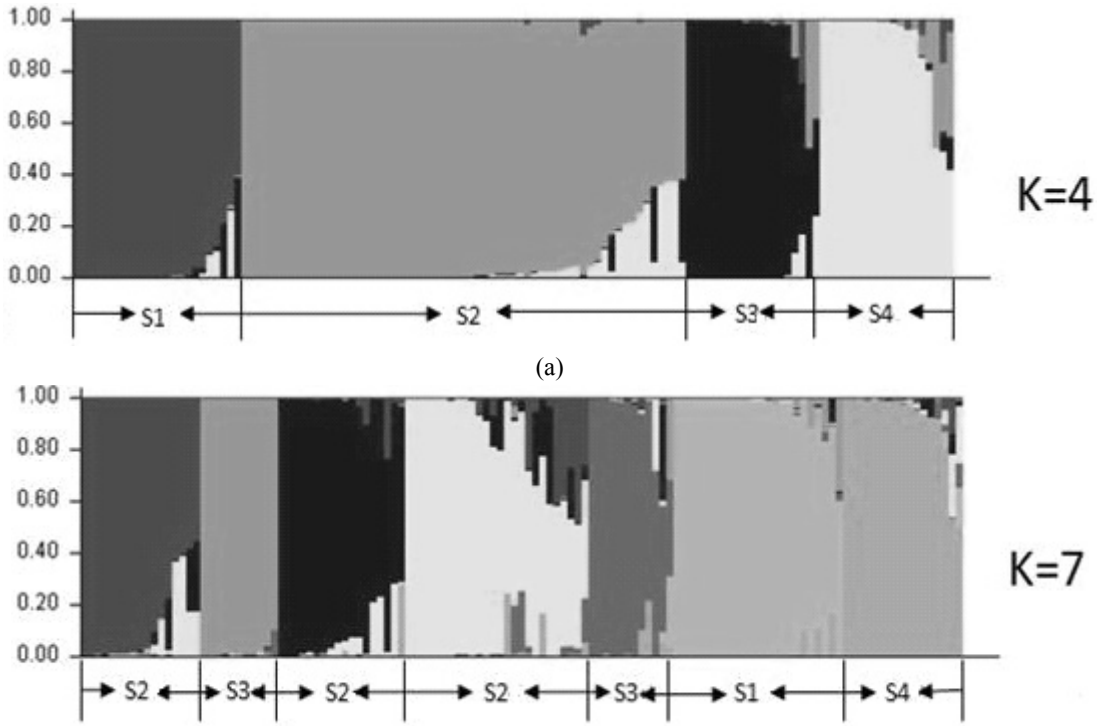


Fig. 3. Model-based clustering for each of the 125 rice landrace accessions examined based on the 29 SSR markers used to build the Q matrix.

specific cluster (Maccaferri *et al*., 2005).The relatively small value of the alpha parameter (α = 0.0365) indicated that most accessions originated from one primary ancestor, with a few admixed individuals (Ostrowski *et al*., 2006).

Of the 125 rice accessions, 113 (90.4%) shared >70% ancestry with one of the four subpopulations and were classified as members of that cluster, whereas the other 12 (9.6%) were admixture forms with varying levels of membership among the five clusters. Cluster 1 was represented by 23 accessions (colored red in Fig. 4) and one admixture (colored black in Fig. 4) that represented accessions collected mainly in Asia (17) and a few in Africa (2), South America (1) and Oceania (1). Cluster 2, with 58 individuals (colored green in Fig. 4) and five admixtures, represented accessions collected mainly in Europe (40) and a few in Africa (3), Asia (9), South America (4) and Oceania (2). Cluster 3 consisted of 16 accessions (colored blue in Fig. 4) and three admixtures mainly from Asia (10), with a few from Africa (1), Europe (2), South America (2) and Oceania (1). Cluster 4 consisted of 16 accessions (colored yellow in Fig.4) and three admixtures that represented accessions collected mainly in Africa (8)

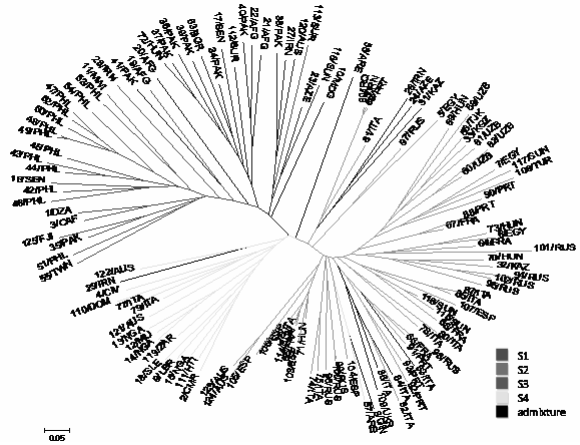


Fig. 4. UPGMA dendrogram based on a genetic distance matrix among 125 rice accessions from 40 countries on five continents.

and a few in Asia (1), Europe (2), South America (3) and Oceania (2). Thus, Clusters 2 and 4 consisted mainly of African and European rice cultivars, respectively. Clusters 1 and 3 were all primarily composed of Asian rice cultivars.

In addition to accessions that were clearly assigned to a single population from which >70% of their inferred ancestry

Table 4. Number of accessions, number of alleles, major allele frequency, genetic diversity, and polymorphism information content of the clusters.

Cluster	N_A[a]	N_A[b]	M_{AF}[c]	PIC[d]
1	23	4.6	0.59	0.49
2	58	6.0	0.56	0.53
3	16	4.6	0.55	0.54
4	16	4.0	0.57	0.49

[a] Number of accessions.
[b] Number of alleles.
[c] Major allele frequency.
[d] Polymorphism information content.

was derived from one of the model-based populations, 12 accessions (9.6%) were categorized as having admixed ancestry.

A comparison of PIC and major allele frequency in the five clusters is shown in Table 4. Clusters 1 and 3 (Asian rice cultivars) had high PIC values and major allele frequency. Cluster 2 (European rice cultivars) had the highest average number of alleles (6.0).

DISCUSSION

Genetic structure has previously been documented in rice (Glaszmann, 1987; Parsons *et al*., 1999; Ni *et al*., 2002), but the present analysis combined a large number of accessions (125) from five different continents with a large number of loci (29). The results show that Asian rice has the highest PIC and number of alleles, followed by African rice, which is consistent with the results of Wang *et al*. (1992). These values are intermediate for European cultivars and low for Oceanian cultivars. This is because rice originated in Asia and later spread to Europe, South America, and Oceania.

Model-based methods, such as those reported by Pritchard and colleagues (Pritchard *et al*., 2000; Pritchard & Donnelly, 2001), use a Bayesian clustering approach in which each group or population is characterized by a set of allele frequencies at each locus along with the likelihood for each K (number of groups). Model-based methods have been widely used to identify population structure for association mapping in human genetics (Pritchard & Rosenberg, 1999; Pritchard & Donnelly, 2001; Pritchard & Przeworski, 2001; Rosenberg *et al*., 2002) and, to a lesser extent, plant genetics (Remington *et al*., 2001; Thornsberry *et al*., 2001).

The model-based structure analysis classified four clusters (S1–S4) in the selected accessions, with similar patterns of groupings of accessions discovered (Fig. 3). The degree of admixture, alpha ($\alpha = 0.0365$), was inferred from the data. When alpha is close to 0, most individuals are from one population, whereas when alpha is greater than 1, most individuals are admixed (Evanno *et al*., 2005; Ostrowski *et al*., 2006). The 12 admixtures found here showed evidence of mixed population ancestry. Because rice is a selfing species, genetic variability is available for breeding programs. Because rice spread throughout Europe, it shaped a defined gene pool, which had the highest average number of alleles (6.0) among the clusters. Clusters 1 and 3 were mainly Asian rice cultivars, which demonstrated that Asian cultivated rice evolved into two groups during the domestication and selection process in the selected core set. And it is commonly recognized that cultivated rice has evolved into two subspecies, *indica* and *japonica* (Wangand & Tanksley, 1989; Nakano *et al*., 1992; Zhang *et al*., 1992; Sano & Morishima, 1992). But the latest research reported that Asian cultivated rice from Korea, China, and Japan, which was selected randomly, was classified into three subpopulations, in which *japonica* divided into two subgroups based on genetic distance (Zhao *et al*., 2009b). Cluster 4 was mainly African rice cultivars. The admixtures shared high ancestry with these (Fig. 3), but the PIC was relatively lower (Table 4).

We conclude that all accessions from the five regions can be divided into four clusters by model-based structure analysis and that three of the clusters are mainly from Asia and the other primarily from Africa and Europe. Rice cultivars from South America and Oceania belong to all four clusters and have not evolved into separate clusters, suggesting that more accessions should be selected. These results can be used to explore the origins of these regional rice cultivars and to design effective breeding programs aimed at broadening the genetic basis of commercially grown varieties.

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